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The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: Methods/validation of a novel approach

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Summary

The influence of ethanol on the in vitro transport behavior of some lipophilic and polar/ionic permeants in hairless mouse skin has been investigated over a 0–100% ethanol/saline concentration range in a two-chamber diffusion cell. The lipophilic permeants were employed in probing the transport enhancing effects of ethanol upon the lipoidal pathway of the stratum corneum, and the polar/ionic permeants were used to quantify the influence of ethanol on the pore pathway of the stratum corneum over the entire range of ethanol concentrations. The following were the important interpretations of the data. The lipophilic permeants (estrone, β-estradiol, and hydrocortisone) were mainly transported via the lipoidal pathway up to around 50% ethanol. The permeation enhancement factor, E, for the lipoidal pathway was calculated from the transport data for the three lipophilic permeants. In order to calculate the E values, it was first necessary to establish the validity of Henry's law by comparing the ratios of permeant solubilities (in different ethanol/saline solutions) to ratios of permeant partition coefficients (in hexadecane/ethanol-saline systems). The calculated E values were found to be about the same for all three permeants: $E = 7.0 \pm 2.0$ at 25% ethanol and $E = 112 \pm 19$ at 50% ethanol. These large enhancing effects of ethanol upon the lipoidal pathway were somewhat surprising, and it is suggested that ethanol ($< 50\%$) may work as an effective 'fluidizing' agent at some locus in the stratum corneum lipid bilayer at or near the polar head plane, but not in the bilayer hydrocarbon interiors. The polar/ionic permeants (tetraethylammonium bromide, mannitol, estrone ammonium sulfate, and vidarabine) all were transported via the pore pathway at all ethanol concentrations. Ethanol up to around 25% had little effect upon the pore pathway; however, at higher concentrations ($\sim 50\%$), ethanol greatly enhanced pore transport and, at very high ethanol levels ($\gtrsim 75\%$), the pore pathway appeared to dominate the transport of all permeants including the lipophilic permeants.

Introduction

That ethanol may significantly alter biomembrane 'fluidity' has been a subject of interest

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to biological and pharmaceutical researchers (Scheuplein and Blank, 1973; Chin and Goldstein, 1977, 1981; Rowe, 1982, 1987; Berner et al., 1989; Kai et al., 1990; Knutson et al., 1990; Kurihara-Bergstrom et al., 1990). This idea has become of even greater interest to pharmaceutical scientists since ethanol has been found to have significant skin penetration enhancer action (Good et al., 1985; Ghanem et al., 1986, 1987a,b; Nathke et al., 1986; Bhargava and Kislalioglu, 1987; De Noble et al., 1987; Higuchi et al., 1987; Yum et al., 1987; Borsadia et al., 1990), and the concept has been recently incorporated into successful transdermal systems for β-estradiol (Campbell and Chandrasekaran, 1983), nitroglycerin (Gale and Berggren, 1986, 1987), and fentanyl (Gale et al., 1986).

Recently the influence of ethanol in aqueous solutions upon the transport behavior of several permeants in hairless mouse skin was investigated (Ghanem et al., 1987b). Permeability coefficients for β-estradiol, hydrocortisone, mannitol, and tetraethylammonium bromide (TEAB) were determined using a two-chamber cell both with full-thickness hairless mouse skin and with tape-stripped skin. The data were analyzed employing a two-layer model shown in Fig. 1 in which the stratum corneum barrier is in series with the epidermis/dermis layer and where the stratum corneum is represented by a lipoidal pathway in parallel with a pore pathway. The stripped skin data allowed 'correcting' for the epidermis/dermis contributions to the permeability coefficients, and this procedure yielded the stratum corneum permeability coefficients. As the data for mannitol and TEAB strongly suggested that these permeants are transported via the pore pathway of the stratum corneum, these two highly polar permeants were used to quantify the pore pathway; the pore pathway contributions were then subtracted from the stratum corneum permeability coefficients to yield the lipoidal pathway stratum corneum permeability coefficient (P_L) values for β-estradiol and for hydrocortisone. Finally, solubility data for the permeants obtained in saline and in ethanol/saline were used as the basis for converting the P_L values to E values, where E is the factor which accounts for the transport enhancement (relative to saline) via the lipoidal pathway resulting from the presence of ethanol. The conversion of P_L values to E values is equivalent to correcting for the activity coefficient difference between the permeant in saline and the permeant in the ethanol/ saline solution.

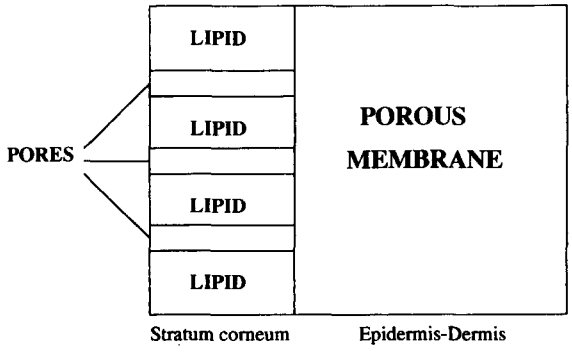

Fig. 1. Schematic diagram of the physical model for data analysis. The stratum corneum consists of parallel lipoidal and aqueous pore pathways and it is in series with the porous epidermis-dermis layer.

The purpose of the present report is two-fold. First, the question of whether Henry's law is obeyed at saturation solubility is important to the meaningful interpretation of the E values; accordingly a method using solvent-hexadecane (a reference oil phase) partition coefficients together with radiotracer level permeation data has been developed to carry out the P_L-to-E conversion on a basis which is independent of solubility experiments. It is to be pointed out that, if Henry's law is not obeyed, this may mean there is solute-solute association and an incorrect E-value may be deduced from the data; relying upon only solubility data to account for the activity coefficient difference between saline and the ethanol/ saline solution is, therefore, not without some risk. The second purpose of this study was to include more permeants with the hope that this would significantly add to the generalizability of the influences of ethanol on the transport behavior of permeants in hairless mouse skin. Two additional highly polar solutes, estrone ammonium sulfate (EAS) and vidarabine, and an addi-

tional lipophilic permeant, estrone, have been added to the investigation.

Considerations in the Determination of the Enhancement Factor, *E*

For the model shown in Fig. 1, the total permeability coefficient, P_T, for a solute is defined as

$$\frac{1}{P_T} = \frac{1}{P_{Sc}} + \frac{1}{P_{D/E}} \tag{1}$$

where P_{Sc} represents the permeability coefficient of the stratum corneum and $P_{D/E}$ is the permeability coefficient of the dermis and epidermis combination. The permeability coefficient of the stratum corneum, P_{Sc}, is assumed to be the summation of a lipoidal pathway and a pore pathway as follows,

$$P_{Sc} = P_L + P_P \tag{2}$$

where P_L denotes the permeability coefficient of the lipoidal pathway and P_P is the permeability coefficient of the pore pathway which may be determined experimentally using highly polar compounds such as TEAB, mannitol, EAS or vidarabine.

If the lipoidal pathway of the stratum corneum is treated as a homogeneous barrier, the permeability coefficient, P_L, may be defined as

$$P_L = \frac{KD}{h} \tag{3}$$

where K is the stratum corneum lipid-to-solvent partition coefficient, D represents the diffusion coefficient in the stratum corneum lipid and h is the effective thickness of the stratum corneum.

Consider now the situation involving a two-chamber diffusion cell experiment where the solvent compositions in the donor and the receiver chambers are the same. Let $P_{L,0}$ denote the permeability coefficient of the solute in the saline case and $P_{L,x}$ be the permeability coefficient of the solute when the solvent is ethanol/saline at a concentration, x. We may then write the following ratio:

$$\left(\frac{P_{L,x}}{P_{L,0}}\right) = \left(\frac{K_x}{K_0}\right) \cdot \left(\frac{D_x}{D_0}\right) \tag{4}$$

where K_x is the partition coefficient when the solvent is ethanol/saline mixture at an ethanol concentration of x, K_0 represents the partition coefficient when the solvent is saline, D_x is the diffusion coefficient in the stratum corneum lipid when the solvent is an ethanol/saline mixture at an ethanol concentration x and D_0 corresponds to the diffusion coefficient in the stratum corneum lipid when the solvent is saline.

We may further write

$$\frac{K_x}{K_0} = \left(\frac{K_x}{K_0}\right)_s \cdot F \tag{5}$$

Here $(K_x/K_0)_s$ is the hypothetical partition coefficient ratio which corresponds to the case where the solute partitioning tendencies in the stratum corneum lipid phase (at constant solute chemical potential) are the same for the saline and for the ethanol/saline cases. In general, however, the solute partitioning tendencies in the stratum corneum lipid phase may not be the same when saline is the solvent and when an ethanol/saline mixture is the solvent. F is the factor which corrects for this difference in the solute partitioning tendencies, and it may therefore have values other than unity.

By combining Eqns 4 and 5, we have:

$$F\left(\frac{D_x}{D_0}\right) = \left(\frac{P_{L,x}}{P_{L,0}}\right) \cdot \left(\frac{K_0}{K_x}\right)_s \tag{6}$$

The right side of Eqn 6 may be determined experimentally. $P_{L,x}$ and $P_{L,0}$ are obtained from the two-chamber diffusion cell experiments and by using Eqns 1 and 2. The ratio $K_R = (K_0/K_x)_s$, may be conveniently obtained in two ways. In the first method, as was done previously (Ghanem et

al., 1987b), this ratio may be obtained from solubility data according to

$$K_R = \frac{S_x}{S_0} \tag{7}$$

where S_x and S_0 are the solute solubilities in ethanol/saline and in saline, respectively. In the second method, which is reported here for the first time, K_R may be obtained by the relation

$$K_R = \frac{\acute{K}_0}{\acute{K}_x} \tag{8}$$

where $\acute{K}_0$ and $\acute{K}_x$ are the hexadecane-saline and the hexadecane-(ethanol/saline) partition coefficients, respectively, for the solute. Hexadecane was found as a suitable 'oil phase' for determining K_R via Eqn 8. It is non-volatile and did not emulsify under experimental conditions (ethanol/saline mixtures up to 50% ethanol).

The advantages in employing both solubility data (Eqn 7) and partition coefficient data (Eqn 8) in determining K_R are the following. K_R may be determined at radioactive tracer levels as well as at solubility saturation using Eqn 8. Thus, for example, if the value for K_R obtained using Eqn 8 at radioactive tracer levels agrees with that obtained using Eqn 8 at solubility saturation and with that determined using Eqn 7 and solubility data, one can be reasonably confident that Henry's law is being obeyed for this particular situation up to solubility saturation and that the value for K_R can be considered to be appropriate and meaningful in the calculation of the enhancement factor. On the other hand, if the agreement between the tracer results and those obtained at solubility saturation is not satisfactory, then Henry's law is not being obeyed in one or both of the solvents and/or something may be wrong with the data, the latter being likely if the K_R values obtained at solubility saturation using Eqns 8 and 7 do not agree well. If it appears that the discrepancy between the tracer results and those obtained at solubility saturation is simply due to Henry's law not being obeyed, then the tracer results may be used in the calculation of E (discussed below).

We may now define the left-hand side of Eqn 6 as the enhancement factor, E,

$$E = F \cdot \left(\frac{D_x}{D_0} \right) \tag{9}$$

The significance of E is that $E = 1$ when solvents have no intrinsic effects upon the membrane or, when solvents are compared, they have the same effect. E accounts both for thermodynamic effects through the factor, F, and for kinetic effects via the factor, D_x/D_0. While E may be determined when data from permeation, solubility, and partition coefficient experiments are available, other kinds of studies (e.g., spectroscopic studies) are needed for factoring out E in terms of F and D_x/D_0.

Materials and Methods

Materials

β-[^{3}H]Estradiol, [^{3}H]hydrocortisone, [^{3}H]-estrone, [^{14}C]TEAB and [^{14}C]mannitol (New England Nuclear, Boston, MA) were used after their purity was checked as specified by the manufacturer's method. [^{3}H]Estrone ammonium sulfate (New England Nuclear, Boston, MA) and [^{3}H]vidarabine (supplied as a gift from Dr David C. Baker, Department of Chemistry, University of Alabama, AL) were purified just before running the permeability experiment by TLC (silica gel sheets, 60F-254, MCB reagents, Gibbstown, NJ) using ethyl acetate : methanol : ammonia (75 : 25 : 2) and chloroform : methanol : ammonia (8 : 8 : 1) as solvents, respectively. Normal saline (McGaw, Irvine, CA) and ethanol (U.S. Industrial Chemical Co., Tuscola, IL) were used to prepare the solvent mixtures.

Solubility determination in ethanol / saline

The solubility data for the permeants in ethanol/saline mixtures were required in the calculation of K_R using Eqn 7 with the assumption that Henry's law is obeyed up to solubility satura-

tion. This assumption was later validated by comparing K_R using Eqn 8, partition coefficient data at trace levels, and K_R using Eqn 7, solubility data.

Excess amounts of unlabeled β-estradiol, estrone (Sigma Chemical Co., St. Louis, MO) or hydrocortisone (Fluka AG, Buchs, Switzerland) were introduced into 1.5 ml polypropylene microcentrifuge tubes containing 1 ml ethanol/saline mixtures and firmly sealed with parafilm (American Can Co., Greenwich, CT). The tubes were shaken at 100 rpm for 72 h in a thermostatically controlled water bath (Model YB-521, American Scientific) at 37 °C and then centrifuged at 2000 rpm (Fischer Micro Centrifuge, Model 235A) for 5 min. The drug solubilities were determined by HPLC from the clear supernatant solutions. The purity of a drug was evident from a sharp single peak. In the case of solubility determinations with β-estradiol in saline, the possibility of non-wetted floating particles was eliminated by filtration using a Gelman Metricel Membrane filter (GA-8, 13 mm, 0.2 μm) presaturated with β-estradiol in saline (to avoid estradiol adsorption on the filter membrane). The experiments were carried out in quadruplicate and repeated.

Determination of permeant partition coefficient

The objective of this study was to verify the validity of Henry's law by comparing K_R values at drug solubility saturation levels using Eqns 7 and 8 with K_R at very low concentrations (at radioactive tracer level, 10 000 dpm/0.1 ml which corresponds to concentrations far below solubility saturation) using Eqn 8. The K_R values were expected to be the same if Henry's law was obeyed in both the aqueous and oil phases (Yalkowsky et al., 1983; Jetzer et al., 1986; Sloan et al., 1986).

n-Hexadecane (99% pure, Sigma Chemical Co., St. Louis, MO) was selected as the oil phase after testing several nonvolatile oils as it did not emulsify under the experimental conditions. 8 ml of hexadecane were pre-equilibrated with 120 ml of ethanol/saline solvent in a tightly closed conical flask, sealed with parafilm and shaken at 100 rpm at 37 °C for 72 h. The oil phase was separated and similarly pre-equilibrated again with a fresh ethanol/saline solvent. A third pre-equilibration was found unnecessary as it yielded partition coefficients comparable to those with the second pre-equilibration. Experiments with [^{14}C]ethanol (as a marker) showed no loss of ethanol during the pre-equilibration process.

The partition coefficient experiment was conducted by adding 0.5 ml of hexadecane (pre-equilibrated with ethanol/saline) to 1.5 ml polypropylene microcentrifuge tubes containing either 0.5 ml ethanol/saline presaturated with unlabeled plus radiolabeled drug to give 10 000 dpm/0.1 ml (for saturated partition coefficient) or only the radiolabeled drug (for tracer level partition coefficient). The tubes were sealed with parafilm, shaken at 100 rpm at 37 °C for 72 h, and centrifuged at 2000 rpm for 5 min. Then 0.1 ml aliquots of both the oil and ethanol/saline phases were pipetted out, separately placed into a 10 ml liquid scintillation cocktail (Optifluor, Packard), and analyzed by liquid scintillation counting (Beckman LS 750). The partition coefficients were calculated from the ratio of the counts in the oil phase and in the ethanol/saline phase. The reproducibility of the experiment was good as the mass balance and the standard deviations were within the experimental error. The experiment was run in quadruplicate.

Solubility of permeants in hexadecane with and without solvent pre-equilibration

The purpose of this study was to provide data for checking and/or correcting the $\acute{K}_x$ and the $\acute{K}_0$ values (and therefore the K_R values) in Eqn 8 for the presence of ethanol and water in the hexadecane phase in the partitioning experiment. 2 ml of hexadecane pre-equilibrated with ethanol/saline solvent (as mentioned earlier) were transferred to a 4 ml glass vial with a slight excess of permeant crystals. The vials were then firmly sealed with parafilm and shaken for 72 h at 100 rpm at 37 °C, followed by centrifugation at 2000 rpm for 5 min. 1 ml of hexadecane was then extracted by shaking with 1 ml of 50% aqueous ethanol for 72 h at 100 rpm in a glass vial firmly sealed with parafilm. The drug content was determined in the ethanol extract by HPLC (Ghanem et al., 1987b). The efficiency of ethanol extraction

of the drug was tested by extracting the oil phase a second time to show that there was no solute remaining in the oil phase. The ratio of this solubility to that obtained in hexadecane without pre-equilibration provided the correction factor for K'_x.

Permeability experiments

For experiments with full-thickness skin, freshly separated male hairless mouse skin (strain SKH-HR1, 8–12 weeks old, Temple University, Pittsburgh, PA) obtained from the abdominal region and freed from adhering fat and other visceral debris was assembled in the two-chamber diffusion cell (Ghanem et al., 1987b). Two pieces of skin (for two permeability experiments) were obtained from each mouse. The donor and receiver compartments were filled with 2 ml of 0–100% ethanol-saline mixture, stirred at 150 rpm and equilibrated at 37 °C. The labeled solute was added to the donor chamber and samples were withdrawn from both compartments at predetermined time intervals after steady state was attained. Usually, 3-μl aliquots were taken from the donor chamber and 100- or 500-μl aliquots from the receiver chamber for liquid scintillation counting. The same volume of the fresh vehicle was added back to the receiver chamber to keep a constant volume. The receiver chamber was always maintained under sink conditions ($\leq$ 10% of the donor concentration). The permeant concentrations in the receiver chamber were corrected for sampling dilution and plotted as a function of time. The permeability coefficients were calculated from the slope of the linear portion of the curve and the average of the donor chamber concentration (Ghanem et al., 1987b).

For experiments with stripped skin, the permeability experiments were carried out in the same manner after stripping the abdominal skin 30 times using 3M Scotch tape (St. Paul, MN).

During the experiment with estrone or β-estradiol as permeants, especially in saline, a significant amount of the permeant was found to be adsorbed to the teflon stirrers. This adsorption problem was eliminated by using stainless steel stirrers (Carpenter stainless type 316L).

The purity of all compounds was checked by thin-layer chromatography (TLC) or HPLC prior to the experiments. All of the permeants were judged to be more than 98% pure, except EAS and vidarabine. These solutes were purified as described under Materials. The possibility that minute radiochemical impurities were important in the transport experiments was ruled out by HPLC or TLC analysis of the receiver chamber at the end of a run.

All of the permeants, except estrone and β-estradiol, were found to be stable during transport experiments and this was checked by analysis of the donor and receiver chambers at the end of the experiment. β-Estradiol is metabolized significantly to estrone, and estrone is moderately metabolized to β-estradiol and to other metabolites during its transport across hairless mouse skin with saline as the solvent. For both estrone and β-estradiol, metabolism is much less important in the presence of ethanol (Liu et al., 1986).

For permeants that undergo significant metabolism during transport through skin (e.g. estrone and β-estradiol), neglecting metabolism and back diffusion of the metabolite may lead to errors in the calculation of $P_{L,0}$ from the permeation data (Liu, 1989). A model based approach was developed to account and correct for the effects of metabolism. The corrected $P_{L,0}$ calculated from the model generally differed somewhat but not greatly from the $P_{L,0}$ calculated from total radioactivity transfer rates from donor to receiver (Liu et al., 1990).

Also recently, Liu et al. (1990) pointed out that, when the volume of the receiver chamber is small and when the permeant is highly lipophilic, a correction for dermis holdup (retention/partition) should be made if the receiver solution is not totally replaced at each sampling. In all of the present experiments, such corrections were applied to the flux data when necessary.

Results and Discussion

Determination of K_R

The results of K_R determinations for estrone, β-estradiol, and hydrocortisone are given in Figs

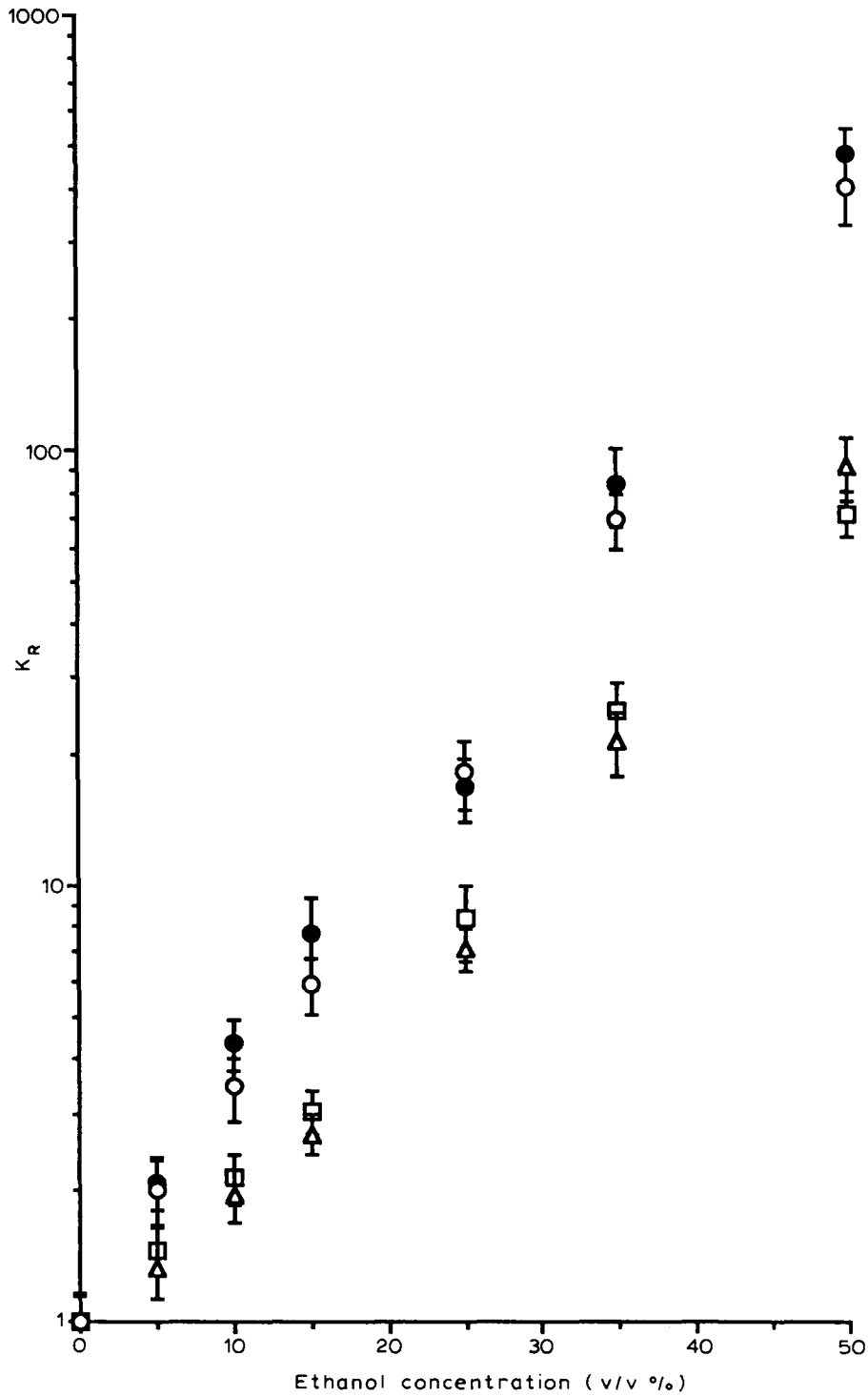

Fig. 2. K_R values for estrone. (□) Raw data from the partition coefficients at trace level and uncorrected for ethanol partitioning in hexadecane; (△) raw data from the partition coefficients at solubility saturation and uncorrected for ethanol partitioning in hexadecane; (●) K_R values based upon solubility data and (○) K_R values based upon the partition coefficients at trace levels and corrected for ethanol partitioning in hexadecane. Each data point represents the mean and standard deviation of 8 determinations.

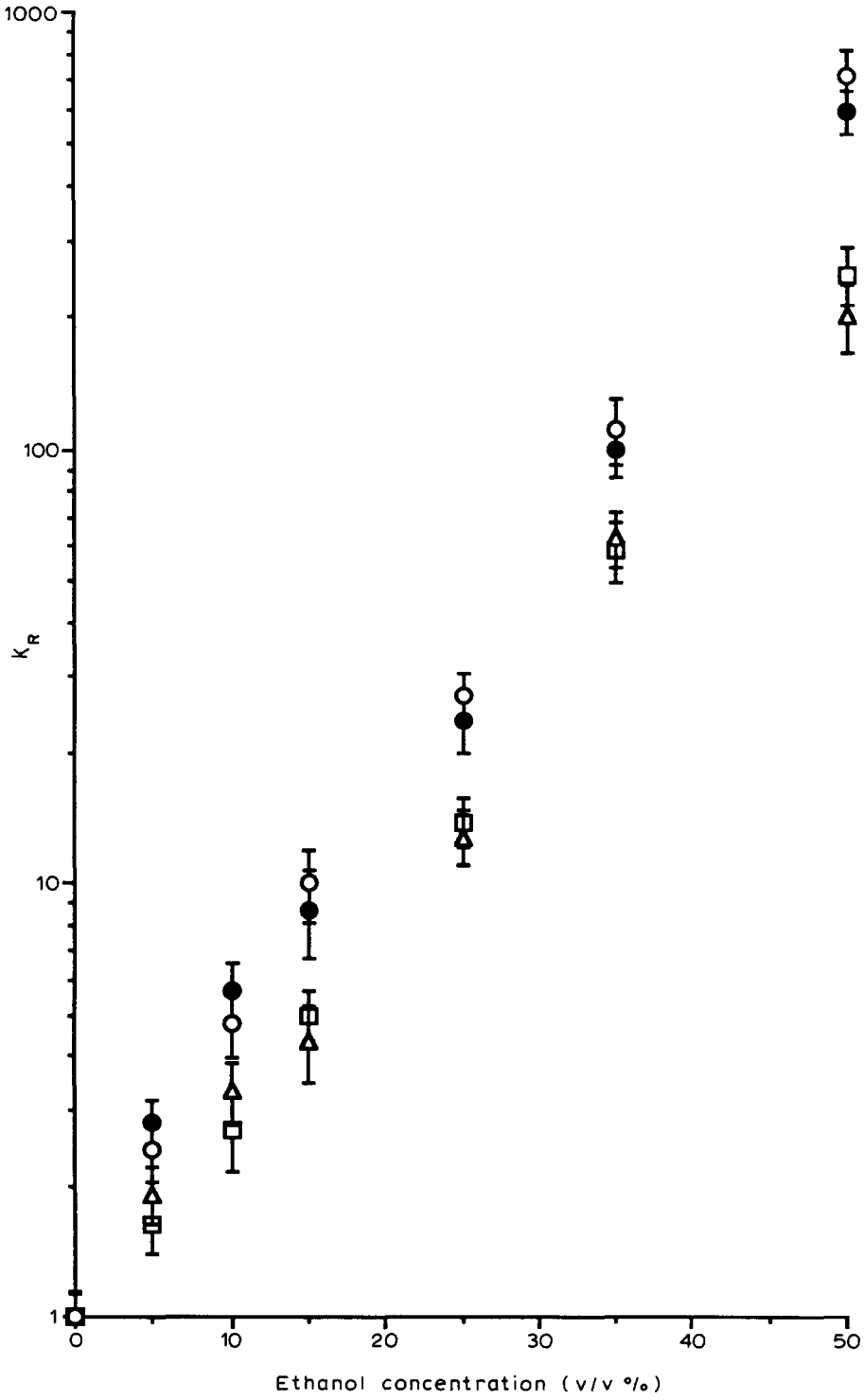

Fig. 3. K_R values for β-estradiol. (□) Raw data from partition coefficients at trace level and uncorrected for ethanol partitioning in hexadecane; (△) raw data from the partition coefficients at solubility saturation and uncorrected for ethanol partitioning in hexadecane; (●) K_R values based upon solubility data and (○) K_R values based upon the partition coefficients at trace levels and corrected for ethanol partitioning in hexadecane. Each data point represents the mean and standard deviation of 8 determinations.

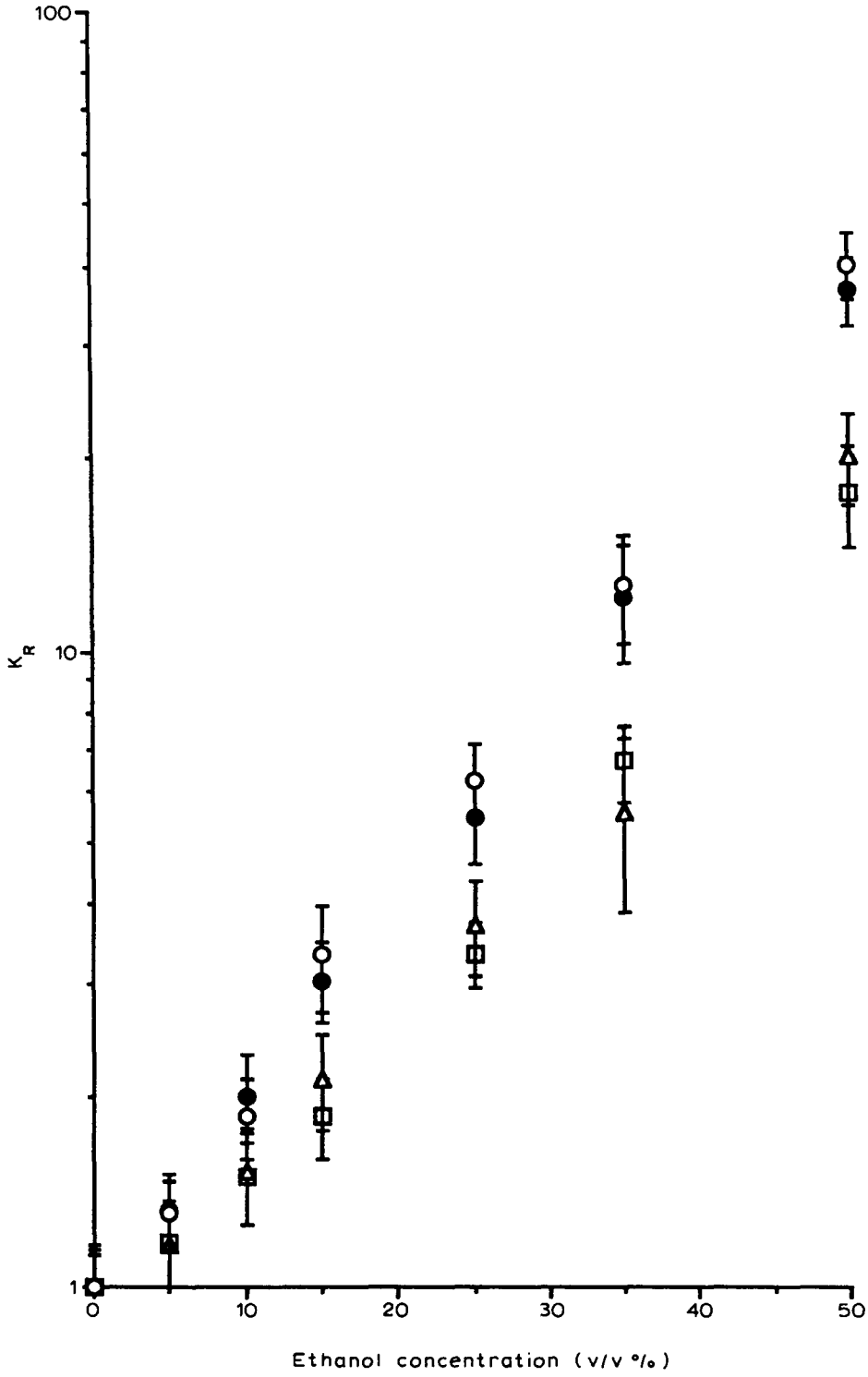

Fig. 4. K_R values for hydrocortisone. (□) Raw data from the partition coefficients at trace level and uncorrected for ethanol partitioning in hexadecane; (△) raw data from the partition coefficients at solubility saturation and uncorrected for ethanol partitioning in hexadecane; (●) K_R values based upon solubility data and (○) K_R values based upon the partition coefficients at trace levels and corrected for ethanol partitioning in hexadecane. Each data point represents the mean and standard deviation of 8 determinations.

2–4. Interestingly, K_R values obtained from the hexadecane partition experiments (Eqn 8) generally agreed rather well for all three solutes as shown by open squares and open triangles for trace and saturation solute levels, respectively, in Figs 2–4. However, these K_R values generally differed, to varying extents, from those obtained from solubility data using Eqn 7 as represented in Figs 2–4 by closed circles.

As it was thought that the differences between the K_R values determined from the solubility experiments and those obtained from the partitioning experiments were due to the transfer of ethanol into hexadecane, a correction procedure was devised utilizing data on permeant solubility in hexadecane with and without pre-equilibration with ethanol/saline. A correction factor, S_R, is defined as $S_R = C_{s,p}/C_s$ where $C_{s,p}$ is the permeant solubility in hexadecane pre-equilibrated with the ethanol/saline solution and C_s is the permeant solubility in hexadecane (without pre-equilibration). The plots of S_R vs ethanol concentration are presented in Fig. 5 for estrone, β-estradiol, and hydrocortisone. The true K_R values for the three permeants may then be obtained from the uncorrected K_R values according to

$$K_R(\text{true}) = K_R(\text{uncorrected}) \cdot S_R \qquad (10)$$

As can be seen in Figs 2–4, the corrected K_R values (open circles) are generally in good agreement with the K_R values obtained from the solubility data (closed circles). It is concluded from these results that (a) the corrected K_R values are reliable figures and (b) Henry's Law is likely obeyed in all of the phases – hexadecane, saline, and ethanol/saline – for all three permeants at solubility saturation in the 0–50% range of ethanol concentration. These K_R values may now be used with Eqn 6 in the calculation of the E values for estrone, β-estradiol, and hydrocortisone for different ethanol concentrations.

Influence of ethanol / saline on the permeability coefficients

Polar / ionic solutes with whole skin

Before we continue with the discussion of the influence of ethanol on the permeation behavior of polar/ionic solutes with whole skin, it should be instructive to review the evidence for the pore pathway in the stratum corneum (see Fig. 1).

First, let us note that, if the proposed model is correct, then from Eqns 1 and 2 we have

$$P_T = \frac{1}{\frac{1}{P_L + P_P} + \frac{1}{P_{D/E}}} \qquad (11)$$

For moderately polar permeants, Eqn 11 becomes

$$P_T \approx P_{Sc} = P_L + P_P \qquad (12)$$

and, for highly polar or ionic permeants, one may argue that $P_L \ll P_P$ and therefore

$$P_T \approx P_P \qquad (13)$$

Ackermann and Flynn (1987) have shown that glucose, urea, glycerol and thiourea all gave saline P_T values with hairless mouse skin of around 3×10^{-8} cm/s; this value is in very good agreement with the saline P_T values obtained in the present study (see Fig. 6) for EAS, vidarabine, mannitol and TEAB (we have not estimated polarities for these permeants; however, they ought to represent a large range). The work of Ackermann and Flynn is significant in that the permeants in their study were all highly polar and represented a 1000-fold range in ether-water partition coefficients, strongly suggesting that for those permeants $P_L \ll P_P$ and $P_T \approx P_P$.

More direct evidence for pores comes from electrical resistance studies of human epidermal membrane (Sims et al., 1991). In these studies, the passive permeability coefficient of mannitol (one of the polar permeants in the present research) was found to be inversely proportional to the electrical resistance for a large number of human epidermal membrane specimens, and the proportionality constant was found to be the same as that for a synthetic porous membrane (Nuclepore membrane). Other direct evidence for the pore pathway has been provided by iontophoresis

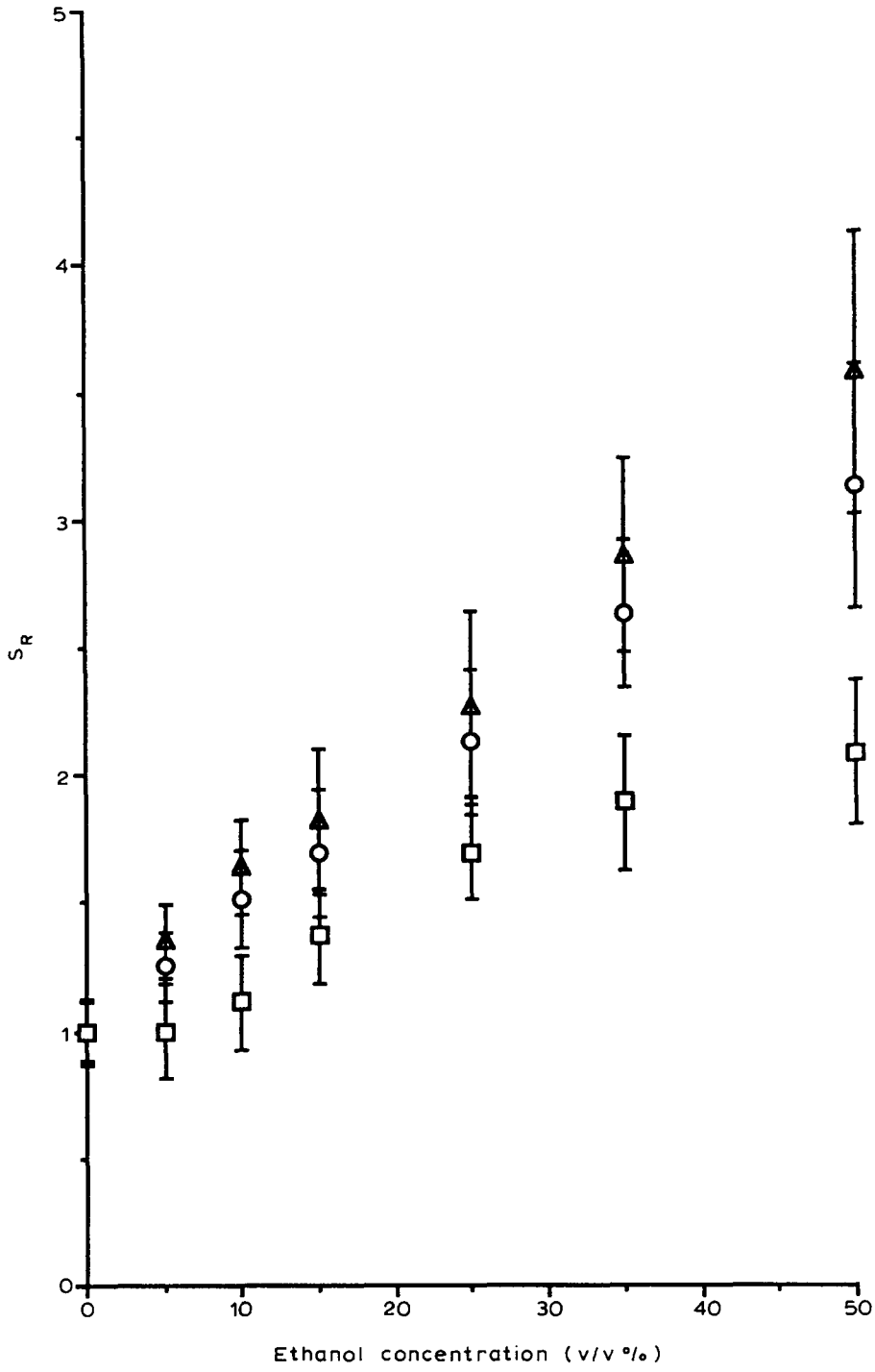

Fig. 5. S_R values for estrone (△), β-estradiol (○) and hydrocortisone (□). Each data point represents the mean and standard deviation of 8 determinations.

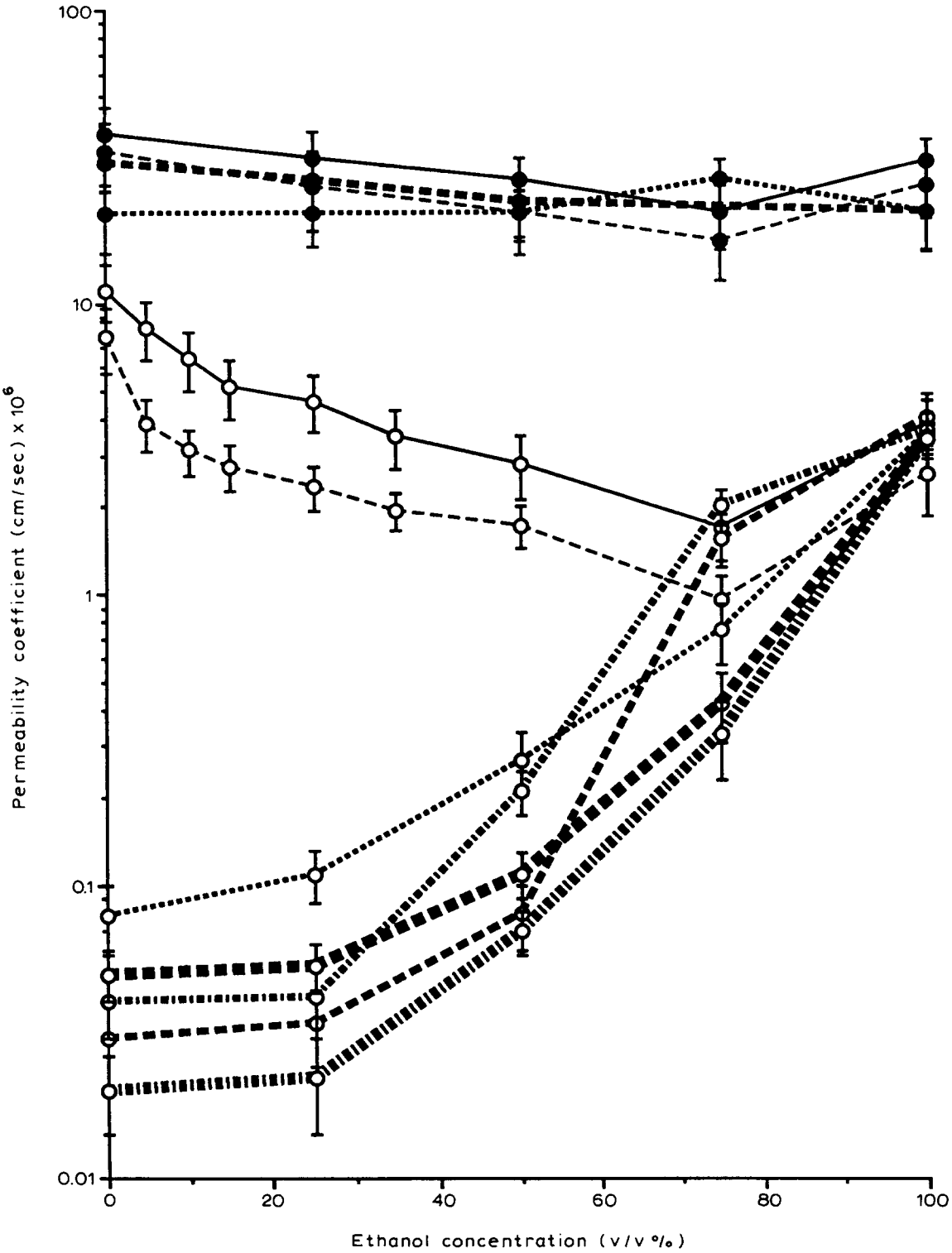

Fig. 6. Permeability coefficients of full-thickness mouse skin (open circles, ○) and stripped skin (closed circles, ●) with estrone (———), β-estradiol (------), hydrocortisone (▪▪▪▪▪▪▪), TEAB (▪▪▪▪), mannitol (▪▪▪▪▪▪), EAS (▪▪▪▪) and vidarabine (▪▪▪▪▪▪). Each data point represents the mean and standard deviation of four determinations.

and electroosmosis studies (Sims et al., 1990) with human epidermal membrane: (a) the Nernst-Planck equation holds for the tetraethylammonium ion when solvent flow corrections are made, and (b) there is convective solvent flow under the influence of an electric field. Also, there are data which clearly show (Ghanem et al., 1990) that there is a strong ionic strength effect on the passive transport of anions and cations across human epidermal membrane; this is interpreted as showing that not only do pores exist but also the pores of human stratum corneum are negatively charged. Finally, further evidence for pores in hairless mouse skin and in human skin may be found in recent studies (Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1987; Pikal, 1990; Pikal and Shah, 1990).

The permeability coefficients of EAS, vidarabine, mannitol and TEAB with whole skin are presented in Fig. 6 as functions of the ethanol/saline concentration. It is first noted that with increasing ethanol the P_T values for all polar/ionic solutes at 25% ethanol are only slightly increased over the saline values, if at all. At 50% ethanol, there are parallel and significant increases in the P_T values for these four permeants. Finally, at 100% ethanol the P_T values for all permeants become nearly the same at around 2×10^{-6} to 4×10^{-6} cm/s. It should be noted here that all polar/ionic solutes investigated (TEAB, mannitol, EAS and vidarabine) behaved quite similarly; up to 25% ethanol, the P_T values were only slightly affected and then, with increasing ethanol concentration, they increased rapidly and reached a common value of $3 \pm 1 \times 10^{-6}$ cm/s.

These consistent results may be interpreted as follows: The highly polar/ionic solutes are transported via pores and, up to 25% ethanol, there may be only modest increases in P_p. At 50% ethanol, however, there is effectively the formation of a substantial amount of new pores and P_p is much greater here than in saline. At 100% ethanol, new pore formation reaches a maximum representing around a 100-fold increase in the P_p values over those in saline. The exact nature of pore pathway(s) is unknown; however, we speculate later on concerning the possible molecular level factors responsible for new pore formation at high ethanol concentrations.

Lipophilic solutes with whole skin

Estrone, β-estradiol, and hydrocortisone are permeants believed to be transported via the lipoidal pathway of the stratum corneum essentially completely (estrone and β-estradiol) or significantly (hydrocortisone). Estrone, a drug more lipophilic than β-estradiol, exhibited permeability behavior similar to that for β-estradiol but with P_T values about 2-fold greater than those for β-estradiol. The P_T values for these two solutes gradually decreased to around one-seventh of the values in saline at 75% ethanol followed by an increase to about one-third of the saline values at 100% ethanol (Fig. 6). On the other hand, the P_T values of hydrocortisone increased slightly up to 25% ethanol, then increased more rapidly with increasing ethanol concentration. The P_T values for all three of these permeants as well as those for the hydrophilic polar/ionic compounds were approximately the same at 100% ethanol, i.e., P_T values of around $3 \pm 1 \times 10^{-6}$ cm/s. It is believed that, at 100% ethanol, pore formation is so extensive that it dominates transport of all permeants, and that the stratum corneum is unable to differentiate between lipophilic and hydrophilic permeants.

Stripped skin studies

The $P_{D/E}$ values (i.e, stripped skin permeability coefficients) of estrone, β-estradiol, hydrocortisone, and TEAB were found to be around $3 \pm 1 \times 10^{-5}$ cm/s in saline and varied only slightly with increasing ethanol concentration up to 100% as shown by the closed circles in Fig. 6. These results are in good agreement with the idea that the dermis/epidermis behaves as a porous membrane for these permeants. The inability of the dermis/epidermis to discriminate between lipophilic and polar/ionic solutes over the entire ethanol concentration range strongly supports the interpretation that the dermis/epidermis behaves like a porous matrix and is sponge-like and capable of accepting whatever solvent compositions in the donor and receiver chambers into its pores (Higuchi et al., 1985).

To examine this model for the dermis/epidermis further, data in Fig. 7 were generated by equilibration of a circular area of stripped abdominal skin (0.7 cm^2) with 2 ml of the radioactive permeant solution in 0–50% ethanol/saline (10 000 dpm/0.1 ml) for 7 h at 37 °C; the solute

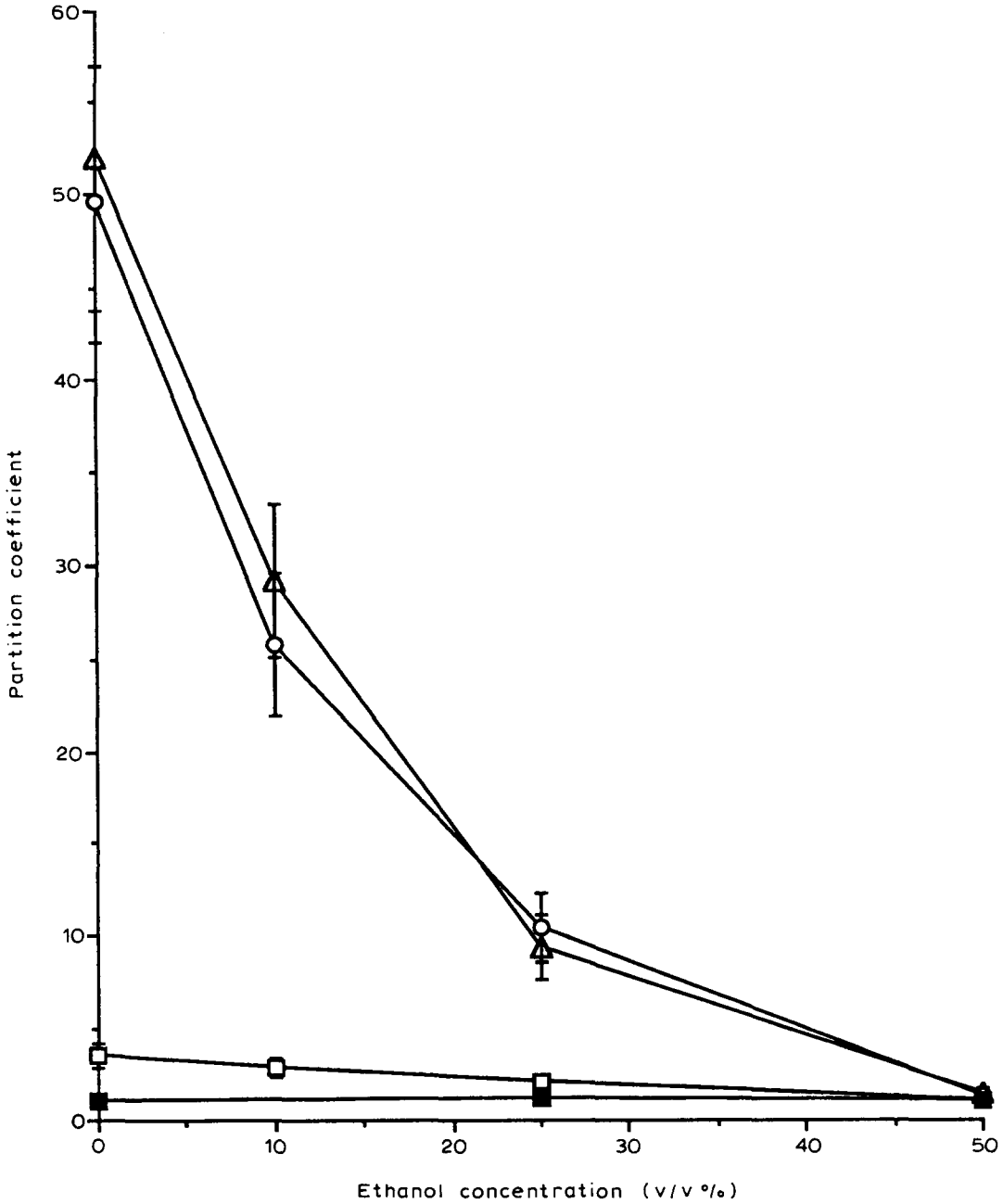

Fig. 7. Partition coefficient with epidermis/dermis (stripped hairless mouse skin) as a function of ethanol-saline concentration for estrone (△), β-estradiol (○), hydrocortisone (□) and TEAB (■). Each data point represents the mean and standard deviation of 4 determinations.

concentrations in the skin (after digestion with 0.3 M sodium hydroxide) and in the solution were determined. It is clear from Fig. 7 that increasing ethanol concentration leads to a significant decrease in the amount of solute partitioned in the tissue (especially the more lipophilic permeants). At 50% ethanol all permeants approached a common value of around unity. Physically, the relationship of these data to the rather constant $P_{D/E}$ values for the dermis/epidermis in Fig. 6 is the following: because the partitioning sites (other than aqueous compartments) are apparently immobile sites, these do not contribute to the steady-state permeant transport and at steady state, diffusion of β-estradiol and the other permeants across stripped skin is well-approximated by a porous membrane model, and the effective partition coefficient pertinent to the transport pathway should be that corresponding to the solvent phase in the pores (Higuchi et al., 1985).

Evaluation of the enhancement factor, E, for the lipophilic permeants

In order to calculate the E values using Eqns 6 and 9, we first calculate P_{Sc} from the experimental P_T values (whole skin P values) and $P_{D/E}$ (stripped skin P values), using Eqn 1. Then the P_L values may be calculated using P_P from polar/ionic solute experiments (i.e., $P_T \approx P_{Sc} \approx P_P$ for polar/ionic solutes) by means of Eqn 2. The E values presented in Table 1 and Fig. 8 were obtained using the K_R values shown in Figs 2–4, and the P_L values obtained from Eqn 2; in the cases of estrone and β-estradiol, permeant metabolism and dermis retention were taken into account when necessary (Liu et al., 1990) in deducing the true P_L value.

Interestingly, the E values for all three lipophilic solutes investigated (i.e., estrone, β-estradiol and hydrocortisone) using the P_P value of TEAB, mannitol, vidarabine or EAS yielded approximately the same E values up to 50% ethanol. The results shown in Table 1 and Fig. 8 are very important as it is seen that E values are not only about the same for all three lipophilic solutes over the entire ethanol concentration range (0–50%), but they are also relatively independent of the polar/ionic permeant used to deduce the P_P value employed in the calculation with Eqn 2.

The possible mechanistic meaning of E

It should be acknowledged that E, the enhancement factor for the lipoidal pathway, is per se of considerable theoretical and practical interest. E represents the 'correction factor' accounting for the deviation of membrane behavior from that for an ideal membrane, i.e. when the membrane is not intrinsically altered by the solvent. It is worthwhile noting that it is relatively recently (Mollgaard and Hoelgaard, 1983; Higuchi et al., 1985, 1987; Ghannam et al., 1986; Twist and Zatz, 1986; Barry and Bennett, 1987; Tojo et al., 1987; Hoelgaard et al., 1988; Touitou and Fabin, 1988) that intrinsic solvent effects have been recognized as being both widespread and generally

TABLE 1

Comparisons of the calculated enhancement factor, E, for estrone, β-estradiol and hydrocortisone based on P_P from TEAB, mannitol (MAN), EAS and vidarabine (VID)

Ethanol (X%)	Enhancement factor (E)											
	Estrone				β-Estradiol				Hydrocortisone			
	TEAB	MAN	EAS	VID	TEAB	MAN	EAS	VID	TEAB	MAN	EAS	VID
5	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3	1.4	1.4	1.5	1.5
10	2.2	2.2	2.2	2.2	2.0	2.0	2.0	2.0	2.2	2.3	2.4	2.2
15	3.0	3.0	3.0	3.0	2.7	2.7	2.6	2.7	3.6	3.7	4.0	3.5
25	5.7	5.7	5.7	5.7	6.1	6.0	6.0	6.0	8.5	9.8	11.3	8.3
35	20.8	20.6	20.7	20.9	20.7	20.4	20.5	20.8	24.3	21.4	32.7	24.2
50	99.5	95.6	98.9	99.7	102.0	96.3	104.4	108.0	140.8	–	142.7	122.7

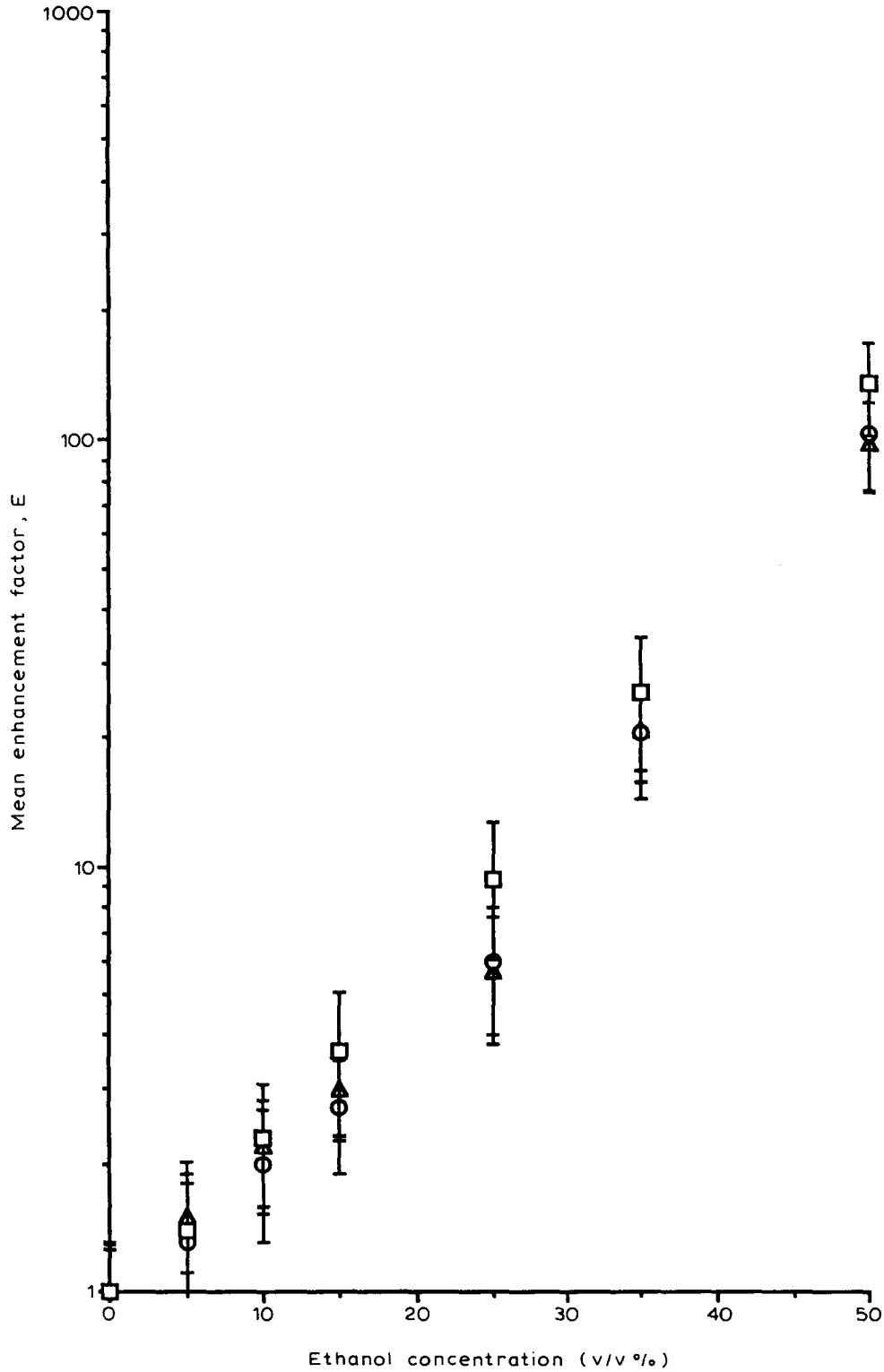

Fig. 8. Mean enhancement factor, E, for estrone (△), β-estradiol (○) and hydrocortisone (□). The bars represent standard deviations accounting for variations in the parameter values used in the calculation of the E values.

important for drug transport across the stratum corneum and idealized skin model membranes.

Regarding the relative importance of F and D_x/D_0, one can only speculate without additional, independent information such as spectroscopic data indicative of local diffusivity and thermodynamic changes in the lipid bilayer phase. The finding that the E values for estrone, β-estradiol, and hydrocortisone are quantitatively similar up to around 50% ethanol suggests that compositional changes in the rate-limiting lipid microenvironment arising from ethanol partitioning might not be of great importance. For example, if there were important 'sponge-effects' (Higuchi et al., 1985; Raykar et al., 1988) in the rate-limiting lipid domains, one may expect the F values for β-estradiol and estrone to increase more than those for hydrocortisone going from saline to 25% ethanol; the present results (Fig. 8) do not support this. It may be suggested, therefore, that the primary effects of ethanol (at least, at low concentrations) relate to D_x/D_0 (i.e., increased solute diffusivity) and, possibly, on a component of F that is polarity independent, e.g., increasing the extent of the disorder but not the polarity in the rate-limiting lipid domains. Clearly, more work is needed on this particular point.

Very recent Fourier transform infrared (FTIR) studies (Krill, 1989; Krill et al., 1991) on the effects of ethanol on the lipid alkyl chain packing, mobility, and conformational order in hairless mouse stratum corneum have provided no evidence for gross lipid alkyl chain fluidization at physiological temperature. At aqueous ethanol concentrations corresponding to E values of around 10 (Fig. 8), Krill et al. (1991) found little evidence for increased lipid chain disorder or increased chain mobility as would be expected of a lipid bilayer fluidizing agent, such as suggested for oleic acid (Golden et al., 1987; Goodman and Barry, 1989). These findings therefore indicate that the simple idea of ethanol causing gross biomembrane fluidity increases may not explain the mechanisms of action of ethanol and the other lower n-alkanols (Kim et al., 1990) where enhancement of the lipoidal pathway for permeation is concerned.

A possible mode of action of ethanol in the enhancement of the lipoidal pathway for permeation may involve the polar head region of the bilayer or the region slightly below this polar head plane or both. Although the evidence suggests the lipid alkyl chains are relatively highly ordered in the stratum corneum, the region of the bilayer that may possess the greatest resistance to diffusion is likely the polar head region and/or the region slightly below (Krill et al., 1991). Also, Anderson and Raykar (1989) have pointed out that permeant functional group contributions to permeant transport across stratum corneum are most consistent with the rate-limiting microenvironment being significantly more polar than pure hydrocarbon, i.e., the polar head region of the bilayer and not the deep hydrocarbon interior. Thus the following should be an attractive possibility for how ethanol may operate to reduce the transbilayer transport resistance (Krill et al., 1991). Ethanol may solvate the polar head regions, intercalating and disrupting the interactions of the polar head groups and the alkyl chains near the polar head plane. This should result in an increase in the effective interfacial area of the bilayer and a likely increase in both the diffusivity and partitioning tendency for a permeant in this microenvironment (and thereby contributing to making both D_x/D_0 and F greater than unity). One may also speculate that interdigitation (Simon and McIntosh, 1984; Knutson et al., 1990) of the alkyl chains in the bilayer interior may accompany the increase in the bilayer interfacial area, thus minimizing possible unfavorable energetic circumstances in the bilayer interior. More work is certainly needed before one can become comfortable with the idea that the above is the likely mechanism of action of ethanol in the enhancement of the lipoidal pathway of the stratum corneum.

Other possible effects of ethanol

The above discussion pertained mainly to the effects of ethanol at low ethanol concentration where up to around 25% ethanol there are no irreversible effects upon the lipoidal pathway and only a modest irreversible influence upon the pore pathway (as compared to the situation at

high ethanol levels). We had previously shown (Ghanem et al., 1987b) that the effects of ethanol are completely reversible at these low ethanol concentrations as far as transport via the lipoidal pathway is concerned. Also, a recent study by Kim et al. (1991) has shown that pretreatment with 30% ethanol for 4 h had no effect on the saline P_T value (and therefore the P_L value) of β-estradiol, but TEAB showed a 2–3-fold increase in its saline P_T ($\simeq P_P$) value. In this study, hydrocortisone exhibited a small increase in its saline P_T value after pretreatment with 30% ethanol; however, when the P_T value was corrected using the TEAB data (and Eqn 2) the P_L value was found not to have changed. These authors therefore concluded that low ethanol concentrations ($\simeq$ 30%) did not irreversibly affect the lipoidal pathway but did cause some irreversible increases in the P_P value.

At high ethanol concentrations, the situation is much more complex. There may be altered or additional pore/polar pathways formed as a result of a combination of changes in protein conformation, reorganization within the lipid polar head regions, or lipid extraction (Kai et al., 1990; Kurihara-Bergstrom et al., 1990). Lipid extraction may take place in conjunction with and/or independently of conformational alterations within protein domains. The present studies have shown that at the highest ethanol levels (i.e., 100% ethanol), the stratum corneum behaves as a purely porous membrane barrier unable to distinguish between a highly lipophilic permeant and a highly polar permeant.

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