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Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities

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Abstract

Recent investigations have shown that human skin permeabilities may not always follow a Gaussian-normal distribution as usually assumed. Permeability coefficients (K_p) for the model hydrophilic permeant, 5-fluorouracil (5-FU), and the model lipophilic permeant, estradiol (ES), were demonstrated to follow more closely log-normal distributions. The present study has evaluated the effect of terpene penetration enhancer treatment on the distribution of 5-FU and ES permeabilities in human skin. Control permeability coefficients (K_p), enhanced permeability coefficients (K_{p_e}) and enhancement ratios (ER) were tested for both Gaussian-normality and log-normality. K_{p_e} and ER data, obtained from 5-FU experiments employing 22 different terpene enhancer formulations and one vehicle control, were pooled using standardised values, to obtain 188 replicate measurements for statistical analysis. Similarly, K_{p_e} and ER data, collected from ES experiments using 10 different terpene treatments and two vehicle controls, were pooled to obtain 69 replicate measurements. Statistical analysis revealed that K_p, K_{p_e} and ER data for 5-FU tended to follow a log-normal distribution. In contrast, the K_p, K_{p_e} and ER data for ES followed, more closely, a Gaussian-normal distribution. Furthermore, the variability of the ES data was smaller in comparison with 5-FU data. It is possible that differences in data distribution may be linked to the physico-chemical properties of 5-FU and ES. The results of this study suggest that, when control permeabilities follow a log-normal distribution, as was the situation for 5-FU, it is likely that enhancer treatment will produce permeabilities and enhancement factors which are also log-normally distributed. In these instances, all data are most accurately represented by geometric means and geometric standard errors.

Keywords: Human skin; Variability; Penetration enhancer; Estradiol; 5-Fluorouracil

1. Introduction

It is well known that human skin permeability is highly variable. Large differences in permeability have been noted between different body sites (Feldman and Maibach, 1967; Scheuplein and Blank, 1971; Elias et al., 1981; Bennett and Barry, 1986) and even at the same site in the same individual (Southwell et al., 1984). Superimposed on this intra-individual variability is an additional large inter-individual variability (Michaels et al., 1975; Southwell et al., 1984). Significant differences exist, for example, between individuals of

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different ages (Roskos et al., 1986) and different races (Berardesca and Maibach, 1990). Differences in skin permeability can be ascribed to structural factors such as stratum corneum thickness, intercellular lipid composition and the density of skin appendages. In vivo, biological factors such as skin metabolism and skin surface temperature may also be relevant.

The variability of human skin permeabilities in vitro has been quantified by Southwell et al. (1984) using a wide range of permeants. It was concluded that drug permeability coefficient measurements would generally be expected to have a mean intra-individual coefficient of variation of approx. 40%, and a mean inter-individual coefficient of variation of 70%. Implicit in most skin permeation studies to date is the assumption that the data are symmetrically distributed about a central mean (i.e., that they are Gaussian-normally distributed). The majority of investigators use arithmetic means and standard deviations to describe their data. However, recent studies at this laboratory have suggested that in vitro human skin permeabilities are not Gaussian-normally distributed (Williams et al., 1992). Frequency distributions of the permeability coefficients of a model hydrophilic permeant (5-fluorouracil, 5-FU) and a model lipophilic permeant (oestradiol, ES) have been shown to be positively skewed with tailing at the high permeability ends of the scales. Logarithmic transformation of the data reduced the degree of skew and produced distributions which approximated to symmetrical Gaussian-normal curves. It was concluded that both 5–FU and ES permeability coefficients were probably log-normally distributed and best summarised using geometric means and geometrically calculated standard deviations. As a result of the log transformation the mean permeability coefficients of 5-FU and ES were reduced 2–3-fold and the coefficients of variation reduced by approximately one third.

Other reports of log-normally distributed skin permeability data are rare. Using results obtained from seven donors and 539 individual samples, Kasting et al. (1992) report that water penetration through split-thickness human cadaver skin has a strong positive skew which is best corrected by log-transformation or near log-transformation of the data. A major problem encountering those wishing to characterise the distribution of skin permeabilities is that most investigations include relatively small (< 10) numbers of measurements. Kasting et al. (1987) overcame the problem of small sample sizes by pooling in vitro flux data from 35 different permeants, and concluded that human skin permeabilities are generally log-normally distributed. However, pooling flux data from permeants with different physico-chemical properties may not have been ideal. Liu et al. (1991) have noted that distributions of in vitro flux for ionic molecules have more positive skew than those for lipophilic permeants.

Whilst the implications of log-normality for the measurements of in vitro drug permeability coefficients are clear, the effects of log-normality on enhancer studies is much less apparent. If, for instance, control drug fluxes are log-normally distributed, can it be assumed that drug fluxes after treatment will also be log-normally distributed? This study investigates the subject of log-normality for in vitro penetration enhancer studies using 5-FU and ES. The frequency distributions of control permeability coefficients (K_p), post-treatment (enhanced) permeability coefficients (K_{p_e}) and enhancement ratios (ER) are tested for log-normality.

2. Materials and methods

2.1. Materials

5-[6-^{3}H]Fluorouracil and [2,4,6,7-^{3}H]estradiol were purchased from NEN (Dupont) Research Products (Dreiech, Germany). Radiochemical purity, as tested by thin-layer chromatography, was 98.0 and 98.5% for 5-FU and ES, respectively. Unlabelled 5-FU and ES, used for the preparation of saturated donor solutions, were purchased from Sigma Chemical Co. (St. Louis, MO); the terpene enhancers, 3-methyl-2-buten-1-ol, geraniol, farnesol (mixture of isomers), nerolidol (mixture of isomers), phytol (mixture of isomers) and β-caryophyllene oxide were purchased from Aldrich (Gillingham, UK). (+)-Longifolene,

(+)-aromadendrene, (+)-β-cedrene, (−)-guaiol, (+)-cedrol and (+)-cedryl acetate were obtained from Fluka (Buchs, Switzerland). *trans*-β-Caryophyllene was purchased from Sigma Chemical Co. (St. Louis, MO). (−)-α-Bisabolol was received as a gift from BASF (Cheadle, UK). The purities of the terpenes, as determined by capillary gas chromatography, have been described in a previous publication (Cornwell and Barry, 1994). Propylene glycol (99%) and dimethyl isosorbide (98%), used in enhancer formulations, were obtained from Aldrich (Gillingham, UK). All other solvents and reagents were of analytical grade.

2.2. Epidermal membranes

Human abdominal mid-line cadaver skin was obtained post-mortem and stored frozen at −20°C in double-sealed evacuated polythene bags (Harrison et al., 1984). Epidermal membranes were prepared by immersing full thickness skin samples, trimmed of subcutaneous fat, in water at 60°C for 45 s; the epidermal membranes could then be gently peeled off the underlying dermis (Kligman and Christophers, 1963). Hairy skin samples tended to tear the membranes and were thus avoided. Permeation studies employing 5-FU as the model permeant used skin samples from 34 different donors. Donors were elderly (mean age = 69.3 ± S.D. 14.4 years) and female (77%). Studies employing ES used skin samples from nine different donors. As for 5-FU, donors were elderly (mean age = 69.7 ± S.D. 14.9 years) and predominantly female (83%).

2.3. Diffusion experiments

Permeation studies employed an automated diffusion system equipped with miniature diffusion cells with flow-through receptor compartments (Akhter et al., 1984). The cells provided a diffusional area of 0.125 cm^2 and were maintained at 32°C. The receptor solution of 0.002% sodium azide was pumped through the cells at 2 ml h^{-1} in order to achieve sink conditions for both 5-FU and ES.

Epidermal membranes were floated, stratum corneum side up, on receptor solution for 48 h before mounting in the diffusion cells, to ensure essentially full hydration.

For 5-FU experiments the drug donor solution was a saturated aqueous solution of 5-FU (14.3 ± S.D. 0.6 mg ml^{-1} at 32°C; mean of four replicate measurements), radiolabelled to an activity of approx. 0.1 mCi ml^{-1}. In ES experiments a saturated aqueous solution of ES (3.6 ± S.D. 0.2 μg ml^{-1} at 32°C; mean of three replicate measurements) was used, radiolabelled to an activity of approx. 0.3 μCi ml^{-1}. 5-FU is a weak acid (pK_a 8.0 and 13.0; Rudy and Senkowski, 1973) and was thus largely un-ionised in double distilled water. ES is also a weak acid (pK_a 10 1; Egar et al., 1972) and, therefore, was also largely un-ionised. The log $K_{octanol/water}$ values for 5-FU and ES have been determined, using radiolabelled drug and a shake-flask method, to be −0.92 and 2.30, respectively (means of three replicate measurements in each case).

For initial control runs with 5-FU, 200 μl of drug donor solution was dispensed into each of the cell donor compartments, which were then covered. Receptor solution was collected over 2-h periods for a minimum of 36 h. In ES experiments 120 μl of drug donor solution was placed in each donor compartment. Receptor solution was collected over 2-h periods for a minimum of 24 h. Since ES was expected to permeate the skin relatively fast, drug donor solutions were replenished every 8 h in order to prevent significant donor depletion. For both drugs 4 ml receptor solution samples were mixed with 5 ml Optiphase 'Hisafe' 3 scintillation mixture (LKB) prior to analysis on a Packard Tricarb-460 liquid scintillation counter.

Following the control runs, the membrane surfaces and donor compartments were rinsed clean of drug and the donor compartments then filled with distilled water. Drug remaining in the membranes was left to wash-out into the donor and receptor compartments over a period of 12 h, whilst replacing the distilled water at regular intervals.

The epidermal membranes were then treated with 150–200 μl enhancer or enhancer formulation for 12 h. After the treatment period enhancers were removed by gently blotting with

tissue paper. In some instances crystals of solid enhancers had to be removed by a single quick rinse with acetone (Bond and Barry, 1988). Drug donor solutions were then reapplied for the post-treatment runs and samples collected as described above for the control runs.

2.4. Calculation of results

Linear regression analysis of the pseudo steady-state diffusion results allowed the calculation of the permeability coefficients (K_p) for both drugs through the epidermal membranes. The activity of the enhancers or enhancer formulations was expressed as a ratio of the K_p values after and before enhancer treatment, i.e:

$$\text{enhancement ratio (ER)} = \frac{K_p \text{ after treatment } (K_{p_e})}{K_p \text{ before treatment } (K_p)} \quad (1)$$

In this way each epidermal membrane sample acted as its own control. Replicate experiments (5–10) were performed for each enhancer or enhancer formulation.

2.5. Pooling of post-treatment permeability coefficients and enhancement ratios

Since most statistical tests on data frequency distributions require large numbers of replicate measurements it was necessary to pool the data obtained for each model permeant. Pooling control K_p data is straightforward, but pooling K_{p_e} and ER values obtained following different enhancer treatments requires the data to be normalised. In the present study K_{p_e} and ER values were pooled for statistical analysis by calculating standardised values. These were obtained for each data value, x, using the transformation:

$$x \rightarrow (x - \mu)/\sigma \quad (2)$$

where μ and σ are the sample mean and sample standard deviation, respectively, of the data set to which x belongs. The effects of the transformation are to centre the pooled data sets around a common mean of 0, and to normalise the dispersion of each data set to a common standard deviation of unity. The transformation preserves the relative distribution of data values. Standardised values therefore allow the investigation of pooled data distributions without distortion.

In this study 5-FU experiments with 22 different terpene enhancer treatments and one vehicle control treatment were pooled, and ES experiments with 10 different treatments and two vehicle control treatments were pooled. The formulations used in the 5-FU experiments were as follows (the number of replicate measurements are shown in parentheses); (+)-aromadendrene (6), (−)-α-bisabolol (5), (−)-α-bisabolol 90% w/w in propylene glycol (8), *trans*-β-caryophyllene (4), *trans*-β-caryophyllene saturated in propylene glycol (18), β-caryophyllene oxide saturated in dimethyl isosorbide (DMI) (5), β-caryophyllene oxide saturated in propylene glycol (12), (+)-β-cedrene (5), (+)-β-cedrene saturated in propylene glycol (17), (+)-cedrol saturated in DMI (5), (+)-cedrol saturated in propylene glycol (7), (+)-cedryl acetate saturated in DMI (8), (+)-cedryl acetate saturated in propylene glycol (12), DMI (4), farnesol (6), geraniol (5), (−)-guaiol (5), (−)-isolongifolol (5), (+)-longifolene (6), 3-methyl-2-buten-1-ol (6), nerolidol (18), nerolidol 90% w/w in propylene glycol (8) and phytol (14). The formulations used in ES experiments were: (−)-α-bisabolol (8), *trans*-β-caryophyllene (6), β-caryophyllene oxide saturated in DMI (7), β-caryophyllene saturated in propylene glycol (6), (+)-β-cedrene (4), (+)-cedrol saturated in DMI (8), (+)-cedryl acetate saturated in DMI (6), DMI (5), farnesol (5), nerolidol (8), nerolidol 90% w/w in propylene glycol (5) and propylene glycol (5). The effects of enhancer treatment on skin permeability towards 5-FU and ES have been described and discussed elsewhere (Cornwell and Barry, 1992, 1994; Cornwell, 1993).

2.6. Testing frequency distributions for log-normality

Raw data and log-transformed data distributions were tested with three statistical tests; probit analysis, the Kolmogorov-Smirnov one sample goodness of fit test and the Davies test. The objective of each procedure was to test the fit of

unlogged and log-transformed data to a Gaussian-normal frequency distribution. If, after log-transformation, a set of data matches a Gaussian-normal distribution then it could be concluded that the original raw data set was likely to be log-normally distributed.

Probit analysis is commonly used for a simple indication of log-normality. The analysis operates on the principle that if a plot of probit values vs sample cumulative frequencies is linear then it is likely that the sample data are Gaussian-normally distributed. Probit values were determined for each sample cumulative frequency value by first obtaining the corresponding normal equivalent deviate, or Z, value. Probit values were calculated by adding 5 to each Z value. Tables of Z values corresponding to various normal curve areas (i.e., percent cumulative frequencies) are published in standard statistical texts. Two examples of probit plots are shown in Fig. 1. Fig. 1A shows probit analysis of standardised K_{p_e} values for 5-FU and Fig. 1B illustrates probit analysis of standardised log K_{p_e} values for 5-FU. Log-transformation improved the linearity of the plot indicating that the data more closely fitted a log-normal distribution. The improvement in linearity is reflected in the least-squares linear regression coefficient which increases from 0.9819 to 0.9916 on log-transformation of the data.

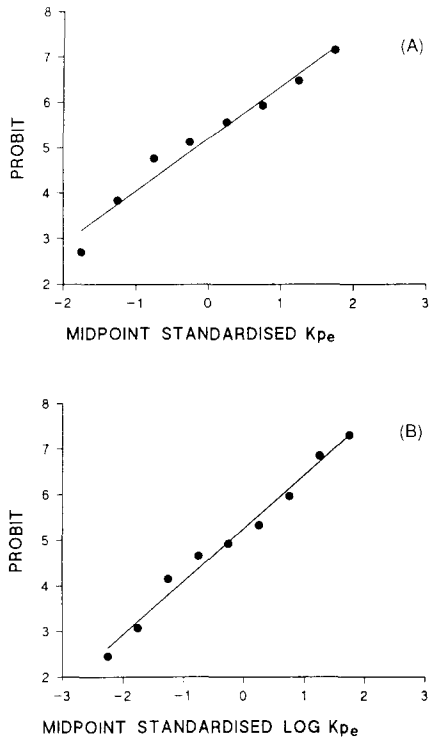

Fig. 1. Probit test on standardised post-treatment permeability coefficients (K_{p_e}) for 5-fluorouracil across human epidermis in vitro; $n = 188$. The line drawn through the data points represents the line of least-squares regression. (A) Unlogged data; (B) log-transformed data.

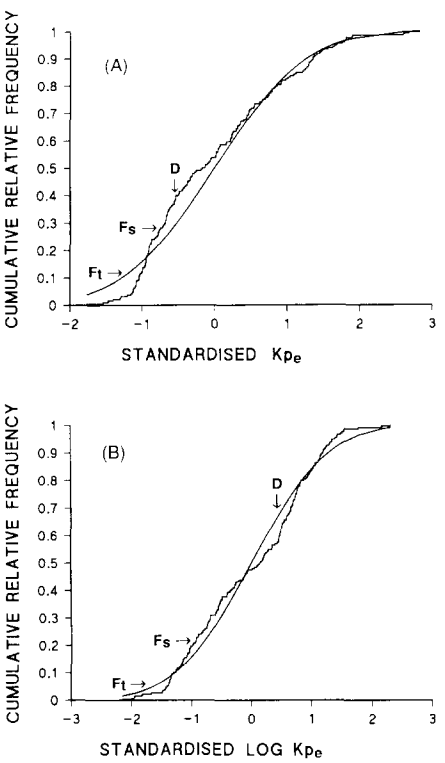

Fig. 2. Kolmogorov-Smirnov one sample goodness of fit test on standardised post-treatment permeability coefficients (K_{p_e}) for 5-fluorouracil across human epidermis in vitro; $n = 188$. F_s and F_t represent the sample cumulative frequency distribution and the theoretical Gaussian-normal distribution, respectively. The point of maximum divergence between F_s and F_t is labelled as D (D is the test statistic, see text). (A) Unlogged data; (B) log-transformed data.

The Kolmogorov-Smirnov (K-S) one sample test measures the fit of a sample cumulative frequency distribution, F_s, to a theoretical Gaussian-normal distribution, F_t. F_s was readily calcu-

lated and was plotted as a stepped line. F_t was determined by first calculating the normal equivalent deviate, or Z, for each value, using a formula identical to Eq. 2. F_t was then determined by obtaining the normal curve area (i.e., cumulative frequency) corresponding to the calculated Z value. Since F_t was based on a perfect theoretical distribution values were plotted as a smooth curve.

The magnitude of the difference between the two distributions was determined by calculating the maximum vertical distance between the two lines. The test statistic, D, was calculated using the formula:

$$D = \text{maximum}\left[\left|F_s(x_i) - F_t(x_i)\right|, \left|F_s(x_{i-1}) - F_t(x_i)\right|\right] \quad (3)$$

where $F_s(x_i)$ is the sample cumulative frequency at the data point, x_i, $F_t(x_i)$ denotes the theoretical/predicted cumulative frequency at datum point x_i and $F_s(x_{i-1})$ is the sample cumulative frequency at the datum point previous to x_i (NB: since the sample distribution was stepped it was necessary to calculate the vertical distance between both the 'edge' and the 'inside corner' of each step, hence the two parts to Eq. 3).

If D exceeds a critical value, D_{crit}, then the two distributions are said not to fit. D_{crit} was determined for the various sample sizes used in this study at a significance level of 0.05. Tables for determining D_{crit} are published in many statistical texts (e.g., Daniel, 1991).

Fig. 2 shows K-S test plots obtained from standardised K_{p_e} values and standardised log K_{p_e} values for 5-FU. The deviation of F_s from F_t was greater for the standardised K_{p_e} data. For standardised K_{p_e} data $D = 0.1043$, exceeding D_{crit} (0.0992). For standardised log K_{p_e} data $D = 0.0991$, i.e., just below D_{crit}. From this test one could conclude that the log K_{p_e} data fitted more closely a Gaussian-normal distribution.

The Davies test for logarithmic distributions involves calculating a coefficient of skewness by:

$$\frac{(\log LQ + \log UQ) - (2 \times \log MQ)}{\log UQ - \log LQ} \quad (4)$$

where LQ is the lower quartile value, MQ represents the middle quartile value (or median) and UQ is the upper quartile value. The effect of log-transforming each quartile is to put the data distribution onto a logarithmic scale. If the distribution is log-normal this will improve its symmetry and decrease its skew. Data are determined to be log-normally distributed if the calculated coefficient of skewness is less than +0.2 (for details see Langley, 1979). Unfortunately, the Davies test could not be used on the standardised data since such data included negative values which could not be log-transformed.

3. Results

3.1. Permeability coefficients

The frequency distributions of both the unlogged and log-transformed 5-FU K_p data are

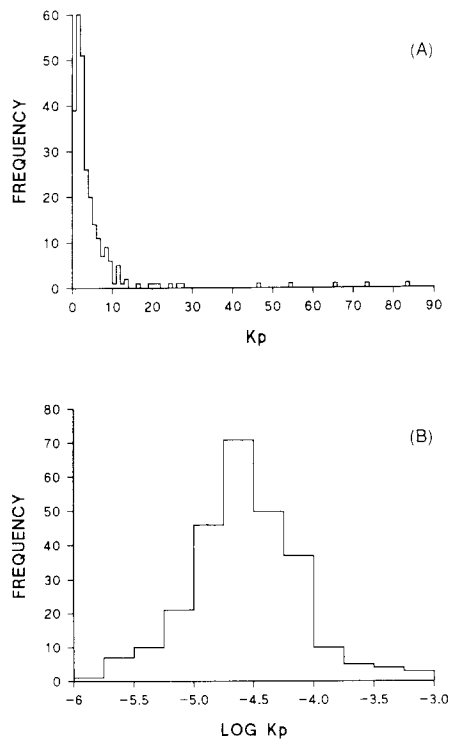

Fig. 3. Frequency distribution of 5-fluorouracil permeability coefficients (K_p) across human epidermis in vitro; $n = 265$, K_p ($\times 10^5$) (cm h^{-1}). (A) Unlogged data; (B) log-transformed data.

shown in Fig. 3. The frequency distribution of the unlogged data shows a strong positive skew with a considerable amount of tailing. Log-transformation of the data appears to remove the skew and produces a symmetrical frequency distribution. The results of the probit analyses and K-S tests performed on the unlogged and log-transformed 5-FU K_p data are summarised in Table 1. Due to space limitations, not all the test plots are shown. Probit analysis demonstrated clearly that only the log-transformed data fit a Gaussian-normal distribution. The probit plot of unlogged K_p data was highly curved, whereas the probit plot of log K_p data was approximately linear. K-S tests also showed that the log-transformed data approximate better to a Gaussian-normal distribution. The calculated D values for the unlogged and log-transformed 5-FU K_p cumulative frequency distributions were 0.2977 and 0.0467, respectively. Since D_{crit} for both tests was 0.0835, the unlogged data are rejected and the log-transformed data accepted as belonging to a Gaussian-normal frequency distribution. The results from the two graphical tests were confirmed by the Davies test which showed that log K_p data for 5-FU have a coefficient of skewness = 0.024, i.e., well below the defined limit of 0.2 and indicating a symmetrical distribution.

3.2. 5-Fluorouracil post-treatment permeability coefficients

The frequency distributions of unlogged and log-transformed 5-FU K_{p_e} values are shown in Fig. 4. The unlogged data distribution shows some positive skew which is corrected by log-transformation. 5-FU K_{p_e} values therefore also appear to be log-normally distributed. The frequency distribution of the log-transformed data appears to be slightly bimodal. The reason for the apparent bimodality is unclear.

The probit analysis and K-S tests on the unlogged and log-transformed 5-FU K_{p_e} values confirmed that the data are log-normally distributed (Fig. 1 and 2). Unfortunately, the K-S test was affected by the slight bimodality of the log-transformed data, and D for this data set (0.0991) was only just below D_{crit} (0.0992). This indicates that the log-transformed data fit a Gaussian-normal distribution only rather loosely. However, since

Table 1
Summary of statistical test results; tests were performed on control permeability coefficients (K_p), standardised post-treatment permeability coefficients (K_{p_e}) and standardised enhancement ratios (ER) for 5-fluorouracil and estradiol across human skin in vitro (n = number of replicates)

Data set	n	Probit analysis		Kolmogorov-Smirnov test			Davies test	
		Regression coefficient	Gaussian-normal?	D_{crit} [a] ($\alpha = 0.05$)	D [b]	Gaussian-normal?	Test statistic	Gaussian normal?
5-Fluorouracil								
K_p	265	0.7749	no	0.0835	0.2977	no		
Log K_p	265	0.9938	yes	0.0835	0.0467	yes	0.024	yes
K_{p_e}	188	0.9819	no?	0.0992	0.1043	no		
Log K_{p_e}	188	0.9916	yes	0.0992	0.0991	yes		
ER	188	0.9859	no?	0.0992	0.1198	no		
Log ER	188	0.9944 [c]	yes	0.0992	0.0629	yes		
Estradiol								
K_p	69	0.8969	no?	0.1637	0.1038	yes		
Log K_p	69	0.9945	yes	0.1637	0.0952	yes	−0.120	yes
K_{p_e}	73	0.9910	yes	0.1592	0.1204	yes		
Log K_{p_e}	73	0.9967	yes	0.1592	0.0784	yes		
ER	73	0.9925	yes	0.1592	0.0935	yes		
Log ER	73	0.9939	yes	0.1592	0.1310	yes		

[a] D_{crit}, critical value of D in a two-sided test with a level of significance of $\alpha = 0.05$.
[b] D, test statistic, see text.
[c] Least-squares regression excluding the first out-lying data point.

the fit to a Gaussian-normal distribution was improved by the log-transformation, it is still possible to conclude that the data are best described as being log-normally distributed.

3.3. 5-Fluorouracil enhancement ratios

In contrast to K_{p_e} data, the ER values for 5-FU showed much clearer log-normality. The frequency distribution of the unlogged 5-FU ER values shows considerable positive skew which is corrected by log-transformation of the data (Fig. 5). The probit analyses and K-S tests confirmed that the data are log-normally distributed (Table 1).

3.4. Estradiol permeability coefficients

The frequency distributions of unlogged and log-transformed ES K_p data are shown in Fig. 6.

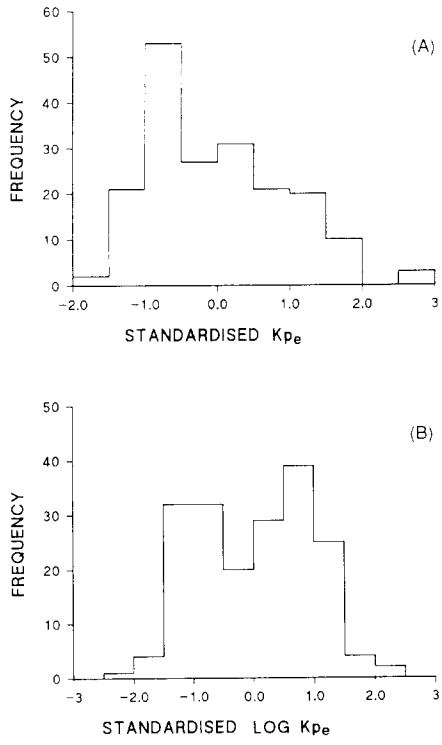

Fig. 4. Frequency distribution of standardised post-treatment permeability coefficients (K_{p_e}) for 5-fluorouracil across human epidermis in vitro; $n = 188$. (A) Unlogged data; (B) log-transformed data.

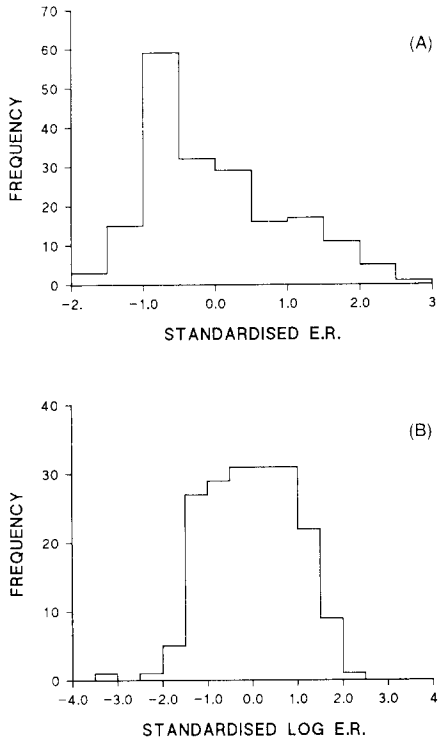

Fig. 5. Frequency distribution of standardised enhancement ratios (ER) for 5-fluorouracil across human epidermis in vitro; $n = 188$. (A) Unlogged data; (B) log-transformed data.

Unlike 5-FU K_p values, ES K_p values appear to exhibit only a weak tendency towards log-normality; the tailing and the skew on the unlogged data are only slight. The probit analyses and K-S tests confirm that the data are only slightly skewed (Table 1).

In marginal cases such as this the subjective nature of probit analysis is exposed. Probit plots for K_p and log K_p data were both approximately linear. No limits for the regression coefficient, or any other measure of linearity, exist. However, it was possible to conclude that the plot was more linear after log-transformation.

In this situation the K-S test is much less subjective since pre-determined limits (or D_{crit} values) have been set. K-S tests on the unlogged and log-transformed data sets confirm that the latter fits a Gaussian-normal distribution slightly better than the former (i.e., $D(K_p) = 0.1038$, $D(\log K_p) = 0.0952$). However, since both D val-

ues were below D_{crit} (0.1637) both distributions could be described as being Gaussian-normal.

The Davies test for logarithmic distributions concludes that ES K_p data could be described as being log-normally distributed (Table 1). However, in view of the lack of skew in the unlogged data the Davies test is not, in this case, absolute proof of log-normality.

3.5. Estradiol post-treatment permeability coefficients

As was noted for the control ES K_p values, the distribution of ES K_{p_e} values appears to be only slightly skewed (Fig. 7). Log-transformation produces only a small improvement in the symmetry of the K_{p_e} data. The probit analyses and K-S tests confirmed that both the unlogged and the log-transformed data can be described as being Gaussian-normally distributed (Table 1).

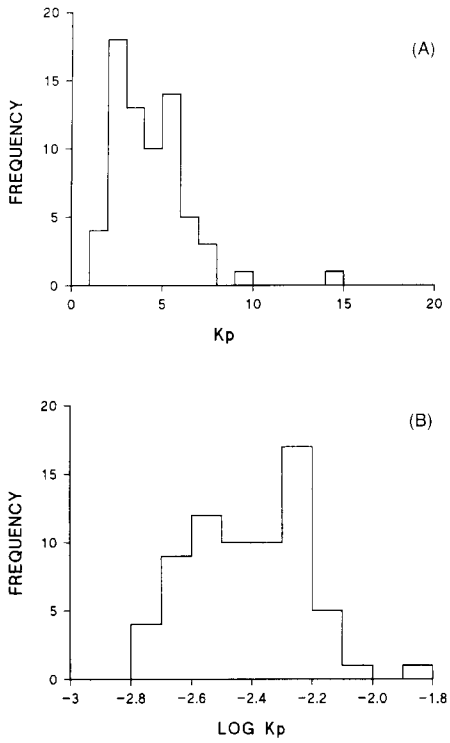

Fig. 6. Frequency distribution of estradiol permeability coefficients (K_p) across human epidermis in vitro; $n = 69$, K_p ($\times 10^3$) (cm h^{-1}). (A) Unlogged data; (B) shows log-transformed data.

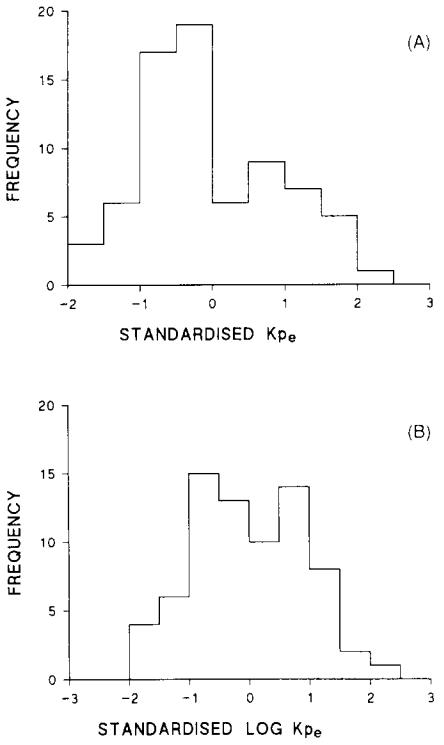

Fig. 7. Frequency distribution of standardised post-treatment permeability coefficients (K_{p_e}) for estradiol across human epidermis in vitro; $n = 73$. (A) Unlogged data; (B) log-transformed data.

3.6. Estradiol enhancement ratios

The frequency distributions of unlogged and log-transformed ES ER values are almost identical (Fig. 8). As for the K_p values and the K_{p_e} values, the probit analyses and K-S tests confirm that both the unlogged and the log-transformed ER data can be described as being Gaussian-normally distributed (Table 1).

4. Discussion

An assumption made in the present study was that each enhancer treatment had similar effects

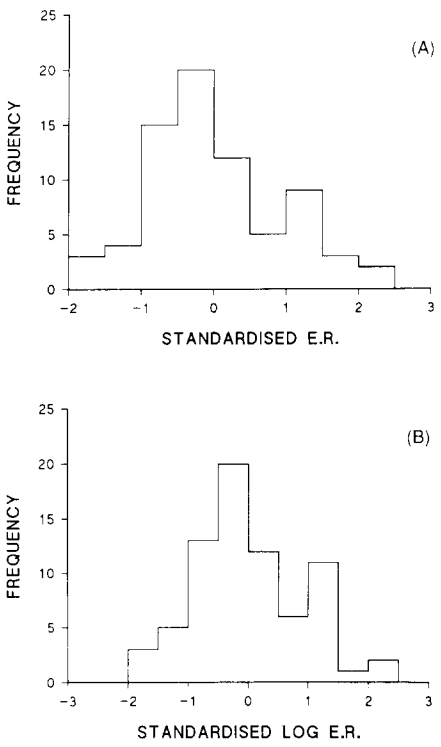

Fig. 8. Frequency distribution of standardised enhancement ratios (ER) for estradiol across human epidermis in vitro; $n = 73$. (A) Unlogged data; (B) log-transformed data.

on the statistical distribution of K_{p_e} values and ER values. Since the primary objective of the original diffusion experiments was to test enhancer actions on skin permeability and not to test enhancer effects on data distributions, only 5–6 replicate measurements were made for each experiment. Many more replicate determinations would need to have been performed in order to test the distribution of K_{p_e} values and ER values following treatment with any particular enhancer.

This study has shown that control K_p values for 5-FU, a model hydrophilic permeant, are probably log-normally distributed. This is in agreement with the previous study by Williams et al. (1992), which also concluded that 5-FU K_p values across human epidermis in vitro are likely to be log-normally distributed. Using data pooled from a series of terpene enhancer experiments, the present study has also shown that 5-FU K_{p_e} values and ER values tend to be log-normally distributed. It would appear, therefore, that K_{p_e} values and ER values follow the log normality of K_p values.

In contrast to 5-FU K_p values, ES K_p values showed only a slight tendency towards a log-normal distribution. In fact, Probit tests and K-S tests fitted both the unlogged and the log-transformed ES K_p data to a Gaussian-normal distribution. This result does not agree with those of the study by Williams et al. (1992) who reported the distribution of ES K_p values to be fully log-normal. The discrepancy between the two studies is possibly due to the lower number of replicates (69 as opposed to 221) used in the present study. However, 69 replicates is still a considerable number and similar trends between the two studies would normally be expected. It is, therefore, also possible that the discrepancy is due to differences in experimental technique between workers.

The frequency distributions of ES K_{p_e} values and ER values were also only slightly skewed. Therefore, again it appears that the K_{p_e} values and ER values follow the distribution of the control K_p data.

Aside from differences in frequency distribution symmetry, 5-FU and ES data also differ in their degree of dispersion. The arithmetic coefficient of variation for 5-FU K_p values in the present study was 185.9% whereas the arithmetic coefficient of variation of ES K_p values was 48.61%. Furthermore, the mean arithmetric coefficients of variation for K_{p_e} and ER measurements with 5-FU were 61.27 and 62.87% respectively, whereas for ES they were 22.93 and 20.66% respectively. Data dispersion and the effect of the log-transformation on data distribution symmetry are linked. If the dispersion of data is high then the change in data symmetry on log-transformation will be large. Conversely, if the dispersion is low the effect of the log-transformation will be small. The latter was probably the situation for ES data, which were so tightly distributed that log-transformation made very little difference to data symmetry.

It is possible that the observed differences in frequency distribution symmetry and data dispersion for 5-FU and ES are linked to their physico-

chemical properties. In a agreement with this theory, Liu et al. (1991) have also shown that the K_p frequency distribution for an ionised/polar drug has more positive skew and tailing than that of a lipophilic drug.

It is likely that despite the large differences in physico-chemical properties between 5-FU and ES, the rate-limiting barrier for both permeants is still the intercellular lipid matrix in the stratum corneum. This has been confirmed by the measurement of diffusional activation energies and the good fit of both permeants' K_p values to a structure-permeability model which is based on membrane properties characteristic of a lipid barrier (Cornwell, 1993). If both permeants encounter the same barriers in the skin the question arises as to why there are still significant differences in frequency distribution symmetry and data dispersion.

As a result of the lipid barrier operating in the stratum corneum the K_p for the hydrophilic permeant 5-FU across human skin is relatively low (geometric mean $K_p = 2.71 \times 10^{-5}$ cm h^{-1}) and the K_p for the lipophilic permeant ES is relatively high (geometric mean $K_p = 3.91 \times 10^{-3}$ cm h^{-1}). Completely removing the stratum corneum by tape stripping increases the K_p of 5-FU over 8000-fold, and the K_p of ES only 40-fold (Williams and Barry, 1991). The tape-stripping experiments demonstrate clearly that since the K_p of 5-FU is very low it can rise, on barrier disruption, much more than can the K_p of ES. It is possible, as a result, that permeable membrane defects (e.g., hair follicles, lipid bilayer defects, artificial tears, etc.) will increase 5-FU permeability much more than ES permeability (i.e., 5-FU flux is much more sensitive to membrane defects than is ES flux). An occasional membrane defect, by this argument, is more likely to produce a higher than expected 5-FU K_p than a higher than expected ES K_p. 5-FU permeability distributions will thus be more disperse and more skewed than ES permeability distributions.

Finally, we can note that this study has used in vitro permeability data and that these were obtained using previously frozen, heat-separated human epidermis. Although it is unlikely that the storage and preparation of epidermal membranes significantly alters the permeability of the majority of skin samples (Harrison et al., 1984; Walker et al., 1984), it is possible that some membrane samples may be slightly damaged. These altered samples would have higher than expected permeabilities and would make the data appear log-normal. Since many experimentalists use epidermal membranes this study remains relevant, but care is needed in transposing these results to the in vivo situation. For example in a recent in vivo study Boddé et al. (1991) have shown that transepidermal water loss (which correlates with permeability towards water) and skin impedance (which measures permeability to ions) are Gaussian-normally distributed.

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