International Journal of Pharmaceutics, 18 (1984) 299–309
Elsevier

IJP 00633

Variations in permeability of human skin within and between specimens

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(Received May 5th, 1983)
(Modified version received September 3rd, 1983)
(Accepted September 30th, 1983)

Summary

Regional differences in the permeability of human skin are well known but few investigators attempt to quantify the variability at specific sites as distinct from site-to-site differences. We assessed the variability at a site, within and between specimens (individuals). We defined the intra-specimen variation (intra-s.v.) at a site as the coefficient of variation for a parameter measured for samples within a specimen; inter-specimen variation (inter-s.v.) was the coefficient for several specimens. We measured the steady-state diffusion of phenol, methanol, octanol and caffeine, and the finite dose diffusion of aspirin and caffeine and performed a vasoconstrictor assay with betamethasone-17-benzoate in 9 vehicles. From these data and from published results, we estimated inter-s.v. and intra-s.v. The overall mean, in vitro estimates of inter-s.v. (66% ± S.D. 25, n = 45) and intra-s.v. (43% ± 25, n = 32) were higher than the mean, in vivo, estimates of inter-s.v. (45% ± 18, n = 114) and intra-s.v. (27% ± 9, n = 4). Inter-s.v. was higher than intra-s.v. both in vivo and in vitro.

Introduction

The diversity of human skin permeability has been noted between specimens and within samples from one specimen or individual. However, few investigators have

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quantified the variability at specific sites, as distinct from site-to-site differences. Most papers reported on features such as age, sex, race, anatomical site, stratum corneum (s.c.) turnover time and appendages (which alter the composition, packing, cellular structure and thickness of s.c.). The blood supply to the epidermis changes the surface temperature of the skin and may alter sink conditions (Feldmann and Maibach, 1967; Taskovitch and Shaw, 1978; Örsmark et al., 1980; Baker and Kligman, 1967a; Elias et al., 1981).

We have quantified the variability of human abdominal skin in terms of its in vitro penetrability to various compounds as determined by a variety of techniques to be used in subsequent work. Initially a simple model penetrant phenol, pK_a 9.98, was chosen because it: (a) is readily and cheaply available; (b) is essentially unionized at physiological pH; (c) is easily analyzed by UV spectroscopy; and (d) has been investigated in aqueous systems (Roberts et al., 1978). Next we used a relatively polar model compound—methanol—a more non-polar model—octanol—and aspirin and caffeine, which may have greater potential for interaction with skin components. Steady-state diffusion and finite dose diffusion techniques were employed. Finally the vasoconstrictor assay assessed human forearm skin permeability to a typical fluorinated steroid, betamethasone-17-benzoate. Vasoconstrictor data have a component arising from skin penetrability although this assessment includes a pharmacological element with its own variability.

Our experiments and published work employed many penetrants and experimental techniques, various sample and specimen numbers and different skin preparations and regional sites. Therefore we chose a simple analysis (coefficients of variation) to compare our data with published values and to establish approximate limits for variations in permeability determined in vitro and in vivo.

Materials and Methods

Diffusion cells (Figs. 1 and 2)

In vitro steady-state flux of phenol. Glass diffusion cells were used (Fig. 1) at 22 ± 1°C. Stratum corneum membranes were supported with stainless steel mesh disks; the effect of the support material and its diffusion layers was shown to be negligible compared to stratum corneum.

In vitro steady-state flux of methanol, octanol and caffeine. Glass diffusion cells (Fig. 2) were at 30 ± 0.5°C.

In vitro finite dose experiments with aspirin and caffeine. Glass diffusion cells (Fig. 1) enclosed unsupported dermatomed skin; the receptor was at 37°C and the donor was at 22 ± 1°C, 60% relative humidity.

Note that the distortion in the donor compartment of the cell in Fig. 1 allows tipping of the cell (to remove air bubbles from receptor) without spillage of donor contents.

Chemicals

Methanol and phenol (BDH Chemicals), caffeine purum (Fluka Agent Fluoro-

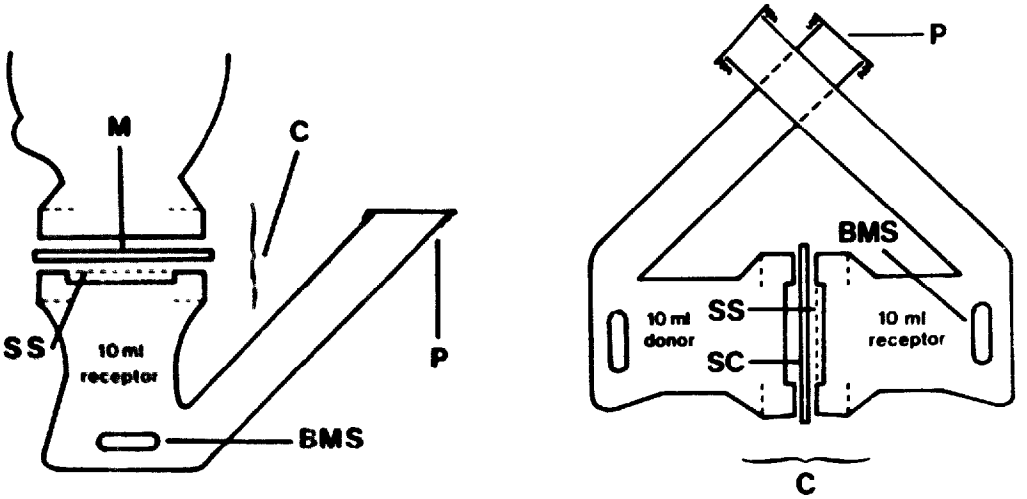

Fig. 1. Glass diffusion cell used for steady-state flux phenol experiments with stratum corneum membranes and for finite dose aspirin and caffeine experiments with dermatomed skin (without support screen). Key: M, membrane; SS, support screen; BMS, bar magnet stirrer; P, sampling port (sealed with parafilm); C, compartments clamped across ground glass surfaces.

Fig. 2. Glass diffusion cell used for steady-state flux—methanol, octanol and caffeine experiments. Key: SS, support screen; SC, stratum corneum; C, compartments clamped across ground glass surfaces; BMS, bar magnet stirrer; P, sampling port (sealed with parafilm).

chem.), octan-1-ol (Aldrich Chemicals). Acetyl [carboxyl-^{14}C]salicylic acid (aspirin) and [^{14}C]methanol (Radiochemical Centre, Amersham), [^{14}C]octan-1-ol (ICN Pharmaceuticals), [^{14}C]1-methyl-caffeine (New England Nuclear). Fisofluor-1 scintillation fluid (Fisons Scientific Equipment). Betamethasone-17-benzoate was a gift from Warner-Lambert (U.K.) and Betnovate Cream (0.1% betamethasone-17-valerate) was from Glaxo (U.K.). 2-Pyrrolidone, N-methyl-2-pyrrolidone, dimethylformamide, acetone, propylene glycol, ethanol (BDH Chemicals), 1-ethyl-2-pyrrolidone, N,N-diethyl-*m*-toluamide (Aldrich Chemicals), dimethylisosorbide (Atlas).

Skin membranes

Midline, human, pale caucasian, elderly (71 years ± 14 S.D.) abdominal skin was obtained at autopsy and was sealed in evacuated plastic bags, frozen and stored at −20°C.

Stratum corneum membranes. S.c. membranes from 22 females and 3 males were prepared by the heat separation method of Kligman and Christophers (1963) omitting the trypsinization step so as to avoid damaging the membrane.

Full thickness dermatomed skin. Sections (430 μm ± 5%) were cut with a Davies Dermatome 7 (Duplex Electro Dermatome) from 8 female skin specimens.

Penetration experiments

(a) In vitro steady-state flux of phenol

Thirty-three diffusion cells (Fig. 1) were prepared with 10 s.c. specimens. Distilled

water (1 ml) in the donor compartment with 10 ml in the receptor section hydrated the s.c. for 16 h. The receptor water was replenished and donor water was removed and replaced with 1 ml of 1% w/v aqueous phenol. We sampled 0.5–1 ml (±0.5%) of receptor fluid (Finpipette) at time 0 then approximately hourly for 7–10 h, restoring receptor sample volume with water Donor solution was replaced approximately every 2 h to avoid depletion. Analytical samples were diluted with water and absorbances at 270 nm, 5 mm path lengths were measured in a Cecil UV Spectrophotometer.

(b) In vitro steady-state flux of methanol, octanol and caffeine

Twenty-four diffusion cells (Fig. 2 with screen supports) were prepared from 15 s.c. specimens, hydrated as above (pH 7.4 buffer for caffeine). The receptor fluid was replenished and the donor chamber was emptied and refilled with 10 ml radioactive solution (approximately 0.3 $\mu Ci \cdot ml^{-1}$ for methanol, 0.07 $\mu Ci \cdot ml^{-1}$ for octanol, 0.5 $\mu Ci \cdot ml^{-1}$ for caffeine). Receptor fluid (1 ml sample) was removed at half- to one-hourly intervals, sample volume was replaced with fresh fluid and the penetrant was analyzed by scintillation counting (Packard Tricarb Scintillation Counter Model 3255).

(c) In vitro finite dose with aspirin and caffeine

Cells (Fig. 1) fitted with unsupported dermatomed skin, were equilibrated for 16 h with 10 ml pH 7.4 aqueous saline buffer at 37°C as receptor and a controlled atmosphere of 22°C, 60% relative humidity above the skin. A dose (12–16 μg) of [^{14}C]-labelled drug was deposited on the s.c. surface from 30 μl acetone. For half of the caffeine cells the donor chamber was sealed with parafilm to occlude and to hydrate the skin. Samples of receptor fluid (1 ml) were taken for 100 h, the volume was restored with buffer, and penetrant was analyzed by scintillation counting.

(d) In vivo vasoconstrictor test

For 10 subjects the blanching response at the forearm was assessed for 0.1% Betnovate Cream and for 0.1% betamethasone 17-benzoate applied in 2-pyrrolidone (2-P), N-methyl-2-pyrrolidone (NMP), 1-ethyl-2-pyrrolidone (EP), dimethylformamide (DMF), N,N-diethyl-*m*-toluamide (DEET), DEET 75% in ethanol, dimethylisosorbide (DMI)—all solvents which are possible penetration enhancers—and with acetone as a control. Application sites were occluded for 6 h (see Barry and Woodford, 1978).

Inter-s.v. was expressed as the coefficient of variation of the total corrected scores for a preparation or solution, for 10 volunteers. Similar inter-s.v. estimates were also calculated for unpublished raw data from seven trials which Barry and Woodford performed. These tests employed various topical steroids formulated as creams, ointments, solutions, foams or gels.

Data treatment

(a) Steady-state flux experiments

Flux (J) was determined by linear regression from plots relating cumulative

amount of compound penetrating per cm^2 of membrane with time (donor depletion or receptor build-up of penetrant was < 10%). The intercept provided the lag time, t_L. The permeability coefficient (k_p) was flux normalized for concentration.

(b) Finite dose diffusion experiments

The cumulative percentage dose of drug penetrating was plotted versus time; penetration rates calculated from tangents were plotted against time. From the first graph the percentage dose penetrated in 48 h was obtained and, from the second, the peak penetration rate was derived.

(c) Inter- and intra-specimen variation estimates

The intra-specimen variation (intra-s.v.) at a site was defined as a coefficient of variation for samples within a specimen for flux, permeability coefficient, lag time, peak absorption rate or percentage dose of a drug penetrated in 48 h.

Inter-specimen variation (inter-s.v.) at a site was designated as the coefficient of variation for a measured parameter (n specimens).

Results

In vitro steady-state flux of phenol

Table 1 reports flux and lag time data for 1% w/v aqueous phenol. Fig. 3 shows typical experimental plots.

For each s.c. specimen in Table 1 with ≥ 3 samples, a coefficient of variation (c.v. ± %) was calculated from flux measurements. Each c.v. provided an estimate of intra-s.v.; the mean was 17% ± 13 (S.D.). Similarly c.v.s were calculated for lag

TABLE 1

STEADY-STATE DIFFUSION OF 1% w/v AQUEOUS PHENOL THROUGH HYDRATED HUMAN ABDOMINAL STRATUM CORNEUM, AT 22°C

Stratum corneum				Mean flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Intra-s.v. [a] (n ≥ 3)	Mean lag time (h)	Intra-s.v. (n ≥ 3)
Specimen	Age	Sex	n				
A	?	F	2	1.66	-	0.72	-
M	78	F	1	2.60	-	0.35	-
J	77	M	1	1.63	-	0.25	-
W	73	F	2	1.38	-	0.40	-
O	70	F	2	0.977	-	0.43	-
C	86	F	9	2.22	± 30	0.93	± 41
N	78	F	3	1.09	± 7	0.57	± 32
D	85	F	4	0.885	± 8	0.85	± 16
I	89	M	4	1.83	± 19	0.60	± 24
Q	53	F	3	0.59	± 14	0.37	± 18

[a] Intra-s.v. = Intra-specimen variation at a site measured as coefficient of variation of flux or lag time for n samples.

times, providing another mean intra-s.v. (26% ± 10 S.D.). The larger lag time variability probably reflects the greater difficulty in experimental measurement of accurate lag times compared to fluxes. Relatively small unavoidable alterations in the determination of the slope of the steady-state plot can markedly change the value of the lag time deduced.

For fluxes for all 10 specimens a c.v. of 42% was calculated for the means, providing an estimate of inter-s.v. A similar estimate of inter-s.v. (36%) was obtained from mean lag time data.

In vitro steady-state flux of methanol, octanol and caffeine

Table 2 reports the mean permeability coefficients (k_p) and lag times for methanol, octanol and caffeine each penetrating 8 different specimens of water conditioned s.c. Fig. 4 provides a typical steady-state plot for octanol. Inter-s.v. estimates for octanol (39%) and caffeine (45%) were close to those obtained from phenol flux (42%) and lag time data (36%). However, methanol k_p data indicated a high value (71%). Lag times for octanol and methanol suggested large inter-s.v. values but as before these high estimates may be influenced by the greater errors in determining lag times compared to fluxes.

TABLE 2

IN VITRO INTER-SPECIMEN VARIATION IN PERMEABILITY OF HUMAN SKIN AT THE ABDOMEN

Steady-state diffusion experiments (n = 8)

Penetrant	Mean [a] $k_p \times 10^3$ (cm·h^{-1})	Inter-s.v. [b] c.v. (± %)	Mean lag time (h)	Inter-s.v. [b] c.v. (± %)
Methanol	1.6	± 71%	0.37	± 58%
Octanol	61	± 39%	0.39	± 74%
Caffeine	1.6	± 45%	4.0	± 45%

Finite dose penetration experiments (n = 3–5)

Penetrant	Mean % dose in 48 h	Inter-s.v. [b] c.v. (± %)	Mean peak rate (% dose·h^{-1})	Inter-s.v. [b] c.v. (± %)
Caffeine	(i) [c] 33	± 34%	2.2	± 63%
	(ii) 69	± 38%	4.9	± 61%
Aspirin	(i) 11	± 58%	0.58	± 46%

[a] k_p permeability coefficient.

[b] Inter-specimen variation at a site measured as coefficient of variation of permeability coefficient, lag time, % dose penetrated in 48 h, or peak penetration rate, for n specimens.

[c] (i) normally hydrated skin; (ii) occlusion hydrated skin.

Fig. 3.

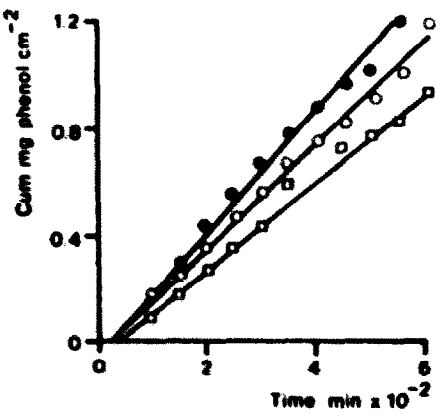

Fig. 4.

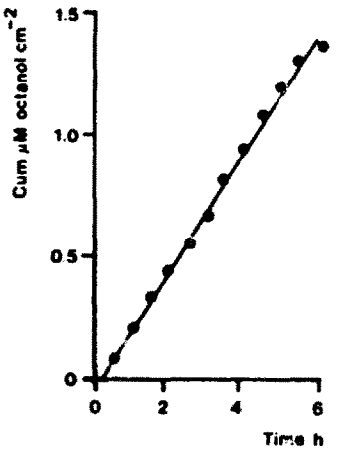

Fig. 3. Phenol steady-state plot for 3 samples from specimen C. Cumulative mg·cm^{-2} $\times 10^3$ penetrating stratum corneum with time, at 22°C, from 1% w/v aqueous phenol into water.

Fig. 4. Octanol steady-state plot. Cumulative μmol·cm^{-2} penetrating stratum corneum with time, at 30°C, from 0.003 M aqueous octanol into water.

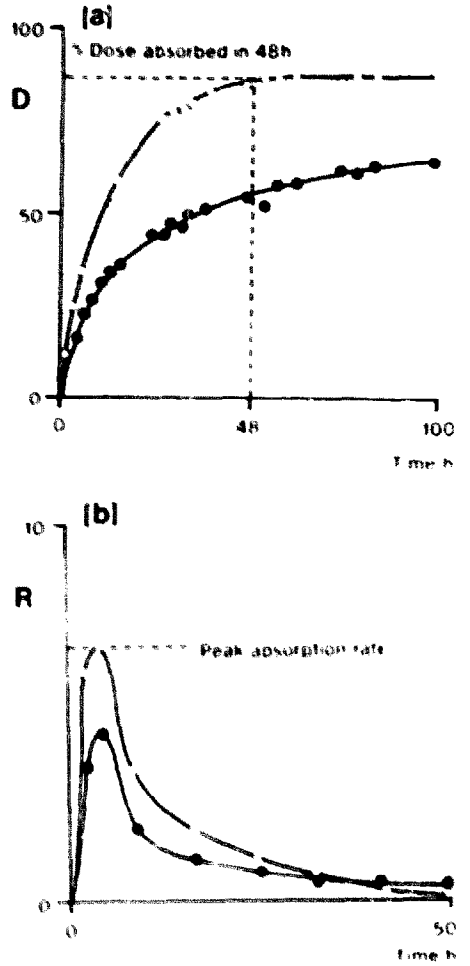

Fig. 5. Finite dose penetration plots for caffeine. Penetration through dermatomed skin, from a solvent deposited film (approximately 4 μg·cm^{-2}) versus time, into aqueous saline buffer pH 7.4 at 37°C. Donor compartment conditions: ●, controlled atmosphere, 22°C and 60% relative humidity; ○, occluded. (a) Cumulative % dose penetrated-D; (b) penetration rate (R, % dose·h^{-1}).

In vitro finite dose with aspirin and caffeine

Table 2 provides mean data for the percentage dose of aspirin or caffeine penetrating in 48 h (from a solvent-deposited film) and the mean peak penetration rates. Fig. 5a shows a typical plot, for caffeine, of the cumulative percentage dose penetrated versus time for controlled atmosphere and for occluded donor conditions. Fig. 5b illustrates penetration rate against time plots derived from the cumulant plots.

When caffeine penetrated fairly dry skin (under a controlled atmosphere) the amounts penetrated in 48 h yielded a typical inter-s.v. estimate of 34%. This did not alter appreciably for hydrated skin (under occlusion). The peak penetration rates of caffeine indicated a higher inter-s.v. of approximately 62%.

Aspirin data for percentage dose penetrated in 48 h showed a fairly high inter-s.v. but we obtained a more typical estimate from peak penetration rates of ±46%.

In vivo vasoconstrictor test

Table 3 lists in vivo, inter-s.v. estimates calculated from our vasoconstrictor test (v.c.) data and from the results of the Barry and Woodford trials. The mean inter-s.v. from our data was ±60% (±17 S.D.) and from the Barry and Woodford trials it was ±32% (±18 S.D.); the overall mean in vivo, inter-s.v. was ±36% (±20 S.D.).

TABLE 3

INTER-SPECIMEN VARIATION AT THE FOREARM FOR STEROID PREPARATIONS MEASURED BY THE VASOCONSTRICTOR TEST; BARRY AND WOODFORD UNPUBLISHED DATA AND THIS WORK—VOLUNTEER PANEL SIZE 10, TOTAL NUMBER OF PREPARATIONS 59

Barry/Woodford trials	Mean inter-s.v. [a] (±S.D., number of preparations)	Inter-s.v. [a] to Betnovate Cream
1	22 (±4.4, 9)	26
2	20 (±2.2, 7)	21
3	26 (±3.1, 7)	22
4	23 (±9.2, 8)	24
5	25 (±4.7, 6)	23
6	57 (±16, 7)	29
7	64 (±8.2, 4)	72
This Work	Mean Inter-s.v. [b] (±S.D., number of vehicles) [c]	Inter-s.v. [a] to Betnovate Cream
	60 (±17, 9)	45

[a] Inter-specimen variation measured as the coefficient of variation of the total corrected scores for a preparation.

[b] Inter-specimen variation measured as the coefficient of variation of the total corrected scores for a betamethasone-17-benzoate solution.

[c] Vehicles given in Materials and Methods section.

Discussion

We can compare our inter-s.v. and intra-s.v. determinations with estimates calculated from published data (Örsmark et al., 1980; Elias et al., 1981; Wallace et al., 1978; Marzulli, 1962; Feldmann and Maibach, 1969, 1970; Rutter and Hull, 1979; Blank et al., 1957; Nilsson, 1977; Lamke et al., 1977; Baker and Kligman, 1967b; Ostrenga et al., 1971; Wurster and Munies, 1965; Mali, 1956) see Table 4.

For each set of data the mean inter-s.v. was higher than mean intra-s.v. Our measurements provided lower mean variabilities than published results both within and between samples. Literature values were derived from experiments using various skin sites but our skin supply for in vitro work was exclusively abdominal. Therefore abdominal skin appears to be less variable in its permeability than other anatomical sites.

Our mean in vitro estimate of inter-s.v. at the abdomen (51%) agreed fairly well with inter-s.v. at the abdomen calculated from published in vitro data of 58% (Blank et al., 1957), 41% (Wallace et al., 1978) and 54% (Elias et al., 1981—for tritiated water). A high value of 94% was obtained for salicylic acid. New-born abdominal skin provided in vivo inter-s.v. estimates of 33% (Rutter and Hull, 1979), 27% and 44% (Örsmark et al., 1980) and 55% (Nilsson, 1977) and adult skin gave inter-s.v. estimates in vivo of 49%, 48% and 45% (Lamke et al., 1977). Therefore abdominal skin does indeed provide lower estimates of inter-s.v. than the average for in vitro data (66%).

In vivo variability estimates were lower on average than those from in vitro data. This difference may be because the skin changes during excision, storage and experimental manipulation.

TABLE 4

SUMMARY OF INTER- AND INTRA-SPECIMEN VARIATIONS AT A SITE IN HUMAN SKIN, CALCULATED FROM: (1) OUR IN VITRO EXPERIMENTS REPORTED IN TABLES 1 AND 2; (2) OUR IN VIVO EXPERIMENT DETAILED IN TABLE 3, AND (3) ALL DATA PROVIDED IN TABLES 1–3 AND IN REFS 3, 5 AND 9–20

Data	Intra-specimen variation mean c.v. (± S.D., n) [a]	Inter-specimen variation mean c.v. (± S.D., n)
(1) our data in vitro	21% (± 11, n = 10)	51% (± 13, n = 14)
(2) our data in vivo	-	60% (± 17, n = 10)
(3) all data in vitro	43% (± 25, n = 32)	66% (± 25, n = 42)
in vivo	27% (± 9, n = 4)	45% (± 18, n = 114) [b]

[a] n = number of c.v. estimates.

[b] This estimate excludes the vasoconstrictor test data from the Barry and Woodford trials in Table 3 because inter-s.v. was deliberately reduced in the design of these trials.

Our data for caffeine (Table 2) show that increasing the hydration of the skin did not appreciably alter the inter-s.v. estimates. Örsmark et al. (1980) measured transepidermal water loss in vivo before and after 1 h of occlusion. Although at the abdomen, this increased hydration increased inter-s.v. from 27% to 44%, there was hardly any effect at the buttock and forearm sites. The v.c. assay for steroid preparations (Table 3) indicated a low inter-s.v. in the first five trials; a panel of screened volunteers were used, chosen for their controlled blanching response. Such selection of individuals probably also eliminated wide variations in permeability to steroids. Trials 6 and 7 showed a higher mean inter-s.v.; during these trials it was necessary to include some unscreened volunteers.

Our study indicated a mean inter-s.v. of $\pm 60\%$ (± 17 S.D.). But the variability in response to Betnovate Cream (used in all v.c. tests) was $\pm 45\%$, a fairly high value compared to estimates from Barry and Woodford's first 6 v.c. tests. Therefore the panel in our assay had a high inter-s.v. relative to most previous panels. Only steroid solutions in NMP, DEET, DEET 75% and PG provided inter-s.v. estimates outside the range of one standard deviation from the mean. NMP reduced the inter-s.v. to $\pm 42\%$, DEET and DEET 75% increased the inter-s.v., relative to the mean, to approximately $\pm 80\%$. This increased variation may be partly due to masking of the blanching response by erythema. PG also provided a high inter-s.v. estimate ($\pm 84\%$); this material is a known irritant at high concentrations.

Our in vivo estimate of inter-s.v. ($\pm 60\%$) was close to our in vitro estimate ($51\% \pm 13$ S.D., information in Tables 1 and 2). Barry and Woodford's data yielded a mean inter-s.v. ($\pm 32\%$) which was lower than our test and closer to our mean value for intra-s.v. ($21\% \pm 11$ S.D. for results in Table 1). The low variability, particularly in the first 5 v.c. trials, was because of the selection of volunteers as described above.

Conclusions

Table 4 summarizes the data discussed in this paper and it identifies the fairly wide limits to be expected for inter-specimen and intra-specimen variation in permeability. The in vitro data yielded larger estimates of variability than did in vivo results. By the very nature of the raw data it was not possible to assess if such a ranking is statistically significant. Inter-specimen variation, both in vivo and in vitro, is higher than intraspecimen variation.

Acknowledgements

The authors thank Dr. A.W. Kemp of Bradford University, U.K., for her statistical advice, Mrs. M. Firth for typing the manuscript and ICI Pharmaceuticals and the Science and Engineering Research Council for support in respect of a C.A.S.E. Studentship for D.S.

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