J. Pharm. Pharmacol. 1992, 44: 371–378
Received July 22, 1991

Quantitative Skin Blanching Assay of Corticosteroid Creams Using Tristimulus Colour Analysis

SUI YUNG CHAN AND ALAIN LI WAN PO

The Drug Delivery Research Group, The School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, Belfast, UK

Abstract—The potencies of four proprietary corticosteroid creams were ranked using blanching measured by a tristimulus colour analyser as an index. Intra-subject variation was measured in a trial using a single subject while in a second study inter-subject variation was quantified using six volunteers. Discriminative parameters were derived from the extended multiple-point chromaticity coordinates $L*$ and $a*$ recorded after application of the steroid creams under occlusion. Analysis of variance of the data using the linear regression model was followed by Tukey's multiple comparison tests. Ranking of the creams using parameters of the pharmacodynamic response corresponded with the generally accepted rank order of the potencies of the corticosteroid creams. It is proposed that this multiple-point skin blanching assay of topical corticosteroids using an internationally accepted clear measurement standard and the subsequent data analysis be adopted as a standard protocol.

The most frequently used bioscreening technique for measuring topical corticosteroid potency is the human skin blanching assay which is a non-invasive, simple, convenient, reproducible, and relatively cheap technique. The earliest report of corticosteroid-induced skin blanching at the site of an intra-articular injection was made by Hollander et al (1950). The precise mechanism by which corticosteroids cause blanching has not been elucidated (Schlagel 1972). McKenzie & Stoughton (1962) first used the blanching phenomenon to develop an index of percutaneous absorption of corticosteroids. The technique has been exploited and refined for the assessment of the potency, bioavailability and bioequivalence of corticosteroid preparations by various investigators (refer to reviews by Barry & Woodford 1986; Haigh & Kanfer 1984). However, many lamented that the induced pallor of the skin had to be graded by eye, making the method subjective and the results less precise for quantitative appraisal (Ashworth 1989; Shah et al 1989). Instrumental methods for measurement of the blanching response provide more objective assessments, but few are available and even these are often difficult to use or are insensitive: for example reflectance spectrometry by Altmeyer & Zaun (1976), Altmeyer & Cremer (1977), Bjerring & Andersen (1987), Conner et al (1990), Dawson et al (1980), Feather et al (1982), Heseltine et al (1964) and Ryatt et al (1982); laser-doppler velocimetry by Amantea et al (1983) and Duteil et al (1990); combination of laser-doppler velocimetry with reactive hyperaemia by Bisgaard et al (1986); thermography by Aiache et al (1980); and tristimulus colorimetry by Király & Soós (1976), Queille-Roussel et al (1990, 1991) and Waring et al (1990).

The present study was conducted to compare objectively the skin blanching response to four proprietary corticosteroid creams of different known clinical potencies (British National Formulary 1991) using a portable tristimulus reflectance analyser.

Correspondence: A. Li Wan Po, Drug Delivery Research Group, The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK.

Materials and Methods

Volunteers

Volunteers gave their written consent after approval was granted by the Research Ethics Committee, The Queen's University of Belfast, to conduct these trials. None of the volunteers had any notable ailment or obvious skin blemishes on their forearms and had not received medication, especially steroids, that would affect the experiment. Any residual differences are randomized through the experimental design used.

Equipment

The Chroma Meter records colour in a three-dimensional space, designated $L*a*b*$, as illustrated in Fig. 1. The luminance $L*$ value expresses the relative brightness of colour ranging from black to white. The $a*$ index is the colour hue related to redness and greenness. The third chromaticity coordinate, $b*$, is the colour range from blue to yellow. The colour of any surface can be specified by a combination of the three values. Changes in any of the indices, as the pharmacodynamic response develops and increases in intensity, indicate shifts in skin colour. The instrument was calibrated using the calibration plate (CD-A43) before each experiment.

Blanching assay

Fixed amounts of each of the coded corticosteroid creams (Table 1) were applied onto the skin of the forearms of volunteers and the blanching responses monitored. The template boundaries of potential sites on the flexor surfaces of the forearms were marked with ink to ensure the precise location of subsequent application of the template holding the creams. The first two centimetres of cream extruded from each tube were discarded. Standard 1 mL disposable syringes were filled with the respective creams immediately before use, to discount interaction between the syringe and the formulation, if any. The amount of cream used is measured by weight difference of the syringe before and after the mass was extruded directly onto the designated Melinex film (ICI Type

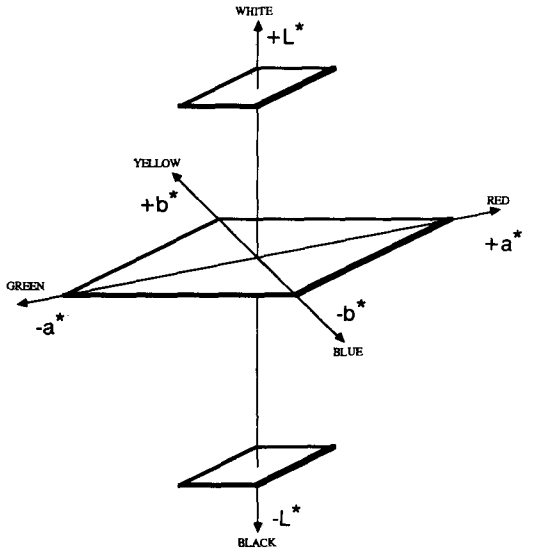

FIG. 1. The $L*a*b*$ colour space.

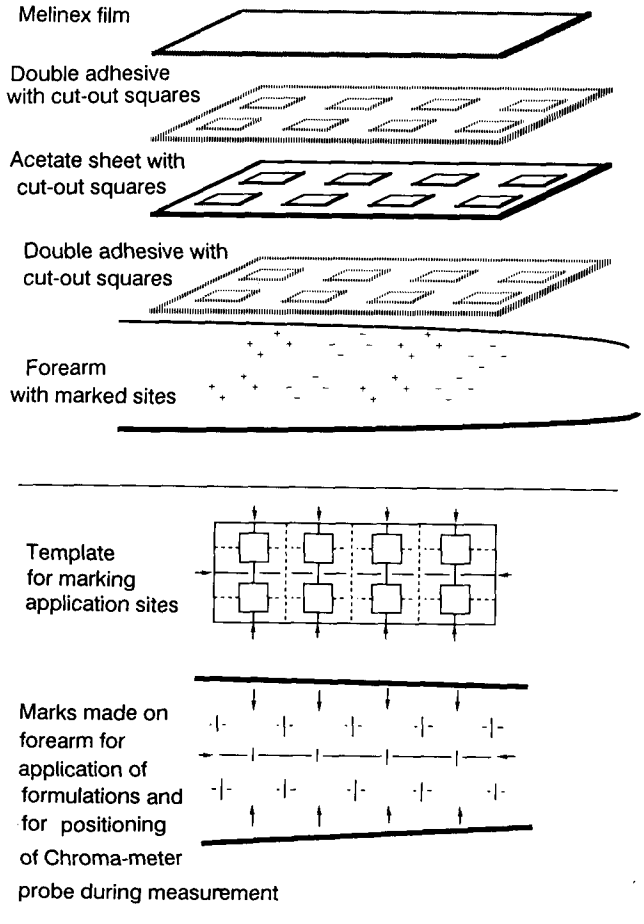

FIG. 2. Template for skin blanching study.

S12-μm) surfaces (area of 1·5 × 1·5 cm) of a template fabricated by layers of PVC film (Schwan Stabilo overhead projection transparency film 0·08 mm thick, A4 size, Cat. No. 7248/100), Melinex polyester film and double-sided adhesive tape (3M Scotch pressure sensitive tape 5 cm wide) (Fig. 2). The Melinex film served as an occlusive barrier. Two trials were conducted. The allocations of formulations to application sites for the single-subject and six-subject experiments were based on a randomized 4 × 4 Latin square design and a completely randomized pattern, respectively. In the first trial, the time of occlusion was for 6 h with a simultaneous application of 10 mg of the respective cream to each of the four sites. In the second trial, a more efficient regimen of a 3 h occlusion period with a larger amount of the preparation (20 mg) was applied on each of the two designated areas per subject. After the period of occlusion, the template was carefully removed and the skin surfaces were gently wiped, washed with soap and lukewarm water and patted dry with soft paper tissues to remove any traces of the creams. The forearm skin occasionally appeared puckered with a transient redness around the application sites, but this normally resolved within 15 to 20 min. The temperature range of the forearm surfaces was 31–33°C, room conditions were 22–24°C and relative humidity 35–40% (measured using Temp squares cat. code LCH SQ, liquid crystal thermometer cat. code 14RT, and humidity strip cat code 14HI, respectively, from Electronic Temperature Instruments Ltd, UK).

The Minolta Chroma Meter was used to record skin pallor of the designated sites, first before the cream was applied (that is, the baseline value), then immediately the cream had been washed off and at successive time intervals. Three consecutive readings were taken at each site at the respective time intervals and their mean values used. The probe of the Chroma Meter was adapted so that the pretreated skin areas to be monitored were at a fixed distance of 1 cm from the end of the probe.

Data treatment

The instrument yielded a quantitative output to two decimal places for each of the three chromaticity coordinates. The data were downloaded onto a personal computer for further analysis to derive the following parameters: area under the response-time curve (AUC) within a fixed time interval, corrected AUC (CAUC), where the baseline chromaticity coordinate was deducted from the successive coordinate values, slope of the cumulative chromaticity coordinate values over a specified time span (Slope), slope of the cumulative differences in chromaticity coordinate values between baseline and the other readings over the stated interval, that is the corrected slope (CSlope).

The AUC and CAUC parameters were computed according to the trapezoidal rule and the two slope parameters were obtained by linear regression of coordinate value or adjusted coordinate value against time. The resulting parameter values were further subjected to statistical treatment to compare the outcome relative to the response to different creams. Analysis of variance using multiple regression was performed on the derived parameters AUC, CAUC, Slope and CSlope to evaluate the variations due to application site, replicate, treatment and subject where appropriate. Treatment means were ranked using Tukey's multiple range test.

Results

For measuring the skin blanching response, chromaticity

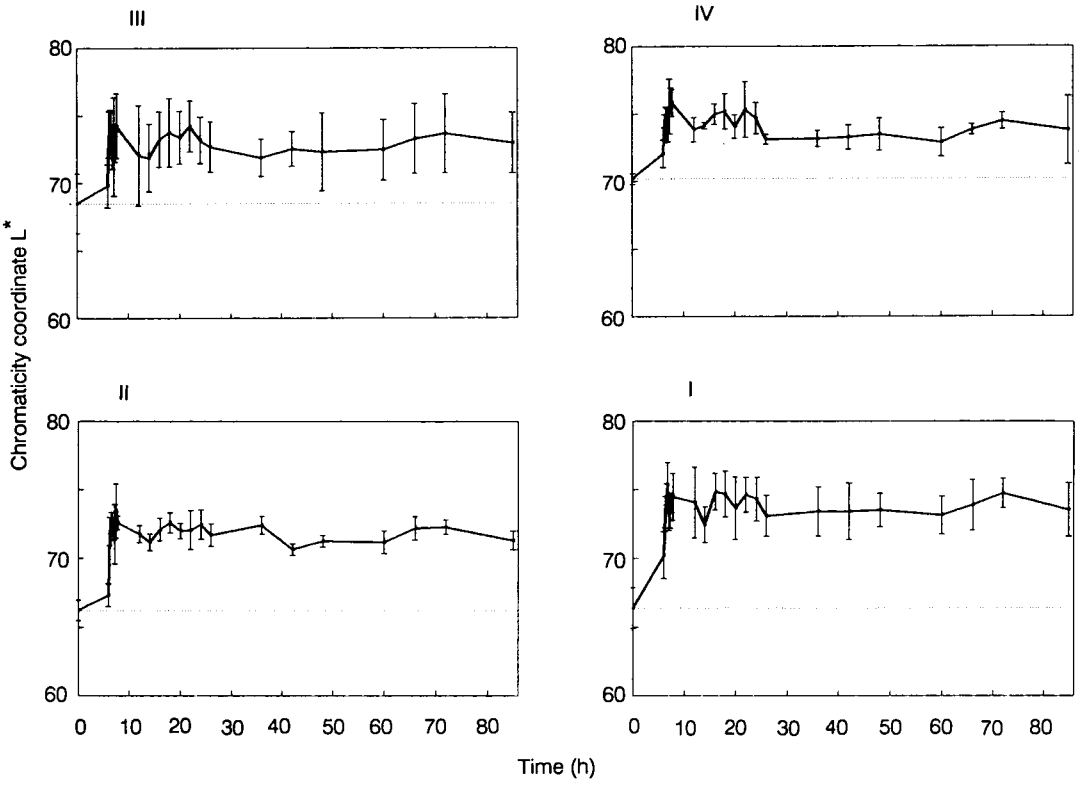

FIG. 3. Blanching effect of corticosteroid creams using the chromaticity coordinate $L*$ in the single-subject study. The error bar represents the standard deviation of measurements on four application sites.

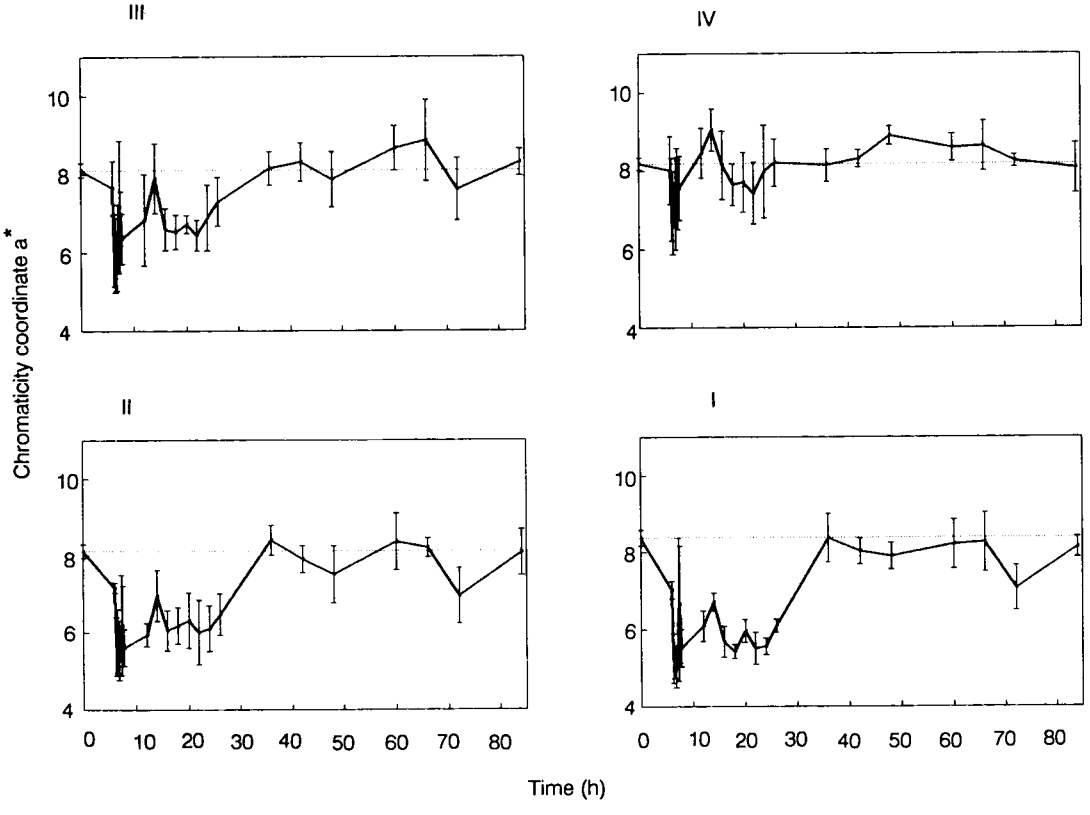

FIG. 4. Blanching effect of corticosteroid creams using the chromaticity coordinate $a*$ in the single-subject study. The error bar represents the standard deviation of measurements on four application sites.

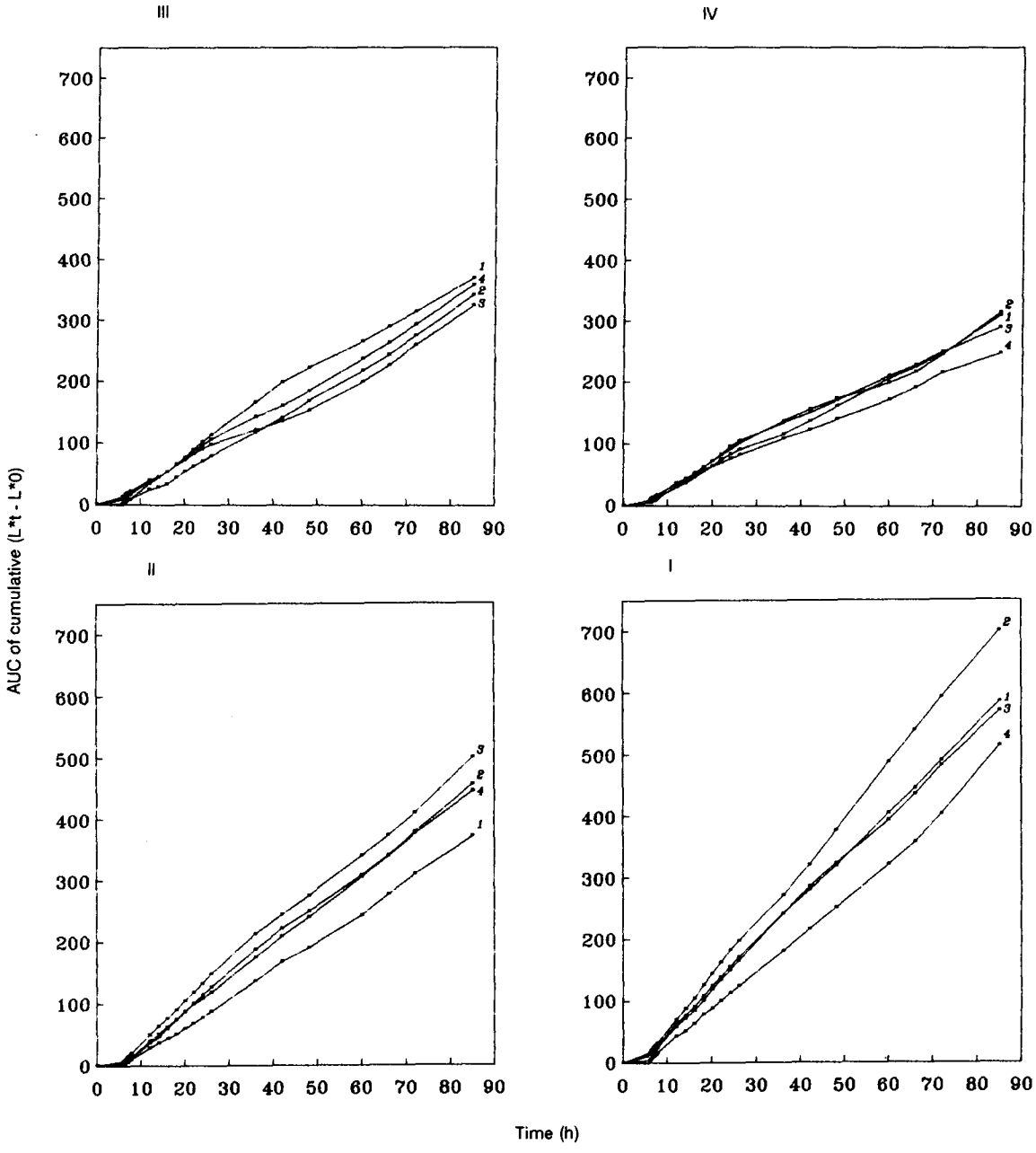

FIG. 5. The corrected AUC of the $L*$ coordinate of the blanching response to corticosteroid creams in the single-subject study. The numbers on the graphs represent the application sites, 1 being the most distal site on the forearm and 4, the most proximal.

coordinates $L*$ and $a*$ were found to be more discriminative than $b*$ as the peak response was registered by negligible difference from the basal $b*$ value. The latter coordinate was therefore omitted in subsequent data analysis. Figs 3 and 4 show the progress of the skin blanching response to the creams using the $L*$ and $a*$ coordinates. As the blanching effect develops, skin pallor becomes lighter and its redness fades. This corresponds to an increase in the $L*$ value and a decrease in the $a*$ coordinate. Therefore the intensity of the blanching response varies directly with the $L*$ coordinate, and inversely with the $a*$ coordinate. Fig. 5 is a representative multiple chart of the corrected areas under the response-time curves (CAUC for adjusted $L*$). The descriptive statistics of the parameters is summarized in Fig. 6. It appears from these graphs that the magnitude of the skin blanching response is in the order of D > C > A > B, which corresponds well to the potencies of the corticosteroid creams used, that is I > II > III > IV. This was confirmed by subsequent inferential statistical analysis.

The analysis of variance for the intra-subject variation (Table 2) and for the multi-subject study (Table 3) reveals those parameters where the four corticosteroid creams were not all equal in their blanching activities ($P < 0{\cdot}05$). Comparisons performed only after a significant global result had been found, were carried out using Tukey's multiple comparison test to differentiate and rank the group means. Table

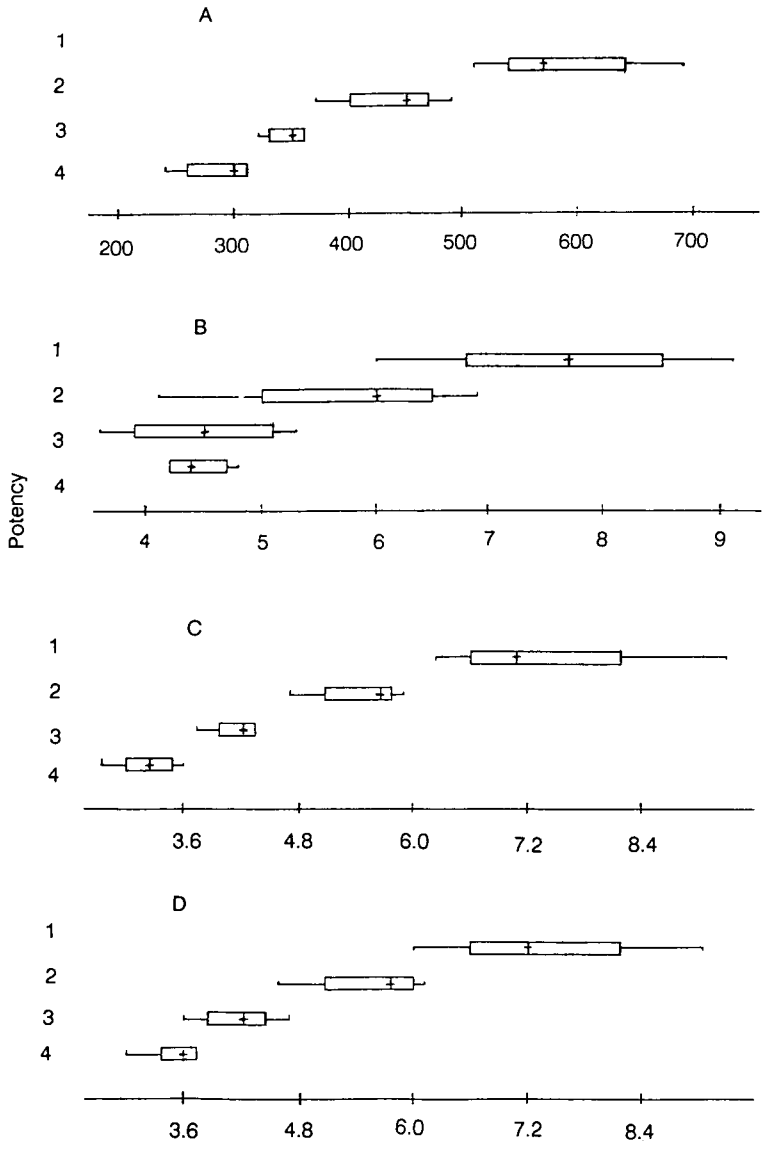

FIG. 6. Box-plot of parameters derived from the L^* chromaticity coordinate of the blanching effect against the potency of corticosteroid cream. A, AUC (0–85 h), B, Slope (6–24 h), C, Slope (24–85 h), D, Slope (6–85 h).

4 for the single-subject study and Table 5 for the six-subject experiment show the rank orders of the effect of the corticosteroid creams, as measured by the different blanching parameters. These rankings were consistent with the clinical potency rankings reported in the literature (British National Formulary 1991).

Table 1. Corticosteroid creams used.

Potency	Code* 1	Code* 2	Proprietary product	Active ingredient
IV	B	A	Mildison lipocream (Gist-Brocades Pharmaceuticals)	Hydrocortisone 1% w/w
III	A	B	Betnovate-RD cream (Glaxo Laboratories Limited)	Betamethasone 0·025% w/w as the valerate ester
II	C	C	Locoid lipocream (Gist-Brocades Pharmaceuticals)	Hydrocortisone 17-butyrate 0·1% w/w
I	D	D	Dermovate cream (Glaxo Laboratories Limited)	Clobetasol propionate 0·05% w/w

* Different sets of codes were assigned to the two trials, 1 and 2, respectively.

Table 2. Analysis of variance of the skin blanching effect of corticosteroid creams of different potencies in a single subject using the linear regression model.

Source	DF	SS	MS	F	P
Parameters derived from chromaticity coordinate a^*					
1. CAUC (0–85 h)					
Treatment	3	21798·38	7266·13	12·60	0·0053
Column	3	1772·40	590·80	1·02	NS
Row	3	351·33	117·11	0·20	NS
Error	6	3460·45	576·74		
Total	15	27382·57			
R-Square = 0·874; root m.s.e = 24·015; mean = −42·247					
2. CSlope (24–85 h)					
Treatment	3	1·65	0·55	5·22	0·0414
Column	3	0·33	0·11	1·05	NS
Row	3	0·13	0·04	0·41	NS
Error	6	0·63	0·11		
Total	15	2·74			
R-Square = 0·770; root m.s.e. = 0·325; mean = −0·132					
3. CSlope (6–85 h)					
Treatment	3	3·60	1·20	14·92	0·0035
Column	3	0·20	0·07	0·81	NS
Row	3	0·06	0·02	0·24	NS
Error	6	0·48	0·08		
Total	15	4·34			
R-Square = 0·889; root m.s.e. = 0·284; mean = −0·545					
Parameters derived from chromaticity coordinate L^*					
4. CAUC (0–85 h)					
Treatment	3	207059·81	69019·94	18·81	0·0019
Column	3	8093·33	2697·78	0·74	NS
Row	3	508·09	169·36	0·05	NS
Error	6	22018·42	3669·74		
Total	15	237679·65			
R-Square = 0·907; root m.s.e. = 60·578; mean = 418·31					
5. CSlope (24–85 h)					
Treatment	3	40·03	13·34	22·06	0·0012
Column	3	2·25	0·75	1·24	NS
Row	3	0·24	0·08	0·13	NS
Error	6	3·63	0·61		
Total	15	46·15			
R-Square = 0·921; root m.s.e. = 0·778; mean = 5·087					
6. CSlope (6–85 h)					
Treatment	3	35·44	11·81	15·30	0·0032
Column	3	1·85	0·62	0·80	NS
Row	3	0·20	0·07	0·08	NS
Error	6	4·63	0·77		
Total	15	42·12			
R-Square = 0·890; root m.s.e. = 0·879; mean = 5·199					

NS represents no significant difference ($P > 0·05$); DF = degrees of freedom; SS = sums of squares; MS = mean square; F = F value; P = probability of observed effect under null hypothesis.

Table 3. Analysis of variance of the skin blanching effect of corticosteroid creams of different potencies in six volunteers using the linear regression model.

Source	DF	SS	MS	F	P
Parameters derived from chromaticity coordinate a^*					
1. Slope (2–3 h)					
Treatment	3	2·97	0·99	0·67	0·0094
Site	3	2·43	0·81	0·55	NS
Subject	5	212·59	42·52	28·68	0·0001
Arm	1	0·71	0·71	0·48	NS
Error	35	51·88	1·48		
Total	47	270·57			
R-Square = 0·808; root m.s.e. = 1·217; mean = 6·07					
2. CSlope (2–3 h)					
Treatment	3	6·95	2·32	6·05	0·0020
Site	3	0·85	0·28	0·74	NS
Subject	5	34·49	6·90	18·01	0·0001
Arm	1	3·86	3·86	10·09	0·0031
Error	35	13·40	0·38		
Total	47	59·56			
R-Square = 0·775; root m.s.e. = 0·619; mean = −0·92					
Parameters derived from chromaticity coordinate L^*					
3. Slope (2–3 h)					
Treatment	3	11·20	3·73	4·45	0·0094
Site	3	0·70	0·23	0·28	NS
Subject	5	144·53	28·91	34·46	0·0001
Arm	1	1·60	1·60	1·91	NS
Error	35	29·36	0·84		
Total	47	187·40			
R-Square = 0·843; root m.s.e. = 0·916; mean = 69·58					
4. CSlope (2–3 h)					
Treatment	3	8·51	2·84	8·44	0·0002
Site	3	0·85	0·28	0·84	NS
Subject	5	16·96	3·39	10·09	0·0001
Arm	1	1·52	1·52	4·52	0·0407
Error	35	11·77	0·34		
Total	47	39·61			
R-Square = 0·703; root m.s.e. = 0·580; mean = 0·832					

NS represents no significant difference ($P > 0·05$).

While no significant difference ($P > 0·05$) was observed between the experimental factor, site, and the cream in the single subject study, significant differences were found between the subject variable and the formulations; and in a few instances, the arm and the formulation in the multiple subject assay.

Discussion

The template system, as proposed by Gibson (1989), was modified for the blanching assay. It is obvious that the ready-made, purpose-built, double-sided adhesive template for direct application would be time-saving and possibly more reliable in preventing the spread of topically applied preparations to adjacent test sites. In the case of occlusive treatments with semi-solid formulations, the weighed preparations are placed onto an assembled template according to the study design and then the whole template is applied directly onto demarcated sites and left for the duration of occlusion. For occlusive studies involving solutions, the template without the occlusive film is fixed onto the skin first, before the test solutions are administered topically and then covered by an occlusive film. For open studies, skin sites are marked using a template which is then removed before the solutions are applied. This prevents the spread of the topical formulations to adjacent sites. In our experiments no obvious blanching was observed beyond the boundaries of individual test sites.

Visual assessment with the naked eye evaluates the composite colours at the test site while measurements of the L^* and a^* coordinates record only the whiteness/blackness and red/green shades of the skin, respectively. The intensity of the L^* coordinate increases with time to reach a peak before levelling off, whereas the a^* coordinate starts at a high value to decrease into a trough and returning to the initial level over time during the course of the blanching response.

In monitoring the progress of the blanching effect, multiple-point measurements over an interval provide more valid summary measures for drawing conclusions, in con-

Table 4. Tukey's multiple range test for comparison* of the skin blanching effect of corticosteroid creams of different potencies in a single subject.

	Corticosteroid potency			
1. a^*: CAUC (0–85 h) DF = 6, m.s.e. = 576·74, q = 4·896, m.s.d. = 58·786	IV	III	II	I
2. a^*: CSlope (24–85 h) DF = 6, m.s.e. = 0·105, q = 4·896, m.s.d. = 0·794	IV	III	II	I
3. a^*: CSlope (6–85 h) DF = 6, m.s.e. = 0·081, q = 4·896, m.s.d. = 0·695	IV	III	II	I
4. L^*: CAUC (0–85 h) DF = 6, m.s.e. = 3669·74, q = 4·896, m.s.d. = 148·29	I	II	III	IV
5. L^*: CSlope (24–85 h) DF = 6, m.s.e. = 0·605, q = 4·896, m.s.d. = 1·904	I	II	III	IV
6. L^*: CSlope (6–85 h) DF = 6, m.s.e. = 0·772, q = 4·896, m.s.d. = 2·151	I	II	III	IV

* A continuous line indicates that comparisons are not significant at the 0·05 level. DF denotes degree of freedom; m.s.e., the mean square error; q, the critical value of studentized range; m.s.d., the minimum significant difference.

Table 5. Tukey's multiple range test for comparison* of the skin blanching effect of corticosteroid creams of different potencies in six volunteers.

	Corticosteroid potency			
1. L^*: Slope (2–3 h) DF = 35, m.s.e = 0·839, q = 3·814, m.s.d. = 1·008	I	II	III	IV
2. L^*: CSlope (2–3 h) DF = 35, m.s.e. = 0·336, q = 3·814, m.s.d. = 0·638	I	II	III	IV
3. a^*: Slope (2–3 h) DF = 35, m.s.e. = 1·482, q = 3·814, m.s.d. = 1·341	IV	III	II	I
4. a^*: CSlope (2–3 h) DF = 35, m.s.e. = 0·383, q = 3·814, m.s.d. = 0·681	IV	III	II	I

* A continuous line indicates that comparisons are not significant at the 0·05 level. DF denotes degree of freedom; m.s.e., the mean square error; q, the critical value of studentized range; m.s.d., the minimum significant difference.

trast to single end-point measurements (Meyer et al 1988). The response-time profile created could be subjected to area under the curve (AUC) analysis, similar to that after systemic administration. The AUC accounts for both the intensity and the duration of action of the pharmacodynamic response (Woodford & Barry 1974). The advantage of using summary measures instead of multiple-point comparisons is well known (Matthews et al 1990). In particular, the erroneous rejection of the null hypothesis is precisely controlled by avoiding multiple pairwise comparisons using standard two-sample tests such as the *t*-test.

The slope parameters used in the present analysis define the trend of the pharmacodynamic response over time and are useful when the functional form of a response is unknown. Slope and adjusted slope values over specified intervals of time can be used to compare the maximal response during the experiment, which is between 30 to 90 min for nicotinate-induced erythema (Chan & Li Wan Po 1992) and 2–3 h after a 3-h occlusion period for the corticosteroid blanching response.

Excellent correlations have been reported between the degree of blanching activity and clinical efficacy of topical corticosteroid preparations (Haleblian 1976; Miller & Munro 1980). Stoughton & Wullich (1990) found that the blanching intensity of a very potent steroid (clobetasol propionate) after an application time of 1 to 1·5 h was equivalent to that after 16 h. However, a visible response to a less potent steroid (betamethasone 17-valerate) cream required a 2- to 8-h exposure. The pharmacodynamic action of corticosteroids is thought to result from binding to glucocorticoid receptors in the dermal tissues (Marks et al 1982). These glucocorticoid receptors may be fully saturated within 1 to 1·5 h of surface dosing with a potent steroid and the remaining drug may pass into the systemic circulation. It is therefore possible to perform the blanching assay with a 3-h occlusion period, and monitoring the effect for a further 3 h, as in the multiple subject trial. This schedule is more efficient and can differentiate between the classes of steroid creams. With a non-occlusive procedure, a longer exposure period might be required as smaller amounts of the drug would have diffused through the skin. The quantitative relationships between topical administration, percutaneous absorption, clearance from site of administration and cutaneous receptor saturation of corticosteroids need to be further investigated.

Naked-eye visual assessment has been successfully used in numerous studies ranging from the potency classification of steroids to the more subtle differentiation of assorted formulations containing a particular steroid at a fixed dose (Barry & Woodford 1986; Haigh & Kanfer 1984). It has been shown that the Chroma Meter can detect the potency variations of different steroid classes. Whether the sensitivity of the Chroma Meter will enable discrimination between corticosteroid formulations remains to be tested. Indeed a rigorous statistical comparison of the performance of the naked-eye assessment and the Chroma Meter methods should be worthwhile. The ease of obtaining data using the Chroma Meter makes efficient data handling essential for the derivation of discriminative parameters and their subsequent statistical analysis. Moreover, the method is non-invasive and involves only one investigator, instead of several trained judges for visual scoring. We have endeavoured to not only present a precise methodology for application of the dosage form and the objective measurement of the ensuing skin pallor, but also to contribute new ideas in the quantification and statistical evaluation of the corticosteroid blanching assay. The authors propose that this multiple-point skin blanching assay of topical corticosteroids using an inter-

nationally accepted colour measurement standard and the subsequent data analysis be adopted as a standard protocol.

References

Aiache, J. M., Lafaye, C., Bouzat, J., Rabier, R. (1980) Measurement of corticosteroids topical availability by thermography. J. Pharm. Belg. 35: 187–195

Altmeyer, P., Zaun, H. (1976) Reflexphotometric determinations of vasoconstriction after topical application of steroids. V. Vasoconstriction phenomenon and tachyphylaxis after repeated steroid application. Arch. Derm. Res. 255: 51–56

Altmeyer, P., Cremer, C. (1977) Results of reflux photometric determinations of vasoconstriction after topical steroid application. Ibid. 257: 281–285

Amantea, M., Tur, E., Maibach, H. I., Guy, R. B. (1983) Preliminary skin blood flow measurements appear unsuccessful for assessing topical corticosteroid effect. Ibid. 275: 419–420

Ashworth, J. (1989) Potency classification of topical corticosteroids: modern perspectives. Acta Derm. Venereol. (Stockh.) 151 (Suppl.): 20–25

Barry, B. W., Woodford, R. (1986) The vasoconstrictor test as a model for developing topical formulation. In: Marks, R., Plewig, G. (eds) Skin Models—Models to Study Function and Disease of Skin. Springer-Verlag, Berlin, Heidelberg, pp 103–112

Bjerring, P., Andersen, P. H. (1987) Skin reflectance spectrometry. Photodermatology 4: 167–171

Bisgaard, H., Kristensen, J. K., Sondergaard, J. (1986) A new technique for ranking vascular corticosteroid effects in humans using laser-doppler velocimetry. J. Invest. Dermatol. 86: 275–278

Chan, S. Y., Li Wan Po, A. (1992) Quantitative assessment of non-steroidal anti-inflammatory topical products. Int. J. Pharm. In press

Conner, D. P., Zamani, K., Nix, D., Millora, E., Almire, R. G., Shah, V., Peck, C. C. (1990) Use of reflectance spectrometry in the corticosteroid skin blanching. Clin. Pharmacol. Ther. 47: 170

Dawson, J. B., Baker, D. J., Ellis, D. J., Grassmam, E., Cotterill, J. A., Fisher, G. W., Feather, J. W. (1980) A theoretical and experimental study of light absorption and scattering by in vivo skin. Phys. Med. Biol. 25: 695–709

Duteil, L., Queille, C., Poncet, M., Ortonne, J. P., Czernielewski, J. (1990) Objective assessment of topical corticosteroids and non-steroidal anti-inflammatory drugs in methyl-nicotinate skin inflammation. Clin. Exp. Dermatol. 15: 195–199

Feather, J. W., Ryatt, K. S., Dawson, J. B., Cotterill, J. A., Barker, D. J., Ellis, D. J. (1982) Reflectance spectrophotometric quantification of skin colour changes induced by topical corticosteroid preparations. Br. J. Dermatol. 106: 437–444

Gibson, J. R. (1989) Topically applied drugs in Type I allergic reactions. In: Maibach, H. I., Lowe, N. J. (eds) Models Dermatol. Karger, Basel, pp 240–248

Haigh, J. M., Kanfer, I. (1984) Assessment of topical corticosteroid preparations: the human skin blanching assay. Int. J. Pharm. 19: 245–262

Haleblian, J. K. (1976) Bioassays used in development of topical dosage forms. J. Pharm. Sci. 65: 1417–1436

Heseltine, W. W., McGilchrist, J. M., Gartside, R. (1964) Comparative vasoconstrictor activities of corticosteroids applied topically. Br. J. Dermatol. 76: 71–73

Hollander, J. L., Stoner, E. K., Brown, E. M., DeMoor, P. (1950) The use of intra-articular temperature measurement in the evaluation of anti-arthritic agents. Ann. Rheum. Dis. 9: 401

Király, K., Soós, G. (1976) Objective measurement of topically applied corticosteroids. Dermatologica 152 (Suppl.1): 133–137

Marks, R., Barow, J. W., Funder, J. W. (1982) Steroid-induced vasoconstriction: glucocorticoid antagonist studies. J. Clin. Endocrinol. Metab. 54: 1075–1077.

Matthews, J. N. S., Altman, D. G., Campbell, M. J., Royston, P. (1990) Analysis of serial measurements in medical research. Br. Med. J. 300: 230–235

McKenzie, A. W., Stoughton, R. B. (1962) Methods for comparing percutaneous absorption of steroids. Arch. Dermatol. 86: 608–610

Meyer, E., Magnus, A. D., Haigh, J. M., Kanfer, I. (1988) Comparison of the blanching activities of Dermovate, Betnovate and Eumovate creams and ointments. Int. J. Pharm. 41: 63–66

Miller, J. A., Munro, D. D. (1980) Topical corticosteroids: clinical pharmacology and therapeutic use. Drugs 19: 119–134

Queille-Roussel, C., Poncet, M., Duteil, L., Schaefer, M. (1990) Corticosteroid vasoconstriction test-quantification of blanching with Minolta Tristimulus Colorimeter. J. Invest. Dermatol. 94: 401

Queille-Roussel, C., Poncet, M., Schaefer, H. (1991) Quantification of skin-color changes induced by topical corticosteroid preparations using the Minolta Chroma-meter. Br. J. Dermatol. 124: 264–270

Ryatt, K. S., Feather, J. W., Mehta, A., Dawson, J. B., Cotterill, J. A., Swallow, R. (1982) The stability and blanching efficacy of betamethasone-17-valerate in emulsifying ointment. Ibid. 107: 71–76

Schlagel, C. A. (1972) Penetration and action of glucocorticoids. In: Montagna, W., Van Scott, E. J. (eds) Advances in Biology of Skin. Vol. XII. Pharmacology and the Skin. Appleton-Century-Crofts, New York, pp 339–356

Shah, V. P., Peck, C. C., Skelly, J. P. (1989) "Vasoconstriction"—skin blanching-assay for glucocorticoids—a critique. Arch. Dermatol. 125: 1558–1561

Stoughton, R. B., Wullich, K. (1990) Relation of application time to bioactivity of a potent topical glucocorticoid formulation. J. Am. Acad. Dermatol. 22: 1038–1041

Waring, M. J., Monger, I., Hollingsbee, D. A., Martin, G. P., Marriott, C. (1990) Evaluation of the Minolta Chromameter CR200 for the assessment of steroid induced skin blanching. Pharm. Res. 7 (Suppl.): 50

Woodford, R., Barry, B. W. (1974) Bioavailability and activity of betamethasone 17-benzoate in gel and cream formulations: comparison with proprietary topical corticosteroid preparation in the vasoconstrictor assay. Curr. Ther. Res. 16: 338–345