The increasing synthesis and use of topical corticosteroid products over the past 30 years has necessitated the development of suitable methods for evaluating the efficacy and potency of new drug entities. Several in vivo models have been developed in this regard using laboratory animals and human subjects (table 1). Generally, these tests measure the difference in the non-immunological inflammatory response to an exogenous inflammatory mediator in the presence and absence of the corticosteroid under test. There are also immunologically based assays and several tests which assess the antiproliferative effects of the drug. Several comparative disease model evaluations have also been developed using human subjects. Most of these assays are non-ideal from one point of view or another: most are invasive methods which require some form of trauma to be induced in the skin and therefore problematic to perform and monitor.

In comparison, the human skin blanching assay for topical corticosteroids is relatively simple to perform and does not require any trauma on the part of the volunteers. This assay makes use of the skin whitening (blanching) side effect of topical corticosteroid application, the intensity of the blanching correlating directly with the drug potency or success of drug delivery through the stratum corneum [1, 2]. The blanching intensity has also been shown to correlate directly to the clinical efficacy of the preparation [3, 4]. In addition the blanching assay may be conducted in a relatively short time (2 days), thereby eliminating the lengthy assessment period of other in vivo assays. The first documented use of the blanching (or vasoconstriction) assay by McKenzie and Stoughton appeared in 1962 [5], although the use of the skin whitening effect of hydrocortisone on
Table 1. In vivo methods used to assess topical corticosteroid efficacy and potency

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Human models</th>
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<tbody>
<tr>
<td>Non-immunological inflammation models</td>
<td>Non-immunological inflammation models</td>
</tr>
<tr>
<td>Croton oil erythema test</td>
<td>Croton oil–kerosene erythema test</td>
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<tr>
<td>Cantharidin test</td>
<td>Ultraviolet-induced erythema test</td>
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<td>Ultraviolet-induced erythema test</td>
<td>Pyrogen erythema test</td>
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<tr>
<td>Immunological inflammation models</td>
<td>Disease models</td>
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<tr>
<td>Hypersensitivity tests</td>
<td>Psoriasis plaque assay</td>
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<tr>
<td>Assessment of antimitotic effects</td>
<td>Poison ivy test</td>
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<td>Hyperplasia models</td>
<td>Assessment of adverse effects</td>
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<td>Atrophy models</td>
<td>Atrophy tests</td>
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<td>Wound healing models</td>
<td>Ammonium hydroxide blister test</td>
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<td>Stratum corneum thickness tests</td>
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<td>Acne tests</td>
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<td>Tests for systemic effects</td>
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</table>

stripped skin was reported by Wells in 1957 [6]. The exact mechanism by which the skin whitening is generated remains unclear, hence the preferred name of blanching rather than vasoconstriction assay [7].

Assay Methodology

The optimized methodology [2] of the skin blanching assay utilizes 12–15 fair-skinned volunteers who are proven responders to topical corticoid application; a small percentage of the population do not demonstrate the blanching side-effect to these drugs (although still respond clinically in the normal way). Full disclosure of information regarding the assay and acquisition of informed consent is practised in the normal way. The subjectivity of the methodology requires 3 independent, trained observers to be used for valid data recording. Investigation has shown that participation in 3 trials provides sufficient experience for a novice observer to become sufficiently skilled in discerning different intensities of skin blanching [8]. The subjectivity of the assay also requires a rigorous double-blind coding protocol to prevent any bias on the part of the observers. The corticoid products to be evaluated are usually coded by a third party not involved in the trial. Four random application patterns are drawn up each comprising 12 application sites. The products to be compared are randomly assigned to these sites so that each formulation is represented an equal number of times and, importantly, applied along the entire length of the forearm [9]. Each arm of each volunteer is randomly assigned 1 of the 4 patterns.

Twelve discrete application sites are demarcated on each arm of each volunteer by the application of 6 self-adhesive labels that have had two 7 × 7 mm squares punched from
their centres. Each square represents one application site and is numbered in a standard manner. The products to be tested are either extruded from a small syringe which has had the needle cut to a length of approximately 5 mm; four 7-mm 'stripes' of formulation extruded in this way approximate 3 mg of formulation [10]. Alternatively, 3 μl of liquid formulation may be applied to each site using a micropipette. After application the formulations are uniformly spread over the entire application site using a glass rod. Once all the sites have been filled, one arm of each volunteer is occluded by covering each site with water-impervious tape, the other arm has its sites guarded using a perspex frame which does not prevent evaporation from the skin or formulation but does prevent accidental abrasion. In this manner both the clinical application modes of occlusion and non-occlusion may be tested. The formulations, tapes and dressings remain on the skin for 6 h after which they are removed and any residual formulation is washed from the skin with water and the arm patted dry. Any erythema or skin puckering from the tape usually subsides within 30 min after removal. The first observation of skin blanching is made 1 h after product removal (7 h after application).

The blanching response is independently assessed by the 3 observers on 10 occasions between 7 and 32 h after corticosteroid application. The intensity of skin whitening is subjectively graded on a 0–4 scale, 0 representing no blanching, 4 representing intense blanching with distinct margins, and 1, 2 and 3 representing the intermediate grades. After the final observation the score sheets are decoded and the scores summated for generation of a blanching profile and statistical analysis. The blanching profile is plotted as response in the form of percentage of total possible score (%TPS) versus time. The TPS is the product of the maximum score per site, the number of observers, the number of sites per arm for each preparation and the number of volunteers. The actual score (AS) is the sum of the frequencies for each grade recorded. The %TPS is the quotient of AS and TPS multiplied by 100. The blanching profiles generated in this way allow normal topical availability comparisons as well as estimations of peak blanching, time to peak and duration of blanching.

In an attempt to assess the precision of this assay methodology, we have conducted a retrospective analysis of all the trials conducted in our laboratories over the last 20 years. We usually include Betnovate cream as a standard formulation into every trial conducted. If the profile obtained for Betnovate has the usual characteristic shape then the credence of the other results is strengthened. The results of several thousand observations in this manner show good precision in both the occluded and unoccluded modes of application [11].

**Results**

This blanching assay methodology may be used for a number of different comparative evaluations: one use has been the screening of new drug molecules for clinical activity [12–17]. When used for this purpose the different characteristic blanching response profiles of betamethasone 17-valerate and fluocinolone acetonide, for example, are evident: the latter has a slower onset, higher peak response and longer duration than the former [18]. This is also a good example for illustrating the misleading results that may be obtained by single-point observation protocols. Any single observation between 0 and 12 h, where the blanching
The blanching assay has been used extensively for potency ranking of corticoid formulations [19–21]. Eumovate (clobetasone butyrate) has been compared to Betnovate and Dermovate in our laboratories [22] and demonstrates the moderate potency of the test formulation when compared to these standards. Interestingly, Eumovate ointment induces higher blanching in the unoccluded application mode compared to the occluded mode, an unexplained phenomenon which is not observed with any other formulations. The blanching assay has also been used for determining optimal application regimens for topical formulations [23].

The blanching assay may be used for comparing topical availability of the same drug from the same type of delivery vehicle [24–28]; comparative topical availability assessments of this type are routinely conducted for regulatory purposes. It is obvious from these studies that the corticosteroid may be delivered to the stratum corneum in very different amounts from two formulations of the same type (creams for example) which both contain the same drug in the same concentration, but differ in the other formulation excipients [18].

The human skin blanching assay may also provide interesting results that influence the clinical choice of drug delivery vehicle. Markedly different topical availabilities of the same corticosteroid are often observed when comparing drug delivery from different formulation types (creams, ointments, gels and solutions) containing the same concentration of drug [29, 30]. One would assume that different vehicles of the same label concentration would deliver similar quantities of drug to the skin. Our experience shows that alcoholic solutions allow much more drug to penetrate the skin than do ointments or oil-in-water creams and lotions. Occlusion greatly enhances drug availability from lotion formulations, but has relatively little effect on the ointment formulation. This is important clinically because these relatively potent lotion and alcoholic solution vehicles are usually applied to the face and scalp, skin which is inherently more permeable than that of many other anatomical regions.

The assay may also be usefully applied to the assessment of penetration enhancer effects on topical corticosteroid availability [31–33]. The effects of penetration enhancers such as oleic acid or propylene glycol may be assessed using the human skin blanching assay. The blanching response results depicted in figure 1 indicate the effect of adding these penetration enhancers to an extemporaneous formulation containing betamethasone 17-valerate. In the case of these results the commercial formulation (Betnovate cream) generated the least blanching in both the occluded and unoccluded application test modes. Even the extemporaneous control formulation which contained neither propylene glycol nor oleic acid performed better than the commercial formulation. The presence of
Fig. 1. Human skin blanching response profiles for extemporaneous cream formulations containing penetration enhancers and commercial Betnovate cream. 

a Unoccluded application mode. 
b Occluded application mode.

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Ranking of Topical Glucocorticoids
propylene glycol in the formulation generated some improvement in drug release compared to the control formulation in the unoccluded application mode; however, neither penetration enhancer demonstrated superiority to the control cream in the occluded mode.

It is interesting to note that a common result observed in our laboratories is repeated in these results: we have almost exclusively found that extemporaneous test formulations perform better than commercial products when compared by the human blanching assay. Furthermore, we have compounded micronized formulations that contain one tenth the drug concentration of commercial products but still elicit greater response profiles due to optimized delivery vehicle microstructure [34]. It must be stressed that long-term stability studies have not been conducted on these extemporaneous formulations and hence we cannot comment on the shelf lives of these products compared to the commercial formulations. However, it seems clear that we should be able to improve the rate and extent of drug delivery by optimizing the chemical and physical composition of the topical vehicle, when compared to products that are already on the market. Such an optimization should have marked clinical, toxicological and financial implications.

**Conclusions**

Although relatively crude in methodology, the skin blanching assay remains an accurate, reproducible and rapid method of assessing topical corticosteroid availability and potency. There has lately been criticism of the subjectivity of the assay and suggestions that optical methods may yield more ‘meaningful’ results [7, 35]. On reviewing the literature one finds that numerous instrumental techniques have been evaluated over the last 2 decades as potential alternatives to the visual assessment of the skin whitening response. These techniques have all demonstrated inferior or equivalent results compared to visual methodology. However, being cumbersome and time-consuming, none of these instrumental methods has replaced visual assessment procedures as a routine practice. As research advances with newer optical instruments [36], the replacement of the current subjective assessment may certainly be justified.

**Acknowledgements**

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