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Assessment of Some Variables Affecting the Blanching Activity of Betamethasone 17-Valerate Cream

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Abstract. The effect of concentration and occlusion time on the ability of Betnovate® cream (betamethasone 17-valerate 0.1%) to produce skin blanching was assessed. Generally, increased concentration or occlusion time produce an increase in the degree of blanching observed, however, a plateau stage is eventually reached where no further increase of blanching occurs.

The blanching assay was first used by *McKenzie and Stoughton* [5] following their observation that treatment with a topical corticosteroid occluded with a plastic film produced pallor of the lesion and of the normal surrounding skin. Since it was first described, this assay has undergone many modifications in an attempt to make it a precise, reproducible and quantitative method for the *in vivo* evaluation of topical corticosteroid formulations. Two of the parameters which are subject to variation are the length of occlusion time and the amount of preparation applied to the skin. Despite considerable variation between different workers in the field, no in-depth study has been made to determine the actual effect of occlusion time and the amount of preparation applied on the degree of blanching produced. For this reason the present study was undertaken. In this study, only one formulation, namely betamethasone 17-valerate cream, was used, consequently the results apply only to that specific preparation. It would however be expected that similar trends would be observed for any topical corticosteroid preparation.

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Materials and Method

Tubes of Betnovate® cream (betamethasone 17-valerate 0.1%), prepared in South Africa, were purchased from a local pharmacy. Four trials were performed using a modified McKenzie-Stoughton blanching assay [1]. Only healthy human volunteers were used. None had received either topical or systemic corticosteroid therapy for at least 6 weeks prior to the studies. The flexor aspects of the forearms were masked producing 12 7-mm square application sites per arm. For each volunteer both forearms were used.

Trial 1. 16 volunteers were included in this trial. A standard mass (approximately 3.2 mg) of Betnovate cream was applied to each application site and all sites were occluded with a non-porous plastic film (Blenderm®). The occlusions were removed at 2, 4, 6 and 8 h after application in a random manner. After each type had been removed, the area was gently washed to remove any cream remaining on the surface of the skin.

Trial 2. Similar to trial 1 except that the occlusions were removed at 8, 10 and 12 h after application.

Trial 3. 11 volunteers were included in this trial. 2, 3, 4 or 5 stripes of cream were applied to the application sites in a random manner. The stripes were extruded from a 1-ml disposable syringe, the needle of which had been cut down to 5 mm in order to facilitate the extrusion of the cream. The syringe was filled immediately prior to use so as to minimise any possible interaction between the corticosteroid and the plastic matrix of the syringe barrel. The mass of preparation applied was determined by differential weighings. 7-mm stripes of cream were extruded onto weighing paper and weighed. 80 weighings were made. The average mass of a 7-mm stripe of Betnovate cream was 0.8 mg. The values ranged between 0.75 and 0.99 mg. Although the standard deviation is high, it is felt that the large number of sites used (66) for each different mass will more than negate this problem. Both forearms were occluded. 6 h after application the maskings and tapes were removed and the forearms washed with soap and warm water.

Trial 4. Similar to trial 3 except that 5, 6, 7 or 8 stripes of cream were applied to the application sites.

For all trials, the arms were evaluated independently using a double-blind technique by 3 observers at various time intervals adequate to establish a blanching profile for each occlusion time or mass applied. The averaged readings of all observers were used to analyse the data. The methods of statistical analysis and calculation of %TPS and area under the curve (AUC) have previously been described [2].

Results and Discussion

Figure 1 depicts the blanching profiles obtained for the 2, 4, 6 and 8 h occlusion times. Figure 2 depicts the blanching profiles obtained for the 8,

10 and 12 h occlusion times. Table I lists the blanching responses for each different occlusion time. These figures represent two different trials and are therefore expressed as a percentage of the 8-hour value to allow for inter-comparison.

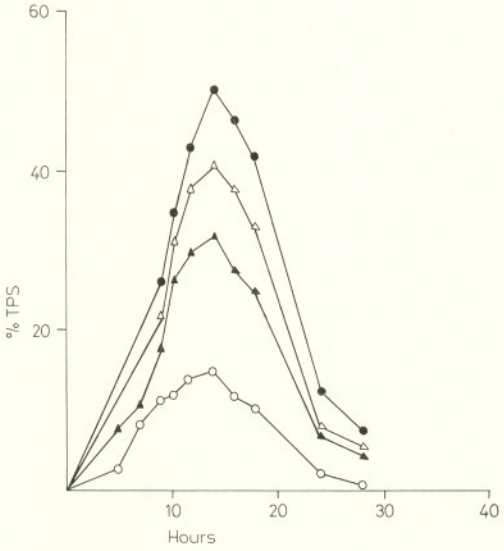


Fig. 1. Blanching profiles obtained for different occlusion times. ○ = 2 h; ▲ = 4 h.; △ = 6 h; ● = 8 h.

Figure 3 depicts the blanching profiles obtained for 2, 3, 4 and 5 stripes of Betnovate cream. Figure 4 depicts the blanching profiles obtained for 5, 6, 7 and 8 stripes of Betnovate cream. Table II lists the blanching responses for each different amount applied. These figures also represent two different trials and are therefore expressed as a percentage of the 5-stripes value to allow for intercomparison.

χ^2 analysis showed statistically significant differences between 2 and 4, 4 and 6 and 6 and 8 hour occlusion times, but no significant differences between the longer occlusion times. It can be seen from figure 2 that a maximum response is obtained from a 10-hour occlusion time; increasing the occlusion time further has no significant effect on the degree of blanching observed. Reference to table I shows that although a maximum blanching response is achieved, the AUC values continue to slowly increase.

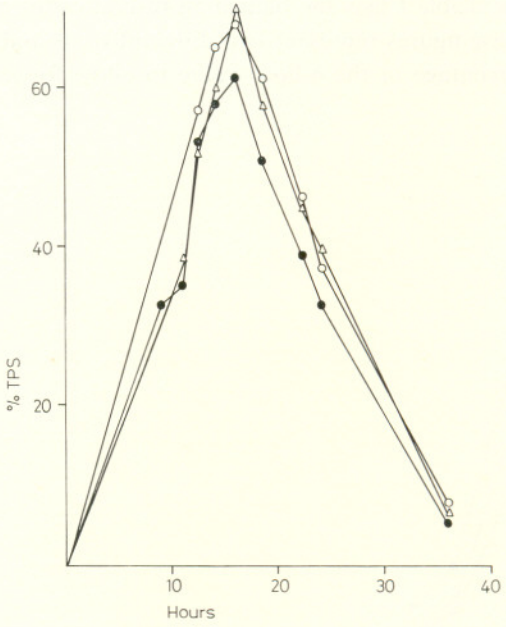
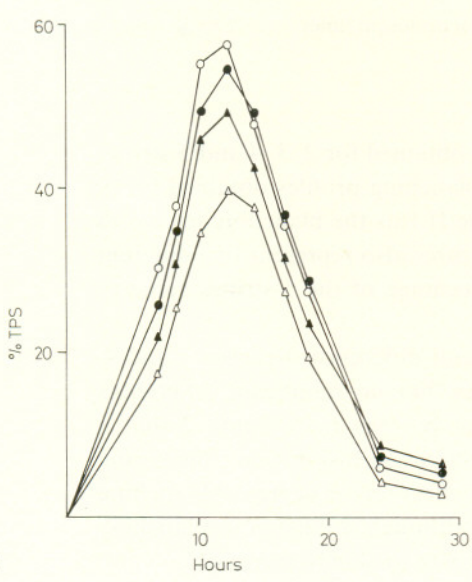


Fig. 2. Blanching profiles obtained for different occlusion times. ● = 8 h; △ = 10 h; ○ = 12 h.



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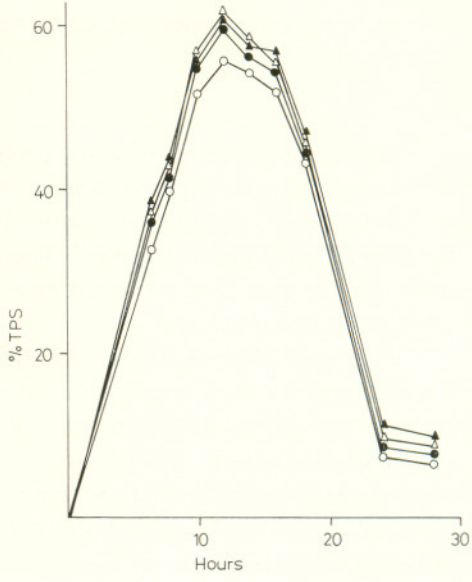

4

Table I. Blanching responses for different occlusion times

	Summed % TPS[1]	AUC[1]
2 h	33	28
4 h	72	63
6 h	88	81
8 h	100	100
10 h	100	108
12 h	95	117

[1] Expressed as a percentage of the 8-hour value.

It is well recognised that the degree of hydration of the horny layer of the epidermis affects its permeability for steroids and their transport through the skin. Occlusion with a non-porous plastic film provides the single most effective mechanism for increasing skin hydration. *Maibach* [4] has shown that hydrocortisone is absorbed in tenfold greater amounts under plastic occlusion when compared to unoccluded. *Sultzberger and Witten* [6] showed that triamcinolone acetonide ointment under plastic occlusion was clinically as effective in obstinate psoriasis as an intralesional injection of the steroid. They attributed the enhanced activity to better contact between the ointment and the skin, more accurate localization of the ointment and increased percutaneous absorption as a result of epidermal maceration.

Hydration results from water diffusing from underlying epidermal layers or from perspiration accumulating after application of an occlusive covering to the surface of the skin. Under occlusive conditions the stratum corneum is changed from a tissue that normally contains little water (5–15%) to one that may contain as much as 50% water, and permeability increases in the order of 4–5 times [3]. Hydration apparently opens up the compact substance of the stratum corneum, thus increasing the rate of percutaneous absorption [7].

It would therefore be expected that the longer the occlusion time, the greater the degree of blanching produced. There are a number of possible

Fig. 3. Blanching profiles obtained for different amounts applied. △ = 2 stripes; ▲ = 3 stripes; ● = 4 stripes; ○ = 5 stripes.

Fig. 4. Blanching profiles obtained for different amounts applied. ○ = 5 stripes; ● = 6 stripes; △ = 7 stripes; ▲ = 8 stripes.

reasons which could account for the reaching of a plateau stage of blanching for betamethasone 17-valerate cream at 10 hour occlusion time. A corticosteroid has an inherent ability to cause blanching. Since most corticosteroids cause different degrees of blanching, the 10-hour occlusion maximum observed here may represent the maximum blanching ability of betamethasone 17-valerate in the particular formulation tested. On the other hand, it may be that the 10-hour occlusion time causes maximum hydration of the stratum corneum. It therefore follows that occlusion times of greater than 10 h will cause no increase in the permeability of the skin, thus no increase in the intensity of blanching. It may also be argued that the ability to observe blanching may be a limiting factor. However, it can be seen from figure 2 that %TPS remains substantially below the maximum possible value.

χ^2 analysis showed statistically significant differences between 2 and 3 and 3 and 4 stripes of Betnovate cream, but all other analyses showed no significant differences between the higher masses. It can be seen from figure 4 that a maximum response is obtained with 6 stripes (approximately 4.8 mg of cream); increasing the mass of cream applied further has no effect on the degree of blanching observed. Reference to table II shows that although a maximum blanching response is achieved, the AUC values continue to slowly increase.

Since the corticosteroid is released from the surface of the cream in contact with the skin, the thickness of the layer of cream applied will become significant. Over a certain thickness, no increased release of corticosteroid would be expected. This critical thickness of the layer of cream may well have been achieved with six stripes of Betnovate cream. It may also be that,

Table II. Blanching responses for different amounts applied

	Summed % TPS[1]	AUC[1]
2 stripes (1.6 mg)	69	64
3 stripes (2.4 mg)	85	85
4 stripes (3.2 mg)	95	95
5 stripes (4.0 mg)	100	100
6 stripes (4.8 mg)	109	109
7 stripes (5.6 mg)	110	110
8 stripes (6.4 mg)	112	113

[1] Expressed as a percentage of the 5-stripe value.

at the concentration level of 4.8 mg per 7-mm square, the maximum blanching activity of betamethasone 17-valerate has been reached.

When the mass of corticosteroid cream is plotted on a logarithmic scale against the AUC value calculated for each mass of cream applied, a typical sigmoid curve is obtained. In view of the fact that the amount of corticosteroid cream applied to the skin does not necessarily reflect the amount of betamethasone 17-valerate diffusing from the preparation, quantitative interpretation of this log dose response curve would be premature.

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