Influence of the oil phase dispersion in a cream on the *in vivo* release of betamethasone 17-valerate

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Corticosteroid delivery to the skin was compared from three extemporaneous cream formulations each containing 0.012% betamethasone 17-valerate (one control, one containing propylene glycol and one containing ß-cyclodextrin as penetration enhancers) and from a commercial cream formulation containing 0.12% betamethasone 17-valerate, utilizing the human skin blanching assay. Additionally, the microstructure of each cream was assessed by scanning electron microscopy. All three extemporaneous formulations showed similar drug delivery rates, and these were equivalent to, or better than, the drug release from the commercial preparation which contained the corticosteroid in a ten-fold greater concentration. The presence of penetration enhancers did not significantly influence drug delivery from the extemporaneous formulations. Examination of the microstructure of each preparation showed considerably finer dispersion of the oil phase in the extemporaneous formulations compared with the commercial product. It is postulated that the increased surface area available for partitioning of the steroid between the formulation and the stratum corneum *is responsible for the improved topical availability of the drug from these formulations and, furthermore, offers a viable method for reducing drug concentration while maintaining or improving pharmacodynamic activity of active constituents in emulsion formulations.*

L'administration de corticostéroïde sur la peau a été comparée pour trois formules extemporanées de crèmes renfermant chacune 0,012% de valérate de 17-bétaméthasone (une formule de contrôle, une formule renfermant du propylène glycol et une autre contenant de la ß-cyclodextrine comme promoteur d'absorption) et à partir d'une crème du commerce renfermant 0,12% de valérate de 17-bétaméthasone, en mettant en œuvre le blanchiment de la peau humaine. En outre, la microstructure de chaque crème a été étudiée en microscopie électronique. Les trois préparations extemporanées ont présenté des cinétiques de libération du principe actif équivalentes ou meilleures que celle de la préparation commerciale qui renferme une proportion dix fois plus importante du corticostéroïde. La présence de promoteurs d'absorption n'a pas eu d'influence significative sur la libération à partir des formules extemporanées. L'examen des microstructures de chaque préparation a révélé une dispersion notablement plus fine dans le cas des préparations extemporanées que dans celui du produit commercial. On peut penser que l'augmentation de la surface disponible pour le passage du stéroïde de la formule au stratum corneum *est la raison de l'augmentation de disponibilité topique du principe actif à partir des formules et qu'en outre, elle représente une méthode réelle pour réduire la concentration tout en maintenant ou en améliorant l'activité pharmacologique des principes actifs contenus dans les émulsions.*

Keywords : Corticosteroid - Cream - Betamethasone - Propylene glycol - ß-cyclodextrin - Blanching - Human skin - Microstructure - Penetration enhancers.

Mots clefs : Corticostéroïdes - Crème - Bétaméthasone - Propylène glycol - ß-cyclodextrine - Blanchiment - Peau humaine - Microstructure - Promoteurs d'absorption.

The efficacy of topical corticosteroid formulations depends primarily on the ability of the corticosteroid to partition from the formulation and diffuse through the *stratum corneum* into the epidermis [1]. Much attention has been given to the inclusion of penetration enhancers in topical corticosteroid formulations as aids to enhance percutaneous penetration [2], but little

attention has been paid to the microstructure of the formulation. It was therefore decided to conduct an investigation of relative corticosteroid release from various cream vehicles in relation to the microtopography of the formulations.

Three extemporaneous oil-in-water creams were prepared, each containing the same concentration (0.012%) of betamethasone 17-valerate, two of the formulations containing the penetration enhancers propylene glycol [2] or ß-cyclodextrin [3]. The *in vivo* drug release of these three preparations and a commercial betamethasone 17-valerate (0.12%) cream were compared using the human skin blanching assay [4, 5]. Scanning electron microscopy was used to establish the morphological structures of the creams.

I. EXPERIMENTAL

1. Delivery vehicles

The oil-in-water emulsions were prepared according to the following formula :
- cetrimide emulsifying wax : 7 g,
- white soft paraffin : 10 g,
- liquid paraffin : 35 g,
- steroid solution : 9 ml,
- purified water : to 100 g.

A 9 ml aliquot of solution 1, 2 or 3 was included in each preparation :
1. betamethasone 17-valerate (0.012 g) dissolved in propylene glycol (9 ml) ;
2. betamethasone 17-valerate (0.012 g) dissolved in ethanol (4.5 ml) and water (4.5 ml) ;
3. betamethasone 17-valerate (0.012 g) and ß-cyclodextrin (0.029 g) dissolved in ethanol (2.5 ml) and water (6.5 ml) ; the flask was sealed and the contents stirred at 25°C for 7 days to allow complex formation.

All chemicals and solvents were obtained from Lennon Limited, Port Elizabeth, South Africa.

The water and oil phases were heated to 60°C on a water bath and the water was then added to the oil phase (to which had been added the steroid solution) with appropriate stirring. After formation of an emulsion, stirring was continued until the temperature of the cream reached 20°C.

The fourth formulation tested was a commercially-available cream containing 0.12% betamethasone 17-valerate (Betnovate® cream, Glaxo Limited, Johannesburg, South Africa) and was purchased from a local pharmacy immediately prior to use.

2. Analytical method

All four formulations were assayed the day after the *in vivo* experiments were performed, utilizing a previously described [6] high performance liquid chromatographic method. The results of these analyses are presented in *table I*.

Table I - Betamethasone 17-valerate content and diameters of dispersed spheres in creams tested (B 17-V = betamethasone 17-valerate, PG = propylene glycol, ß-CD = ß-cyclodextrin).

Formulation	% B 17-V found	Diameter of spheres
Betnovate®	0.12030	24 to 40 µm
B 17-V cream	0.01173	5 to 15 µm
B 17-V + PG cream	0.01175	4 to 10 µm
B 17-V + ß-CD cream	0.01171	4 to 16 µm

3. *In vivo* blanching measurement

A total of 12 healthy male and female Caucasian subjects was selected from a panel of volunteers known to show a blanching response. All subjects had previously taken part in similar experiments, and written, informed consent was obtained from each subject. The volunteers were not using any form of systemic medication and had not received topical corticosteroids for at least six weeks prior to the investigation.

The creams were applied to the flexor aspect of the forearm in one of four different arrangements, each application pattern being chosen randomly to avoid any bias during observation. On each forearm 12 sites were demarcated by applying six self-adhesive labels from which two 7 x 7 mm squares had been punched. Since four preparations were being evaluated, each preparation was applied to three sites per arm. A uniform quantity of each cream, equivalent to approximately 3.2 mg [7], was applied to the sites by extrusion from a syringe in a double-blind fashion. The areas close to the wrist and elbow were avoided [8]. The creams were evenly spread with a glass rod prior to occlusion or protection of the sites. All the sites on one forearm of each volunteer were occluded while the sites on the other arm were left unoccluded but protected with a plastic grid to prevent accidental removal of the formulations. Occlusion was attained by means of individual strips [9] of non-porous plastic tape (Blenderm, 3M Company, South Africa) placed over the self-adhesive labels.

The demarcating labels, plastic coverings and occlusive dressings were removed 6 h after application and any residual cream was removed by gently washing the forearms with soap and warm water and patting dry.

Assessment of the blanching responses was performed independently by three experienced observers at 7, 8, 9, 10, 12, 14, 16, 18, 28 and 32 h after application [10] using a 0 to 4 scale where 0 = normal skin, 4 = marked, intense blanching over the whole application site, and the values of 1, 2 and 3 representing the respective grades of blanching between these two extremes [11]. Each cream was applied to three sites on each forearm of 12 volunteers resulting in the induced blanching being assessed 108 times for each formulation at each observation time in both the occluded and unoccluded modes of application.

The percentage of the total possible score (% TPS) was calculated [5] and plotted against time (in hours) after application to produce full blanching profiles. The trapezoidal rule was used to calculate the area under the curve (AUC) values [5]. Chi-squared analyses were performed on the graded responses of the preparations being compared and on direct comparisons between application sites [12]. Statistical analyses were performed at the 95% level of significance on the pooled results of all three observers.

4. Electron microscopy

A scanning electron microscope (model JSM 840, Jeol Corporation, Japan) fitted with a cryo-SEM accessory (model CT 1000, Hexland Limited, United Kingdom) was used to establish the microstructure of the creams. Samples were quench-frozen in sub-cooled liquid nitrogen, freeze-fractured and sputter-coated with a thin layer of gold in the cryo chamber and then transferred to the microscope cryo stage for observation.

II. RESULTS AND DISCUSSION

1. *In vivo* blanching

Figures 1 and 2 depict the blanching profiles of four creams tested in the unoccluded and occluded modes, and the AUC values are listed in *table II*. In all experiments of this kind conducted in our laboratories, a standard preparation (Betnovate cream) is included to monitor the assay procedure. In this investigation the standard preparation produced the expected results, *i.e.* a lower AUC value in the unoccluded mode and a higher maximum blanching value and narrower peak in the occluded mode [13]. This gives credence to the results obtained for the experimental formulations.

Table II - Blanching profile details of creams tested (key : see *table I*).

	Max %TPS	% change*	AUC	% change*	T_{max} (h)
Occluded					
Betnovate	63.4	52.0	984	36.1	14
B 17-V cream	62.0	18.6	904	3.6	14
B 17-V + PG cream	54.2	-3.0	822	-17.3	14
B 17-V + ß-CD cream	58.8	3.3	870	-7.4	14
Unoccluded					
Betnovate	41.7		723		14
B 17-V cream	52.3		877		14
B 17-V + PG cream	55.8		964		16
B 17-V + ß-CD cream	56.9		934		14

* Percentage change in occluded data compared with unoccluded data.

The most striking feature of the unoccluded blanching profiles is that the three extemporaneous formulations containing 0.012% betamethasone 17-valerate all induce a higher degree of blanching than Betnovate cream which contains ten times the amount of corticosteroid. These differences are statistically significant at most of the reading times during the time

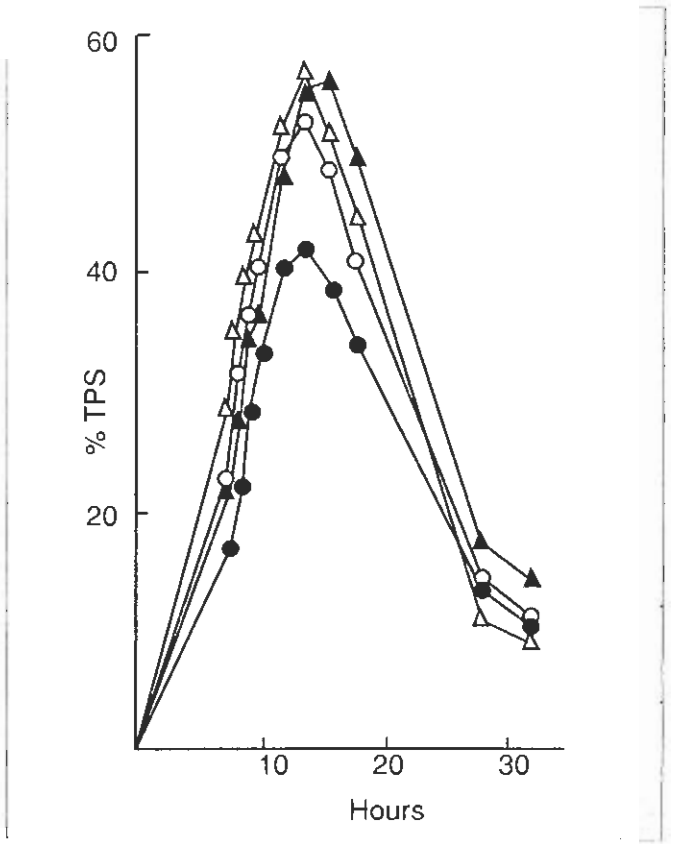

Figure 1 - Blanching profiles of creams tested in the unoccluded mode (● : Betnovate ; ▲ : B 17-V + PG cream ; Δ : B 17-V + ß-CD cream ; ○ : B 17-V cream).

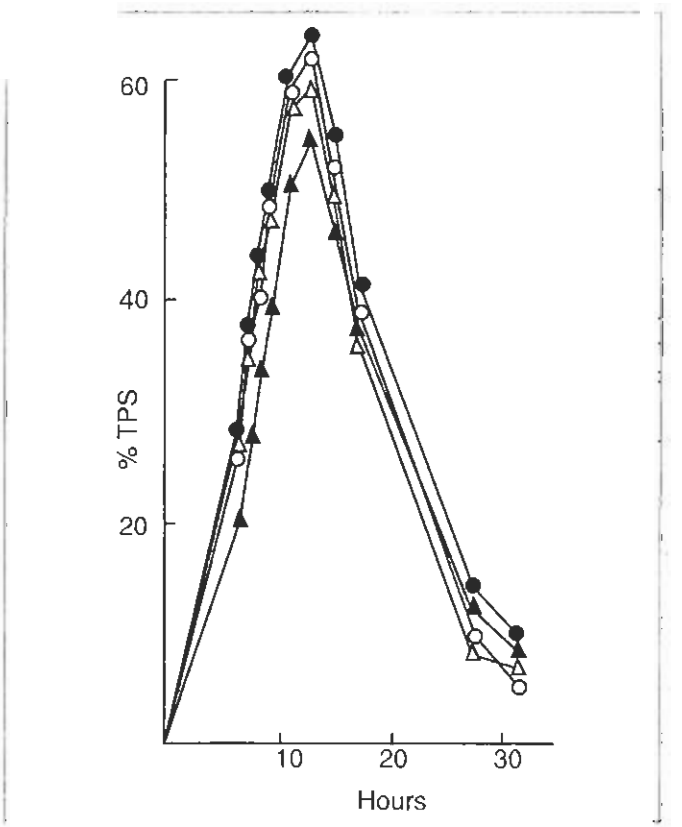

Figure 2 - Blanching profiles of creams tested in the occluded mode (● : Betnovate ; ▲ : B 17-V + PG cream ; Δ : B 17-V + ß-CD cream ; ○ : B 17-V cream).

span of the experiment. This clearly demonstrates the superior release characteristics of the extemporaneous base and emphasizes the significance of vehicle design and delivery for optimum pharmacological response. Since it has now been shown conclusively that the intensity of blanching is directly related to clinical efficacy [5, 14], the inference is that the creams containing 0.012% betamethasone 17-valerate in this particular extemporaneous vehicle should be clinically more efficacious than the commercial cream containing 0.12% when applied to the skin without additional occlusion.

With regard to the efficacy of propylene glycol and ß-cyclodextrin as penetration enhancers, reference to *figure 1* shows that they produce an increase in the induced blanching compared with the extemporaneous preparation without enhancers. The inclusion of propylene glycol produces a base which seems to have a slightly delayed action with regard to the release of betamethasone 17-valerate. Statistical analysis of the blanching responses produced by the three extemporaneous preparations indicates that they are not significantly different throughout the course of the experiment. The extemporaneously prepared cream base therefore appears to deliver the corticosteroid to the skin at a maximal rate which is only marginally affected by the inclusion of penetration enhancers.

It is well known that occlusion masks vehicle effects [8, 12] and this is clearly demonstrated in *figure 2*. Occlusion causes hydration of the *stratum corneum* thus reducing its barrier properties [15] and allowing better penetration of the corticosteroid. This is accompanied by more rapid drug clearance from the site of action. Occlusion also prevents evaporation of volatile components from the formulation after application to the skin [16]. The delivery environment of the drug is, therefore, little altered during the application period. Generally a higher maximum value of blanching and a narrower response profile are observed in the occluded mode [13]. *Table II* lists the maximum % TPS values, the time to maximum blanching and the AUC values for each preparation in each application mode. Also listed are the percentage changes in maximum % TPS and AUC values between the two application modes. Although the commercial 0.12% betamethasone 17-valerate formulation shows an increase in both maximum % TPS (52%) and AUC (36%) values in the occluded mode, the relative blanching responses elicited by the extemporaneous formulations are variable.

Bearing in mind that the commercial formulation contains ten times the drug concentration of the extemporaneous formulations, it is to be expected that any improvement in the drug delivery conditions, such as occlusion of the application sites, would greatly augment percutaneous penetration of the corticosteroid. This is evident from the relatively large percentage increases in maximum % TPS and AUC values in the occluded mode. However, it is interesting to note that even occlusion of this concentrated formulation does not produce a significantly superior drug delivery system than that achieved by non-occlusion of the weaker extemporaneous formulations. This observation further reinforces the vitally important role played by formulation design in the achievement of optimal drug delivery.

Occlusion of the extemporaneous formulation without enhancers increases drug absorption. The additional moisture in the *stratum corneum*, or prevention of formulation component evaporation, therefore improves drug delivery from this control formulation. The inclusion of penetration enhancers causes a slight decrease in drug delivery. It is possible that the enhancers may react with the higher moisture content to produce a partitioning system that is less favourable for drug movement from the formulation. The humectant properties of propylene glycol and the sugar moieties of the ß-cyclodextrin suggest that favourable interaction with water molecules is possible. Exogenous water in the formulation and endogenous water from the horny layer that cannot evaporate because of occlusion may be reacting with the enhancers in some manner to reduce the partitioning potential of the drug in these delivery systems.

It must be borne in mind, however, that the differences in the drug delivery rates from the different extemporaneous formulations are small and, in the majority of cases, are not statistically significant. Explanation of the exact rank order of blanching is, therefore, less important than attempting to explain why extemporaneous formulations with ten-fold lower drug concentrations produce equivalent or superior pharmacodynamic response compared with a commercial formulation of much higher corticosteroid concentration.

2. Microstructures of the creams

The electron micrographs (*figures 3 to 6*) show the hydrophilic (continuous) phase and the lipophilic (spherical dispersed) phase for the four creams studied, and demonstrate quite

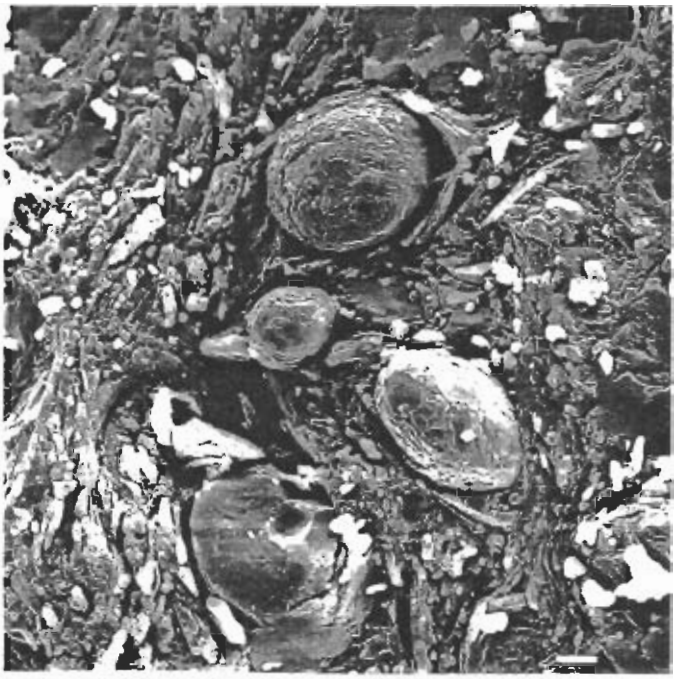

Figure 3 - Scanning electron micrograph of freeze-fractured Betnovate® cream ; bar 10 µm.

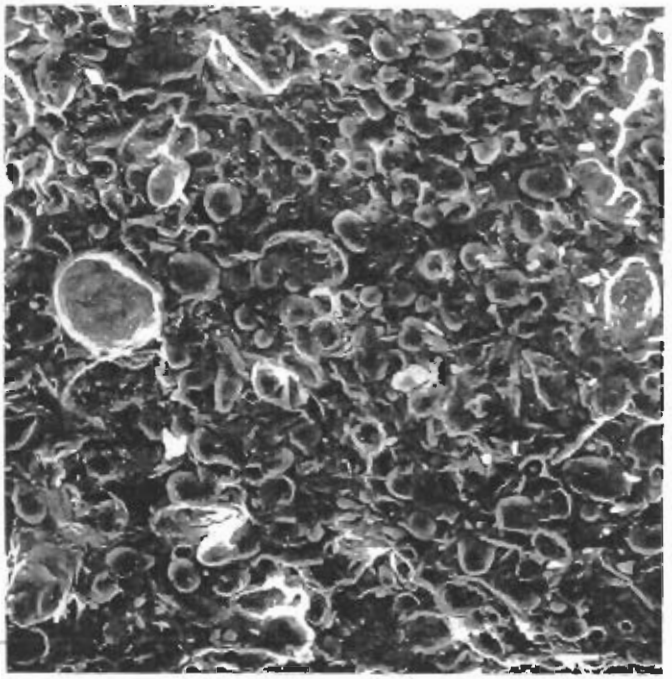

Figure 4 - Scanning electron micrograph of freeze-fractured betamethasone 17-valerate cream : bar 10 µm.

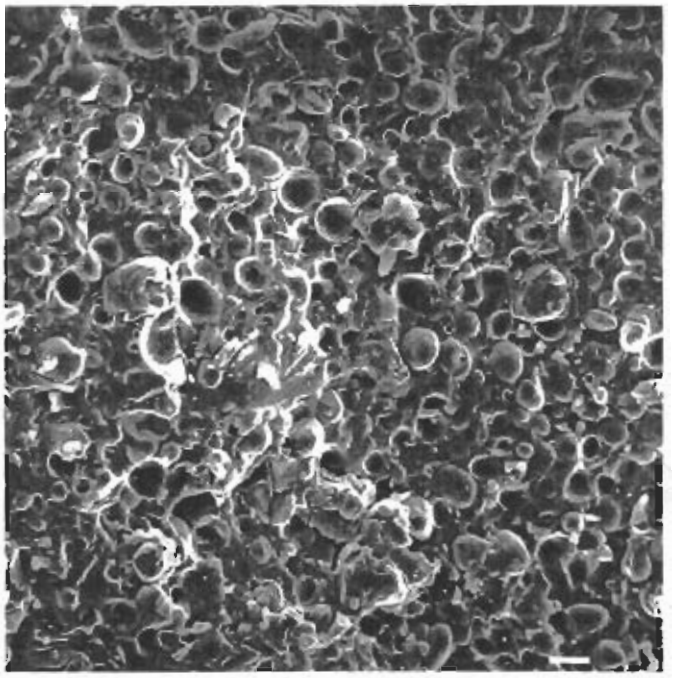

Figure 5 - Scanning electron micrograph of freeze-fractured betamethasone 17-valerate plus propylene glycol cream : bar 10 µm.

clearly the finer dispersion of the oil phase in the three extemporaneous formulations compared with Betnovate cream. lists the diameters of the largest and smallest lipophilic dispersed phase spheres as measured from the micrographs. It is interesting to note that the largest dispersed sphere of the extemporaneous formulations is smaller than the smallest dispersed sphere of the commercial product. There is approximately a four-fold difference in the average diameters of the dispersed spheres of the two cream types. It is not known whether the volume of the dispersed phase in the commercial formulation is the same as that in the extemporaneous formulations.

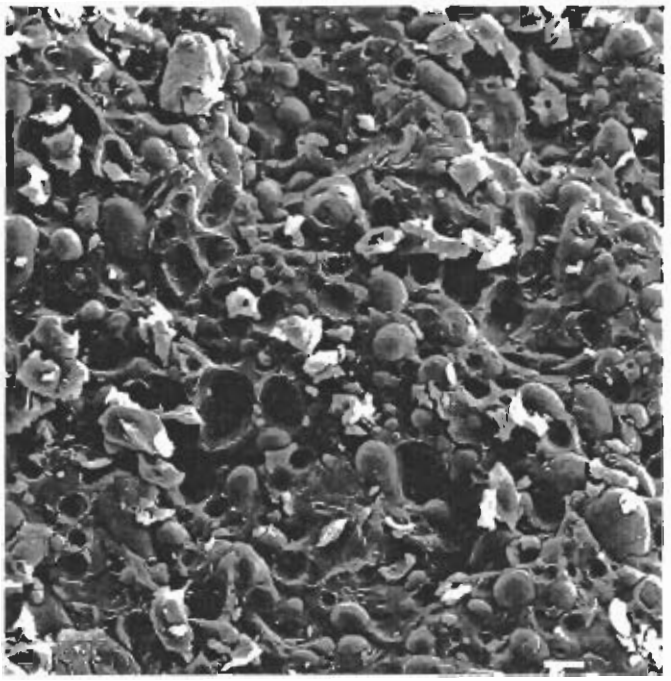

Figure 6 - Scanning electron micrograph of freeze-fractured betamethasone 17-valerate plus ß-cyclodextrin cream : bar 10 µm.

However, since both preparations are oil-in-water creams, the volumes of the dispersed phases will be similar and, therefore, it is obvious that the greater number of smaller spheres in the extemporaneous formulations will produce a greater *stratum corneum*/dispersed phase contact area. This increased surface area would produce greater skin contact with the corticosteroid which is, theoretically, contained within this phase. This increased skin contact could well give rise to the enhanced blanching produced by the extemporaneous formulations.

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The extemporaneous base used here shows excellent release characteristics for betamethasone 17-valerate, and the inclusion of propylene glycol or ß-cyclodextrin has little effect on these release characteristics. It is further concluded that the observed blanching differences between the extemporaneous formulations and the commercial cream are almost certainly related to the microstructure of the creams which demonstrate a large number of small dispersed spheres for the extemporaneous formulations and fewer, larger dispersed spheres for Betnovate' cream. The greater surface area for partitioning afforded by the former is thought to enhance drug absorption significantly. However, since the manufacturer of the commercial product was, understandably, not prepared to disclose the formula of Betnovate cream", differences in activity coefficient and partition coefficient of the drug in the commercial and extemporaneous formulations cannot be assessed.

No long-term stability analysis has been conducted on the extemporaneous formulations. Although the commercial pro-

duct is known to maintain its potency and stability for a stated shelf life, the same cannot be said for the compounded creams. However, the concept of being able to reduce formulation drug concentrations dramatically while maintaining or improving pharmacodynamic activity by adequate micronization of the dispersed phase appears to offer an alternative method of improving topical drug delivery without additional penetration enhancers. ◆

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial assistance from the Rhodes University Council and the Foundation for Research Development. The assistance of the Electron Microscopy Unit of Rhodes University is also acknowledged.

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