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Activity and bioavailability of a new steroid (Timobesone acetate) in cream and ointment compared with Lidex and Dermovate creams and ointments and Betnovate cream

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Summary

This study investigates the vasoconstrictor activities and bioavailabilities and, by inference, the clinical anti-inflammatory efficacies of cream and ointment formulations of a new steroid, Timobesone acetate (an etianic acid derivative of betamethasone in which the terminal carbon (C-21) of the corticoid side-chain has been replaced by a thiomethyl group and the 17α-hydroxy by an acetate ester group). These preparations were compared with selected commercially available steroid creams and ointments (Lidex, Dermovate and Betnovate) in the occluded and non-occluded blanching tests in 10 volunteers. From the vasoconstrictor data and the absence of any untoward effects, it was concluded that the Timobesone formulations were active, bioavailable and showed promise for future clinical use.

Introduction

Although topical corticosteroids are widely employed in dermatology, the continual use of potent preparations may lead to a variety of side-effects. In general the

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risk of side-effects increases with the potency of the preparation used (Burton, 1983). The new corticosteroid under investigation in this study (Timbosone acetate) is one synthesized by Syntex Research in their search for novel effective steroids with more favourable ratios of desirable to undesirable effects. Timobesone acetate is an etianic acid derivative of betamethasone in which the terminal carbon (C-21) of the corticoid side-chain has been replaced by a thiomethyl group and the 17α-hydroxy by an acetate ester group. Preliminary in-house screening (vasoconstriction and animal studies) has shown that this steroid has topical anti-inflammatory activity in the range of the most potent topical corticosteroids, while systemic corticoid activity in animal models is in a range similar to that of hydrocortisone.

The aim of this study was to investigate the vasoconstrictor activities and bioavailabilities and, by inference, the clinical anti-inflammatory efficacies of Timobesone cream and ointment formulations. The term "bioavailability" is used here to refer to the relative absorption efficiency for a steroid as determined by its release from the preparation, followed by its penetration through the epidermis into the dermis to produce the characteristic blanching effect. It is accepted that a knowledge of the onset time, intensity and duration of corticoid-induced pallor may be used both to assess the activity of a steroid and its bioavailability from different vehicles, as determined from a pharmacological response (Barry, 1976). The Timobesone preparations were compared with selected commercially available steroid creams and ointments in the occluded and non-occluded blanching test described by Barry and Woodford (1974, 1975). Full details of these procedures, including the reproducibility of the occluded assay over several years of use and in vivo/in vitro correlations, have been published elsewhere (Barry, 1983; Barry and Woodford, 1978).

Materials and Methods

Topical corticosteroid preparations

Seven tubes (randomly coded A–G) were supplied to the investigators by Syntex Research containing: Timobesone cream (0.05%), Timobesone ointment (0.05%), Lidex cream (fluocinonide, 0.05%), Lidex ointment (fluocinonide 0.05%), Dermovate cream (clobetasol propionate, 0.05%), Dermovate ointment (clobetasol propionate, 0.05%) and Betnovate cream (betamethasone 17-valerate, 0.1%). Timobesone acetate is 9α-fluoro-11β, 17α-dihydroxy-16β-methyl-17β-methylthiocarbonyl-androsta-1,4-dien-3-one 17-acetate (Fig. 1). The Lidex (U.S.A.) and Dermovate (U.K.) commercial preparations were included for comparison and Betnovate cream (U.K.) was employed as a standard positive control in the work.

Subjects

Ten volunteers were selected from an experienced panel as those demonstrating consistency of response to Betnovate cream. None had received topical corticosteroid application for at least 3 months prior to the study and none had previously shown any adverse reaction (e.g. erythema) to topical steroids. All 10

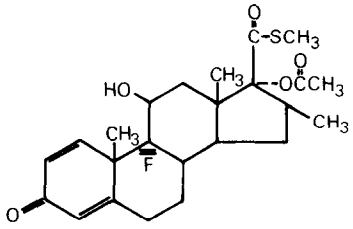

Fig. 1. Structural formula for Timobesone acetate.

subjects were healthy adults of legal age able to give informed consent and willing to attend all reading times: none was pregnant.

Methods

The topical corticosteroid preparations were stored in a cool, dark place. Immediately before applying formulations to each volunteer approximately 15 mm of preparation were expelled from each tube and rejected. A senior laboratory technician then transferred sufficient of each formulation to coded vessels: the investigators then received only the coded vessels so as to maintain double-blind conditions. The code was broken only at the completion of the study to allow calculation of the results.

Five mg of each preparation was applied at two sites on the washed flexor surface of each forearm to a total of 14 sites per forearm. The application sites consisted of 7×7 mm areas punched out from double-sided adhesive Blenderm tape, to which the formulations were applied using previously-designed randomization charts. Half the sites on each forearm were occluded with water-impervious Melinex film for 6 h: the remaining sites were left unsealed and exposed to the air. A perforated plastic shield was then secured over all 14 sites. Thus, each of the seven formulations under test was applied in both the occluded and non-occluded mode on each forearm of all 10 volunteers.

The shields, tapes and films were removed after 6 h and the areas were washed with soap and water at body temperature and were dried. Readings were taken in a double-blind manner after 10 min from washing the sites and after 1, 2, 3, 6, 18, 26, 42, 66, 74 and 90 h to provide data points for skin blanching at 6, 7, 8, 9, 12, 24, 32, 48, 72, 80 and 96 h after application.

Assessment of pallor was made according to a 0–4 scale with half-point ratings for intermediate readings:

0 = normal skin
1 = slight vasoconstriction
2 = more intense vasoconstriction
3 = generally even vasoconstriction
4 = more marked vasoconstriction with very distinct blanching

Ten volunteers provided a total of 280 application sites.

In order to assess steroid retention in the skin, the application sites were reoccluded with Melinex film for 6 h, 8 days after commencement of the study.

Pallor assessment was made according to the above scale 1, 3, 5, 18 and 24 h after removal of the films to provide data points for skin blanching at 7, 9, 11, 24 and 30 h after re-occlusion commenced.

Adverse reaction reporting

The study was designed to include facilities for the reporting of adverse reactions. In fact, no adverse reaction of any kind was observed in any volunteer during, or following, this work.

Results

The individual results for each volunteer were collected on separate sheets. At this stage the investigators did not break the code for formulations A–G except to ascertain from the laboratory technician which tube contained Betnovate cream; this information was required in order to produce the "corrected % total possible scores" (see below).

For each formulation the data for all volunteers were converted to percent of the total possible score for each time period, i.e. maximum score per site = 4; for 2 arms and 10 volunteers this equals 80. As an example, the 6-h reading for Lidex ointment (occluded study) was 53.5 out of a possible 80 which is 66.9%. Now all single application vasoconstrictor studies performed by us include Betnovate cream as a standard preparation. It is, therefore, possible to relate the values obtained for Betnovate cream in this occluded trial to those obtained in the comprehensive studies on proprietary corticosteroid preparations published by Barry and Woodford (1974, 1975). In the present trial the correction factor is given by: summed % total possible score for Betnovate cream in the paper by Barry and Woodford (1974) divided by the summed % total possible score for Betnovate cream in this trial = 393.333/382.500 = 1.028. Hence all occluded values were multiplied by this factor. However, the original studies performed by Barry and Woodford (1974, 1975) were made using the occluded blanching test only, therefore there is no corresponding unification procedure for a non-occluded study.

Figs. 2–8 express graphically the results of the vasoconstrictor assays. Because the curves for the occluded preparations have been drawn using data unified in terms of a "standard Betnovate cream" the figures may be compared directly with those published previously (for a review, see Barry, 1983).

The data (combined occluded and non-occluded study) were submitted to an analysis of variance, which is used to compare more than two sample means (Goldstein, 1964).

A computer was used to facilitate calculation and the computer program was arranged so that the data could be analyzed without transformation or in one of five (x^{-1}, $x^{-\frac{1}{2}}$, log x, $x^{\frac{1}{2}}$ and x^2) transformations (Tukey, 1957). Tests for non-additivity (Harter and Lum, 1962) showed that both the square-root transformation and non-transformed data gave very low and virtually identical T-values. The square-root transformation values were used in the statistical analysis (Table 1): identical results

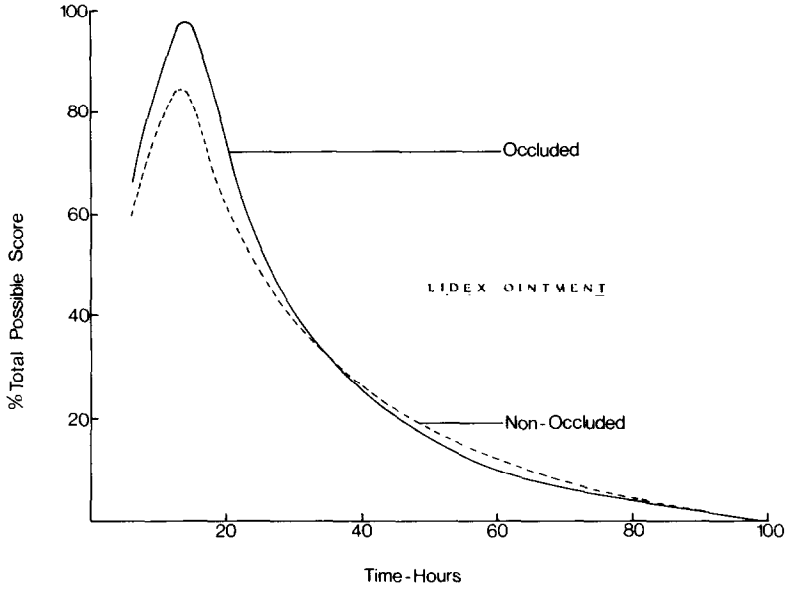

Fig. 2. Blanching curves for Lidex ointment.

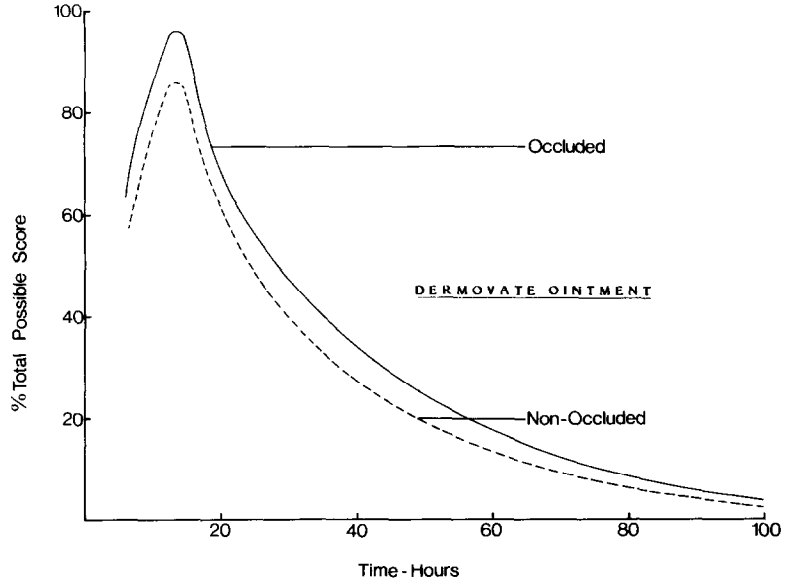

Fig. 3. Blanching curves for Dermovate ointment.

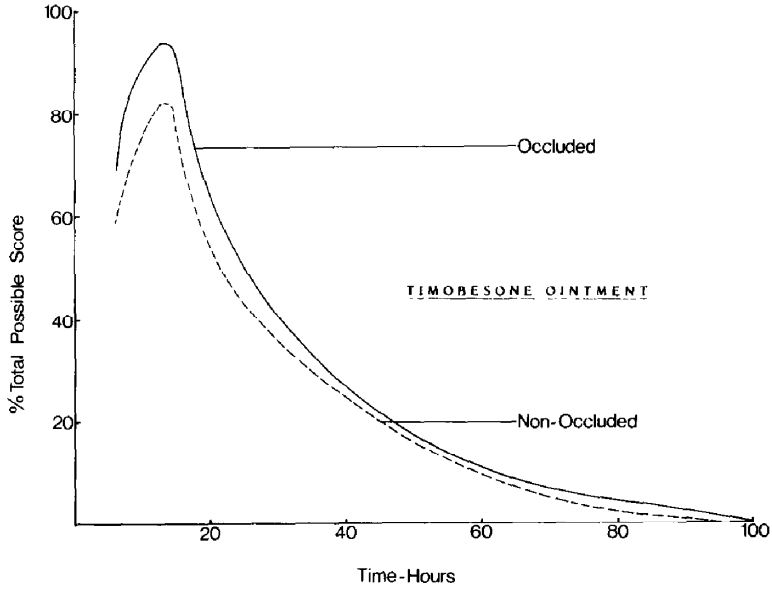

Fig. 4. Blanching curves for Timobesone ointment.

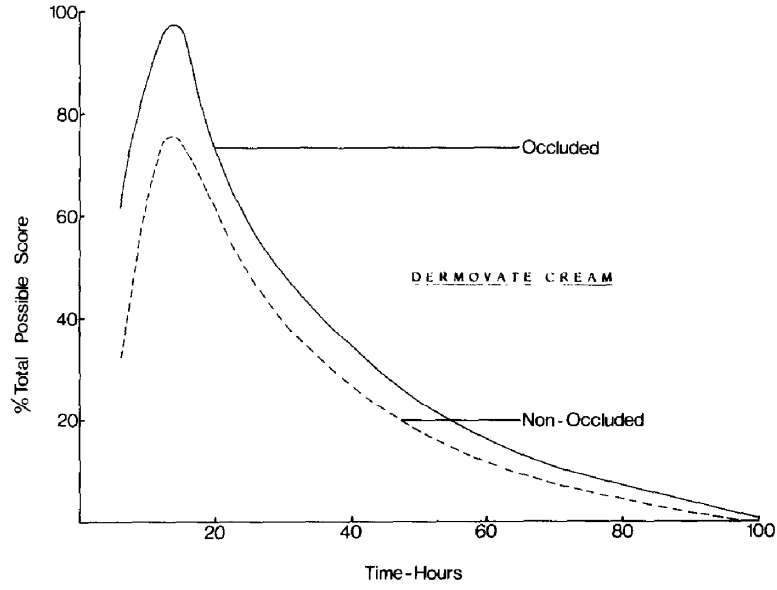

Fig. 5. Blanching curves for Dermovate cream.

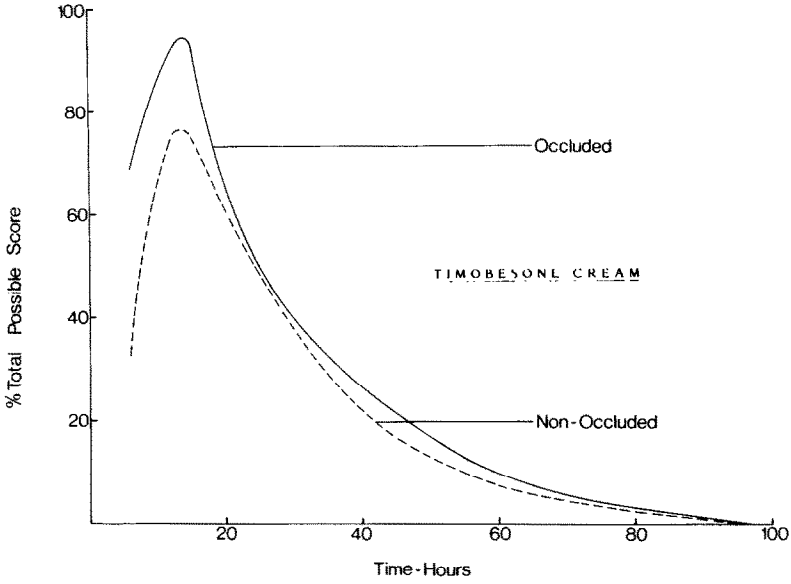

Fig. 6. Blanching curves for Timobesone cream.

were obtained employing the non-transformed data.

Calculation of the minimum significant range value k using the Studentized Range Test (Goldstein, 1964) permitted comparison of the corticosteroid formulations. At the 5% significance level, $k = 0.53$. If the $T_m/10$ mean values of two formulations (Table 1) differ by more than 0.53 there is a significant difference at the 5% level between those preparations.

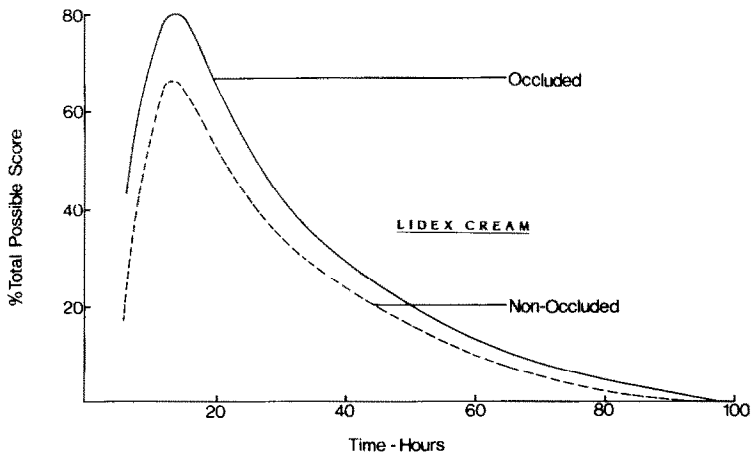

Fig. 7. Blanching curves for Lidex cream.

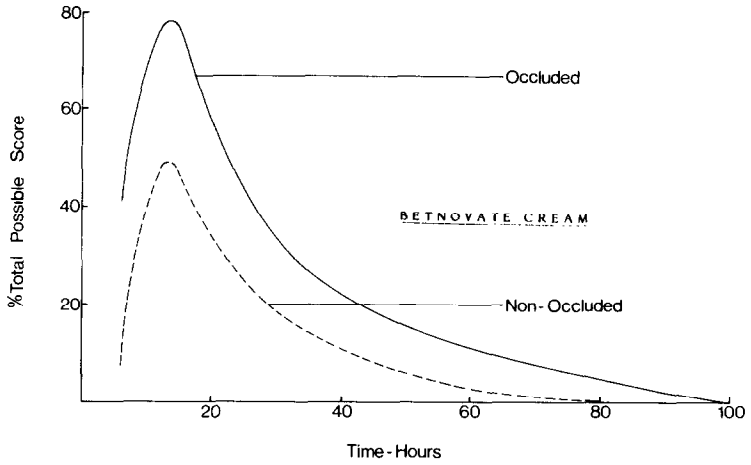

Fig. 8. Blanching curves for Betnovate cream.

TABLE 1
BLANCHING RESPONSE TO CORTICOSTEROID PREPARATIONS LISTED IN ORDER OF AREA UNDER THE CURVE VALUES (OCCLUDED THEN NON-OCCLUDED)

Preparation	Application conditions	Area under the curve [a] % × hours	Summed % total possible score [b]	$T_m/10$ mean value [c]	Summed % total possible score on re-occlusion [d]
Dermovate cream	Occluded	3040	541	6.47	47
Dermovate ointment	Occluded	3010	541	6.47	35
Lidex ointment	Occluded	2600	518	6.34	12
Timobesone ointment	Occluded	2530	524	6.38	22
Lidex cream	Occluded	2490	418	5.68	14
Timobesone cream	Occluded	2470	509	6.29	14
Betnovate cream	Occluded	2160	393	5.50	8
Dermovate ointment	Non-occluded	2530	464	6.08	26
Lidex ointment	Non-occluded	2440	471	6.12	11
Dermovate cream	Non-occluded	2280	376	5.46	28
Timobesone ointment	Non-occluded	2170	451	5.99	13
Timobesone cream	Non-occluded	2130	381	5.50	9
Lidex cream	Non-occluded	1920	302	4.88	8
Betnovate cream	Non-occluded	1080	181	3.76	4

[a] Obtained by planimetry of the blanching profile.

[b] The corrected % total possible scores (occluded study) and % total possible scores (non-occluded study) summed for all volunteers over all reading times.

[c] The $T_m/10$ mean value is the square-root transformation of sum of scores (T_m) divided by the number of volunteers (10). The minimum significant range value k = 0.53 ($P = 0.05$), i.e. if the $T_m/10$ values of two preparations/application conditions differ by more than 0.53 there is a significant difference between those preparations/application conditions (see Results).

[d] Re-occluded for 6 h, 8 days after commencement of the experiment; as b above.

TABLE 2
STATISTICAL ANALYSIS OF OCCLUDED AND NON-OCCLUDED STUDIES, TREATED AS SEPARATE EXPERIMENTS LISTED IN ORDER OF $T_m/10$ VALUE

Preparation	Application conditions	$T_m/10$ mean value [a]	Preparation	Application conditions	$T_m/10$ mean value
Dermovate cream	Occluded	6.57	Lidex ointment	Non-occluded	6.12
Dermovate ointment	Occluded	6.56	Dermovate ointment	Non-occluded	6.08
Timobesone ointment	Occluded	6.47	Timobesone ointment	Non-occluded	5.99
Lidex ointment	Occluded	6.42	Timobesone cream	Non-occluded	5.50
Timobesone cream	Occluded	6.38	Dermovate cream	Non-occluded	5.46
Lidex cream	Occluded	5.76	Lidex cream	Non-occluded	4.88
Betnovate cream	Occluded	5.58	Betnovate cream	Non-occluded	3.76
	$k = 0.46$			$k = 0.51$	

[a] The $T_m/10$ mean value is the square-root transformation of the corrected sum of scores (T_m) divided by the number of volunteers (10). See footnotes to Table 1.

It may also be valuable to examine the occluded results and the non-occluded results separately. Table 2 shows that statistical analysis. The occluded data are derived from the corrected sum of scores and the non-occluded values from the sum of scores: the square-root data were the marginally-preferred transformation.

Because the re-occlusion values showed obvious differences between the volunteers no statistical analysis was performed on these results.

Discussion

As for previous studies (e.g. Barry, 1976, 1983; Barry and Woodford, 1974, 1975, 1978; Barry et al., 1984; Woodford and Barry, 1982; Bennett et al., 1985), we used three parameters to compare the activities of the steroids in their various formulations — the area under the blanching curve (AUC) as measured by a planimeter, the summed % total possible score, and the square-root transformation of the sum of the scores divided by the number of volunteers ($T_m/10$) — as defined in the Results section. The analyses are shown in Tables 1 and 2.

All of the corticosteroid formulations produced obvious vasoconstriction, the rank order of the preparations being similar irrespective of the blanching parameter (area under the curve or summed % total possible score) employed (Table 1). The area under the curve value for Betnovate cream was in accord with our published values (Woodford and Barry, 1977; Barry and Woodford, 1978). The data for Dermovate cream and ointment also agreed well with published work (Barry and Woodford, 1974, 1975).

All formulations produced similar blanching curves with a maximum after approximately 12–14 h, followed by a sharp decline (Figs. 2–8). Each non-occluded profile was very similar to the corresponding occluded graph and often the non-oc-

cluded values were less than the corresponding occluded data (most obviously so with Betnovate cream).

When the occluded and non-occluded studies were treated as one experiment (Table 1), all other formulations employed in both application modes were statistically superior to Betnovate cream under non-occluded conditions ($P = 0.05$). Whereas Lidex ointment, Dermovate ointment and Timobesone ointment were statistically equivalent when applied in the occluded and non-occluded modes, Dermovate cream, Timobesone cream, Lidex cream and Betnovate cream produced statistically better results when tested under occlusive conditions than when applied non-occluded. The dramatic improvement conferred by occlusion is especially noticeable in the case of Betnovate cream. These results may be rationalized by realising that ointment formulations, by their very nature, are occlusive preparations and we thus expect little *additional* effect on occluding these application sites with Melinex film.

When the occluded results were examined separately (Table 2), all formulations except Lidex cream were superior to Betnovate cream.

When the data were analyzed under non-occluded conditions only, Timobesone ointment was more active than Lidex cream, Betnovate cream and Dermovate cream; no other preparation was more active (Table 2). Timobesone cream was more active than Lidex cream or Betnovate cream, although as might be expected it was less potent than some ointment formulations (Lidex and Dermovate).

Under occluded conditions, both Timobesone preparations were better than Lidex cream and Betnovate cream and similar to the remaining formulations.

All preparations elicited some skin blanching on re-occlusion but not every volunteer demonstrated pallor in all cases. Such results indicate the presence of a steroid reservoir within the stratum corneum.

From these experiments, we can conclude that all the preparations tested were active and bioavailable, providing prominent blanching curves typical of potent or very potent corticosteroid formulations. Blanching curves produced under non-occluded conditions were generally similar to those elicited under occlusion but the scores were generally lower in the non-occluded mode. This concurs with the general observation that occlusion (and thus hydration) of the stratum corneum promotes the percutaneous absorption of most compounds. All preparations, when applied under occluded or non-occluded modes, elicited pallor on re-occlusion. This was greater under occluded than non-occluded conditions (because hydration promotes reservoir formation).

Most investigators accept that the vasoconstrictor response may be taken to be a good predictor of steroid efficacy in the clinic (Barry, 1983). The data presented here, and the absence of any untoward reaction from any preparation in any volunteer, give confidence in the formulation of these Timobesone preparations.

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