COMPARATIVE BIOAVAILABILITY AND RANKING OF TOPICAL CORTICOSTEROID FORMULATIONS

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OBJECTIVES OF THIS STUDY

Numerous experiments in recent years have indicated differences in the bioavailability of corticosteroids from seemingly identical topical dosage forms. The human blanching assay was utilized in this study to assess the comparative blanching activities of various locally manufactured proprietary corticosteroid preparations.

The first experiment was performed to assess the relative blanching activities of six semi-solid preparations containing the same concentration of betamethasone 17-valerate. The preparations used were Betnovate cream and ointment, Persivate cream and ointment and Celestoderm-V cream and ointment. This was followed, in the second experiment, by the investigation of the blanching activities of two lotions containing betamethasone 17-valerate (Betnovate and Celestoderm-V) and a lotion containing betamethasone 17,21-dipropionate (Diprosone).

The third experiment involved a study of six semi-solid proprietary corticosteroid-containing formulations, *viz.* Dermovate (clobetasol propionate) cream and ointment, Betnovate (betamethasone 17-valerate) cream and ointment and Eumovate (clobetasone butyrate) cream and ointment. This investigation was prompted by claims in advertisements in the medical media that Dermovate is therapeutically more efficacious than Betnovate which is more efficacious than Eumovate.

The penultimate experiment in this study served the purpose of finding a corticosteroid-containing preparation that falls into the moderately potent group of corticosteroid formulations, as described in the United Kingdom MIMS. This preparation was used in the final experiment which was undertaken to ascertain the potency category of Florone (diflorasone diacetate) cream and ointment.

CONTENTS

CHAPTER 1

INTRODUCTION

Since the discovery some 30 years ago that hydrocortisone was effective in the treatment of dermatoses (1,2), the use of topically applied corticosteroids has dramatically changed the approach of the physician to the treatment of various skin disorders (3), with corticosteroids probably being the most frequently prescribed and efficacious compounds in modern skin therapy (4,5). A number of reports have discussed the widespread use of corticosteroid-containing topical preparations in the United States of America and the United Kingdom (3,6,7) while in South Africa the medical practitioner can choose from the 85 available preparations (8), depending on the required vehicle, concentration of the corticosteroid and whether or not additional pharmacologically active compounds are desirable.

The popularity of topical corticosteroid treatment has prompted researchers to study the uses and side effects of these drugs and the various possible methods of assessing their potency and biopharmaceutical properties.

1.1 PHARMACOLOGY

Topical corticosteroids are used extensively in medicine today for their non-specific anti-inflammatory action in the management of various acute and chronic dermatological conditions, where corticosteroid treatment has the particular virtue of shortening the acute early phase of the disease (9,10).

Various types of eczema are effectively controlled by the topical administration of corticosteroids and are, in fact, the main indications for topical corticosteroid therapy (11). In a study by Fry (12) skin disorders were the second most common minor illnesses,

with approximately 30% of these being eczema. Examples of dermatological problems that usually respond well to topical corticosteroids include allergic contact dermatitis, atopic eczema, primary irritant dermatitis, psoriasis (especially of the face and flexures) and seborrhoeic dermatitis. Others, including acne cysts, discoid lupus erythematosis, lichen planus, lichen status, nail disorders and psoriasis (mainly of the palms, soles, elbows and knees) usually present more of a problem, requiring highly potent preparations, occlusion or intralesional therapy (9,13).

1.1.1 Mode of Action

The physiological actions of corticosteroids allow them to be divided into two major groups, *viz.* mineralocorticoids and glucocorticoids. It has thus far been impossible to dissociate the glucocorticoid and anti-inflammatory properties of synthetic topically applied corticosteroids, although almost all of the unwanted activity of the mineralocorticoids has been eliminated (14).

Topical corticosteroids are primarily effective because of their anti-inflammatory, antipruritic and vasoconstrictive actions (15). It is common knowledge that topical corticosteroids have these effects, but their actual mechanism of action remains unresolved and has been the subject of much speculation. The degree of anti-inflammatory activity of corticosteroids appears to be quantitatively related to the concentration of hormonally active corticosteroid present at the inflammatory site (16). A series of experiments performed by Nakamura (17) showed that the corticosteroid concentration after intravenous injection is higher in inflamed skin than in normal skin. These experiments, which included topical administration, led the author to suggest that corticosteroids act directly on the cells of the lesions of dermatoses, not indirectly through their action on blood vessels. Although corticosteroids have some suppressive action on the blood vessels, this does not appear to be the main mechanism of action in the treatment of dermatoses. It has been suggested (11) that the vasoconstrictive effect acts in parallel to the other anti-inflammatory effects by

blocking circulating mediators of inflammation from the local site, although there is as yet no evidence to support this concept.

The inflammatory response is a complex one and it is generally accepted that corticosteroids influence many aspects of this response (18). The possibility also exists that systemic and topical administration of corticosteroids may have different mechanisms of action due to the systemic action being limited by topical application. It should also be noted that corticosteroids exert an effect on allergic, immunologic and inflammatory reactions, possibly implying that their effects on the different body systems may be part of the mechanism of action of some, but not all, of these effects (18).

Some of the suggested mechanisms of anti-inflammatory action include the decrease in membrane permeability by a lysosomal membrane stabilizing effect (19-21), the inhibition of prostaglandin biosynthesis (22,23) and inhibition of the formation, release and action of endogenous mediators of inflammation by the corticosteroids attaching themselves to tissue receptors (9,20,24-27). The vasoconstrictor activity decreases serum extravasation, swelling and discomfort (28). Corticosteroids are also immunosuppressive and may inhibit allergic reactions by preventing the production of antigen/antibody complexes (28).

It is a well established fact that corticosteroids exhibit an antimitotic effect on various cells in the body (29-31) and it appears as though this may be a factor of enzyme inhibition (32). Marks *et al.* (33) demonstrated a decreased uptake of thymidine in corticosteroid pretreated skin, probably reflecting decreased epidermal DNA systhesis due to steroid treatment. This is probably an important factor in the antimitotic effect of topical corticosteroids which may explain their well documented effectiveness in the treatment of psoriasis (11,30,34-41).

1.1.2 Side Effects

As with most forms of medication, attempts to increase the effectiveness of corticosteroid therapy, whether by enhanced bioavailability (by improvement of the drug delivery system or by occlusion) or by increasing the inherent potency of the drug, lead to the incidence and severity of side effects becoming increasingly more significant (11,42). The side effects of topical corticosteroids can, in general, be divided into two categories, *viz.* local effects and systemic effects (9,28).

1.1.2.1 Local Side Effects

The most common side effect of topical corticosteroids is most likely skin atrophy, caused by damage to the dermal collagen (28,42). Application of corticosteroids over a prolonged period has been found to cause thinning of the epidermis and dermal tissue (11,28,42-46). Based on histological studies, the appearance of atrophy and transparency of skin has been found to be due to thinning of the epidermis and stratum corneum as well as effacement and shrinkage of the papillary dermis (43). Changes in animal and human skin thickness suggest that both the dermis and epidermis are implicated in the skin thinning caused by corticosteroids (47). Dermal changes involve thinning of the reticular layer, often associated with the degeneration of elastic fibres which, in the upper part of the dermis, are found to be thin and sparse with a more compact and dense network in the deeper layers as compared to normal skin (48). An accompanying decrease is found in the diameter of collagen fibrils and in the number of fibroblasts (48). *In vitro* studies on human foetal cells have further helped to elucidate the mechanism of skin atrophy by indicating a decrease in the synthesis of hyaluronic acid, collagen and sulphated glycosaminoglycan after treatment with corticosteroids (49). Thinning is therefore suggested to be a function of cell size (rather than cell number) accompanied by a decrease in the number of cell layers comprising the epidermis (48,50). Two of the abovementioned studies have further shown a positive dose response effect with the corticosteroids used (49,50).

Another well documented side effect of topical corticosteroids is telangiectasia (9,42,43), which is often found within the area of atrophy (42) and the severity of which correlates with the severity of the atrophy (43). Data obtained from two independent studies on corticosteroid-containing creams indicate that the rank order of blanching correlates with the severity of atrophy and telangiectasia caused by these preparations (43,51). (It should be noted that the experiments were performed about seven years apart with different proprietary preparations).

The site and method of application also have an effect on the side effects due to differences in percutaneous absorption, side effects increasing with an increase in percutaneous absorption (11,28,42). Other local side effects include striae, allergic reactions (probably due to a constituent of the preparation other than the corticosteroid), perioral dermatitis, rebound pustulation (for example after cessation of treatment of rosacea with topical corticosteroids) and ophthalmic effects (9,28,42,52-56).

1.1.2.2 Systemic Side Effects

Topical corticosteroids have the potential to reduce endogenous cortisol levels either by direct action on adrenocortical function or by inhibition of ACTH production by the anterior pituitary lobe (9,11,42,57-59). Clinical manifestations have not been reported in a very large number of cases, although there have been reports of iatrogenic disease (28,58,60-62).

Cushing's syndrome and a depression of cortisol levels have been reported in patients after use of topical corticosteroids for the treatment of psoriasis and other dermatoses as well as in healthy experimental volunteers (61-66). Some experiments have shown no cortisol suppression in normal subjects and patients with alopecia, whereas cortisol suppression was observed in the case of psoriatic patients, suggesting enhanced percutaneous penetration through psoriatic skin (66,67). It has further been noted in the clinical situation (60,61,65) that the penetration of corticosteroids appears

to decrease as the dermatitis clears, thus suggesting that the problems associated with percutaneous absorption are temporary, occurring mainly in the initial stages of treatment. The potential for topical corticosteroids to produce a decrease in plasma cortisol levels should therefore be considered during treatment and is especially important when occlusion is required (9,11,42,65,66) and in the case of children (6,11,42,65,68,69) where percutaneous absorption occurs more readily and where the surface to weight ratio is greater than that for adults. Hepatic dysfunction should also be considered as this has been found to increase the possibility of systemic effects of topical corticosteroids (70).

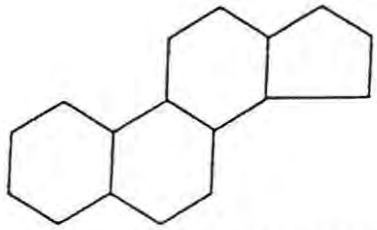
It should be noted, however, that although the morbidity of these effects is significant they occur in a small number of patients, are generally reversible and are probably of little significance in outpatients where the amount of corticosteroid applied is not excessive and occlusion is normally not used over large body areas for prolonged periods of time (61,64-66,71).

As mentioned previously, increasing the therapeutic efficacy of a drug will more than likely lead to increased incidence and severity of side effects (42). The most common and, in most cases, most effective means of increasing the efficacy of topical corticosteroids has been the inclusion of a halogen atom (see section 1.2). This has led to the terms "fluorinated" and "halogenated" being used to describe the superiority of the newer corticosteroid molecules over hydrocortisone and "fluorinated steroids" being most often implicated in the various side effects (42,44,72). The terms "fluorinated", "halogenated" and "strong" should, however, not be used synonymously (65,73). Hydrocortisone 17-butyrate (72), desonide (73-75) and budesonide (76) do not contain halogen atoms and have been shown to elicit similar or superior responses to halogenated corticosteroids in blanching assays (51,73,75-78) and clinical trials (72-75). Their side effects (both local and systemic) have also been found to be similar to or more severe than those of the halogenated corticosteroids (47,50,52,65,72).

1.2 STRUCTURE ACTIVITY RELATIONSHIPS

The relationship between structure and functional activity is of major importance in topical corticosteroid therapy.

The basic structure to which all steroids are related is that of fully reduced phenanthrene to which is fused a five-membered ring structure, giving rise to a cyclopentanoperhydrophenanthrene (perhydrocyclopentanophenanthrene) nucleus consisting of three six-membered rings and a five-membered ring, fused to form the basic nucleus depicted in figure 1.2.1 (20,79,80).



cyclopentanoperhydrophenanthrene

Figure 1.2.1

Some of the essential structural features required for glucocorticoid and anti-inflammatory action include (3,16,20,79-81):

a) a double bond between carbon atoms 4 and 5,
b) a - $CO.CH_2OH$ moiety at carbon 17,
c) a ketone moiety at carbon 3 and
d) an oxygen function in the β configuration at carbon 11; this is normally an hydroxyl group.

The constant drive to find a molecule with an increase in anti-inflammatory activity and a reduction in unwanted effects gave rise to several alterations in the synthetic analogues of cortisone. The introduction of a 1:2 double bond into the molecule led to an increase in glucocorticoid and anti-inflammatory action and a decrease in mineralocorticoid side effects (9,16,20,81,82). The synthesis of the 9-α and 6-α halogenated derivatives (20,82,83) had a major influence in enhancing both glucocorticoid and mineralocorticoid effects, the latter effect being overcome by the

introduction of a substituent at the 16 position, e.g. the β-methyl in betamethasone (9,81) and the 16,17-acetonide in fluocinonide (82). In addition to decreasing the unwanted mineralocorticoid effects the 16,17-acetonide has been found to increase the corticosteroid-induced blanching (83). Chloronation of the 21- hydroxyl in clobetasol 17-propionate resulted in an increase in lipophilicity and has given rise to an extremely powerful topical corticosteroid (84). Halogenation (especially fluorination) has therefore given rise to some very potent topically active steroids, but the terms "fluorinated steroid" and "potent steroid" can no longer be used synonymously, since some of the non-fluorinated compounds undoubtedly fall into the potent group (see section 1.1.2.2). An interesting exception to the effect of fluorination is seen in the comparison of triamcinolone acetonide and desonide (73). The structure of these compounds is identical except for the absence of the 9-α fluorine atom in desonide. Desonide, however, penetrates the skin to a greater extent (73), elicits a similar blanching response to triamcinolone acetonide (73,75) and was found not to be statistically different in a clinical trial using a preparation containing a 50% lower concentration of desonide than triamcinolone acetonide (75).

Apart from the structure of the corticosteroid affecting its inherent anti-inflammatory activity and ability to elicit blanching, the ability of the molecule to penetrate the stratum corneum is also affected by its structure (80), although these two effects may be divorced from each other (85). This can be seen from the findings that betamethasone 17-valerate elicits a much more potent blanching response than its parent alcohol (85,86) yet they are almost identical in their penetration characteristics (85). Hydrocortisone and its acetate penetrate to a greater extent than fluocinolone acetonide, although the latter is more potent pharmacologically (83,87). Similarly, fluocinolone acetonide penetrates the skin approximately 14 times as effectively as fluocinolone alcohol, but is 125 times more effective in the blanching bioassay (85).

The stratum corneum is known to be an effective barrier to most compounds (see section 1.3). It is, however, to some extent

permeable to both water soluble and lipid soluble compounds, especially after hydration, the activation energies for lipid soluble molecules being lower than for water soluble molecules (88). Increasing the polarity of the molecule has been found to cause a decrease in permeability and the converse can therefore be assumed to be true, although Ponec and Polano (89) have found, in *in vitro* studies, that the amount of corticosteroid that penetrated the epidermis after application in an ethanolic solution decreased with decreasing polarity of the corticosteroid. Osamura (90) points out that polarity and lipid solubility of corticosteroids are not the only factors influencing the extent of penetration through the epidermis. In an experiment using betamethasone 17-valerate, hydrocortisone 17-butyrate 21-propionate, clobetasol 17-propionate, hydrocortisone and betamethasone, the polarity of the betamethasone 17-valerate was between that of the other two esters and the parent alcohols. Betamethasone 17-valerate, however, penetrated through the epidermis to the greater extent. The blanching activities of these corticosteroids in descending order were clobetasol 17-propionate, hydrocortisone 17-butyrate 21-propionate, betamethasone 17-valerate, betamethasone and hydrocortisone. An early study by McKenzie (91) showed that the absorption of corticosteroid salts was different to that of the parent alcohol when compared by using the blanching assay. Intradermal injections of dexamethasone and its phosphate derivatives elicited a similar blanching response, whereas the relative activity of the alcohol was far superior to that of the salt after topical application, a phenomenon suspected to be due to the difference in penetrability of the two compounds. In a comparison between betamethasone 17-valerate and the parent alcohol, no significant differences were found in the percutaneous absorption through human leg skin or the abdominal skin of the hairless mouse (both *in vitro* studies), but the concentration of betamethasone 17-valerate able to elicit a blanching response was 300 times lower than that of the parent alcohol (85). Conversely, fluocinolone acetonide and fluocinolone acetonide acetate have similar penetration abilities and both penetrate better than fluocinolone alcohol which also has a much reduced blanching activity than the esters (85). Although the fluocinolone esters have similar penetration abilities, fluocinolone

acetonide acetate was found to be 5 times more potent than fluocinolone acetonide in the blanching assay (83,85). Comparisons other than those of parent alcohol versus ester have shown similar results. Hydrocortisone and hydrocortisone acetate have been found to penetrate to a greater extent than fluocinolone acetonide, with the latter, however, eliciting a superior blanching response (83,87). Similarly, fluocinolone acetonide was absorbed to a vastly greater degree than betamethasone 17-valerate, but their blanching response was similar (85).

The increase in lipophilicty by the removal or masking of hydroxyl groups increases the topical effectiveness of corticosteroids (81). This substitution is normally at the 17 and/or 21 positions. The length and position of these side chains are of the utmost importance as can be seen in the comparisons of betamethasone 17-valerate and 21-valerate (9,86) and by comparing different esters in the 17 position of hydrocortisone and the 17 and 21 positions in betamethasone (86) in the blanching assay. It has been found that the acetates of topically applied corticosteroids are more efficient vasoconstrictors than the parent alcohols, whereas the phosphates are usually less effective (91). This experiment also showed that the vasoconstrictor action of two of the corticosteroids was equal after intradermal injection, indicating that the difference in topical effect is probably due to differences in their absorption.

The structure of the drug will affect its oil/water solubility which will in turn affect its ability to penetrate the stratum corneum, the diffusion of a drug through the skin being directly proportional to its lipid solubility and inversely related to its polarity (88,90,92,93). It has been suggested therefore that both the solubility of the drug and its diffusivity through the stratum corneum play a part in percutaneous absorption. It would appear from the results obtained by Scheuplein *et al.* (92) that the more polar molecules are not rigorously excluded from the membrane, but rather more firmly bound within it. An increase in the number of polar groups was also found to cause a decrease in the diffusion coefficient. He therefore concluded that the reduced permeability

of the polar steroids does not arise from a limited solubility within the membrane, but from their decreased mobility due to stronger chemical binding. Generally, drugs possessing a dual solubility in both oil and water require the least hydration of the barrier for percutaneous penetration, followed by drugs soluble only in water, whereas those soluble only in oil appear to require the greatest hydration of the stratum corneum to penetrate (94).

Although penetration of the corticosteroid molecule is essential for it to exert physiological and pharmacological action (see section 1.3) it is, to a large extent, the inherent pharmacological activity of the molecule that allows one steroid to be classed as superior to another (87,90).

1.3 PERCUTANEOUS ABSORPTION

It is generally accepted that drugs, such as topical corticosteroids, which have a specific effect on viable tissue, usually have their site of action at a point below the lower border of the stratum corneum and therefore have to penetrate the stratum corneum to be effective (9,93-95). Ostrenga (96) concluded that the similarity between the profiles for vasoconstriction and penetration is striking. This and other experiments have suggested that the rate-controlling step is in the skin barrier and that the efficacy of a topical preparation is directly related to the ability of a drug in a particular vehicle to penetrate the skin barrier (9,80,96,97). If however, the skin is damaged and the barrier is thus absent, the release of the drugs from the vehicle becomes the rate-limiting step (82). In the clinical situation it is therefore reasonable to assume that the rate-controlling step will shift from the latter to the former as the healing process proceeds (98). This assumption is strengthened by the observation that the depression of endogenous cortisol levels becomes less significant a short while after therapy with topical corticosteroids has commenced (60,61,65).

The stratum corneum is thus the first and often principal barrier encountered by a corticosteroid after application to the skin (11,88,92,99-101). It has been found that the isolated stratum corneum is almost as impermeable as the entire skin (101,102). There are numerous factors which affect the percutaneous absorption and therefore the efficacy and blanching ability of topically applied corticosteroids.

1.3.1 State of the Stratum Corneum

1.3.1.1 Intact or Damaged

Whether the stratum corneum is intact or not is a major dividing line in dermatological therapy and investigations (94). Penetration has been shown to be greatly enhanced when the skin is abraded, broken or inflamed (103) as is the case in numerous dermatoses (97,104). It has been shown that the percutaneous absorption of radioactive hydrocortisone is approximately 3 times more rapid in the initial stages of absorption through irradiated skin (105). Removal of the stratum corneum by stripping with cellophane tape has also been found to enhance the absorption of certain topical corticosteroids (87,97,106). Regeneration of the barrier has been found to be just beginning 25 hours after stripping and was complete 72 hours after stripping (106). Feldman and Maibach (107) found that removal of the stratum corneum by stripping doubled the penetration of hydrocortisone, but caused a considerable change in the excretion rate curve, suggesting the existence of a second barrier residing in the Malpighian and basal layers of the skin. This second barrier will be of less importance in intact skin due to the barrier efficiency of the stratum corneum, but may well be significant in the case of damaged skin. It is the diffusion of corticosteroids through this second barrier that gives rise to systemic side effects, the ideal situation thus being retention of the drug in the epidermis with only slow migration to the dermis (81).

1.3.1.2 Hydration and Temperature

The cells of the stratum corneum contain structural lipids and water soluble substances which allow hydration of this tissue (101). The major constituents and their approximate percentages in hydrated stratum corneum are water 76%, protein 20% and lipid 5% (99). The amphoteric protein of the stratum corneum is hygroscopic and softens when it contains sufficient water (94), the stratum corneum therefore always being partially hydrated *in situ* (108). Additional hydration appears to increase the rate of passage of all substances which penetrate the skin (9,82,94,97,100,109). It has been suggested that hydration may increase the size of the pores in the membrane (94,110). Hydration of the skin can be artificially induced by means of the vehicle (94) or by the application of an occlusive dressing (111,112). Occlusion can increase the water content of the stratum corneum from the normal 5 - 15% to as much as 50% (109).

Although plastic dressings had been successfully employed in topical dermatological therapy, McKenzie and Stoughton (111) and McKenzie (91) were the first to attempt to quantitate the effect of occlusion by using the blanching assay. No attempts were made to grade the intensity of pallor, but the corticosteroid concentrations that were required to produce a blanching response were 100 times smaller when applied under occlusion. It has also been shown that some corticosteroids which produced no blanching unoccluded, did in fact show vasoconstrictive properties when applied under occlusion (91). Conversely it has been found that hypohidrosis or anhidrosis, whether due to a disease state or artificially induced, caused a decrease in blanching after the application of topical corticosteroids under occlusion (113).

Occlusion with plastic film has thus far been found to be the single most effective mechanism for increasing penetration (9,87), a fact that has been demonstrated in numerous experiments since the original observations of McKenzie and Stoughton (87,111). The effect of occlusion time has been studied and for a particular betamethasone 17-valerate preparation it was found that maximum blanching was

attained with a 10 hour occlusion period (114). In an experiment to compare the urinary excretion of radiolabeled hydrocortisone after topical application to normal skin, stripped skin, occluded skin and a combination of these, it was found that penetration was greatest for stripped skin in the occluded mode followed by unstripped occluded, followed by stripped unoccluded with unstripped unoccluded showing the least penetration (107).

It is reasonable to assume that this increase in penetration is clinically significant as many corticoid unresponsive dermatoses became responsive solely with the addition of the occlusive dressing (87). Occlusion has been found to force the corticosteroid into the deeper layers of the stratum corneum, thus forcing it closer to the living epidermis and therefore closer to the site of anti-inflammatory action (115).

Besides hydration, occlusion also has the effect of increasing the skin temperature (109,111). It has been shown that the penetration rates of some substances are altered by a change in temperature (116) and it is possible that this is also true for topical corticosteroids. The permeability change produced by an increase in temperature is, however , probably slight relative to the permeability changes resulting from increased hydration (82), as *in vitro* experiments have shown that the permeability of the stratum corneum is only slightly affected by temperatures below $60^{o}C$ (97).

1.3.1.3 Thickness and Regional Variation

It was shown as early as 1956 that the thickness of the skin has an effect on the rate of absorption of topically applied hydrocortisone, in that the corticosteroid took 6 hours to penetrate lichenified skin to the same extent that it had penetrated normal, thinner skin after 2 hours (105). A comprehensive study of the regional variation in percutaneous penetration has shown that hydrocortisone is absorbed at different rates and to a different extent from various anatomical sites (117). Absorption, expressed relative to absorption through the skin of the ventral aspect of the forearm, varied from a trace

for the heel to a 42-fold increase for the scrotum. The foot showed lower penetration whereas the various regions of the head showed greater penetration than the forearm. The stratum corneum of the palms and soles is known to be much thicker than that of other parts of the body (118,119). The possible role of structural variability of the stratum corneum of different body areas should not be excluded (108).

Foetal and infant skin have been reported to be more permeable than adult skin (97). Percutaneous absorption of topical corticosteroids therefore occurs more readily in children than in adults (69). A review by Keipert (69) outlines absorption in infancy and childhood as compared to adults as well as absorption through the membranes of the eye and mouth and after the use of nasal sprays.

1.3.2 Concentration of Steroid

The concentration of the corticosteroid applied to the skin has been found to affect the amount of drug penetrating the skin, the rate of penetration and the degree of blanching. The effect of concentration can be demonstrated by a simplified version of Fick's Law (97) :

$$Js = Kp\Delta Cs$$

where Js = steady state flux of solute
Kp = permeability coefficient
ΔCs = concentration difference of solute across the membrane.

The blanching assay shows that, as expected, increasing the concentration of the corticosteroid leads to an increased degree of blanching, up to a certain point at which a plateau is reached (104,120,121). Similar results were obtained after applying different amounts of a cream containing the same concentration of betamethasone 17-valerate (114). It should be noted, however, that in this experiment the actual thickness of the cream applied possibly presented a physical barrier between the corticosteroid in the outer part of the base and the skin surface. Diffusion of the drug from the outer layer of the vehicle is thus required for continued

percutaneous absorption (122,123). Barry and Woodford (51) have, on the other hand, found that no significant differences in the blanching response were observed when between 3 mg and 8 mg of the preparation were applied, while the plateau effect described in the abovementioned experiment was observed with the application of 4,8 mg of cream. The size of the application sites was equal for both sets of experiments. It has further been found that as the concentration of an agent increases on the surface area, there is a definite decrease in the percent of applied material that will penetrate the skin (104). Clinical evidence, however, does suggest that increasing the concentration of the corticosteroid increases the therapeutic response (124).

The abovementioned plateau effect may be explained in a number of ways. Closely related to the concentration of the drug in the vehicle is the time of application. The concentration of the drug in the vehicle will decrease as the drug passes through the skin and may increase to some extent as the aqueous phase of the vehicle evaporates (94). It has also been suggested that the rate of percutaneous absorption can decrease as the tissue becomes saturated with the drug (106). In the case of the blanching assay it has been suggested that the maximum degree of blanching may have been reached at the plateau stage (114). The amount of undissolved corticosteroid in the base has also been implicated in the relationship of concentration versus vasoconstriction, undissolved drugs not being able to diffuse out of the vehicle (82,125,126). An *in vitro* study of the release of different concentrations of betamethasone 17-valerate from a water-propylene glycol mixture into isopropyl myristate indicated an increase in release rate with increased corticosteroid concentration (125). This possibly indicates that the plateau is of biological origin. Another possible explanation is that the relevant receptor in the skin became saturated at a certain point, an increase in the number of steroid molecules thus having no further effect (120).

It is, however, probable that the maximum observed blanching is due to a combination of the above effects with the predominant effect

depending on the specific experimental variables, e.g. the inherent blanching ability of the steroid (Altmeyer and Zaun (120) found no concentration dependency in the case of hydrocortisone) or the vehicle (alcoholic or semi-solid) into which the corticosteroid is incorporated.

1.3.3 Mechanism of Percutaneous Penetration

As previously mentioned, the therapeutic activity of a topically applied corticosteroid relies on its ability to penetrate the stratum corneum. There has been much speculation over the mechanism of percutaneous penetration and various conclusions have been drawn over the last 25 years (118). When a corticosteroid comes into contact with the skin it has three potential routes of entry into the subepidermal tissue, *viz.* via the hair follicles, via the sweat ducts or across the continuous stratum corneum between these appendages (118). The last route would involve the movement of the molecules either through or between the cells of the stratum corneum.

A study of the percutaneous absorption of radiolabeled hydrocortisone showed an accumulation of the corticosteroid in the orifices of glands and in hair follicles (105). However, the rate of appearance of the corticosteroid in the cutis seemed to be uniform, indicating that the percutaneous absorption was not greater via the gland orifices and hair follicles than through the other cells of the stratum corneum. Shelmire (94), in his review, draws the conclusion that there can be little doubt that drugs which penetrate the stratum corneum do so most easily at the follicular ostia, a view which could certainly not be agreed with today. He further stated that all drugs, regardless of solubility, penetrate the skin in exactly the same manner and by the same route. Several workers (88,92,97,117) have, however, concluded that the follicular component of absorption would be less prominent in substances which are rapidly absorbed (e.g. ^{32}P tributyl phosphate) when compared to a poorly absorbed substance like hydrocortisone, where all penetration routes are significant. The very small percentage (0,1 - 1,0%) of hair follicles and sweat glands should also be borne in mind (100),

leading to the possible assumption that the transepidermal route is the principal mode of entry (97), but Barry (97) does state that in the case of corticosteroids the shunt pathways may be dominant in both the transient period and during steady-state diffusion, a point of view also suggested by other workers (99,127). Scheuplein *et al.* (92) have further stated that the large molecular volume and polyfunctional character of corticosteroids have a profound influence on skin permeability and Barry (97) concludes that the polarity of a molecule could affect the mechanism of penetration.

Occlusion, which is known to increase the amount of corticosteroid absorbed through both intact and stripped skin, does not alter the shape of the absorption rate curve, suggesting that it has no effect on the mechansim of penetration (107), although occlusion is thought to affect the structure of the stratum corneum (94,110).
The regional variation in percutaneous penetration has provided some interesting data (117). Absorption of hydrocortisone through the stratum corneum of the forehead and scalp (where follicles are larger and more numerous) is greater than through the skin of the foot (where the stratum corneum is thicker). The authors suggest that absorption occurs both transepidermally and through hair follicles, with greater absorption being attributed to the increase in the size and the number of hair follicles. The inconsistency of this conclusion with respect to absorption through the skin of the palms and scrotum is however noted, the former having a thick stratum corneum, no hair follicles and three times as many sweat glands than other skin sites, but being less permeable than other skin sites (103,117). The possibility of structural specialization of the stratum corneum in these regions should be considered (117).

It is generally accepted that percutaneous penetration of topical corticosteroids is by passive diffusion rather than by a specialized active transport system (93,96,99,100). Corticosteroids are thought to maintain steady state penetration of the skin following a lag period (92,100), which may last from several hours up to a few days in the case of the more polar corticosteroids (101). The steady state penetration through the stratum corneum is considered by some

workers (82,92,100) not to be primarily intercellular or intra-appendageal, but a combination of the two, while others (128) maintain that after steady state diffusion has been established the dominant diffusion mode is probably no longer intra-appendageal, but occurs through the matrix of the stratum corneum. Another opinion is that transient penetration occurs via the shunts (100,128) and that steady-state transport occurs directly through the cells (100).

Cognizance should be taken of the fact that most of these experiments were conducted on a healthy human skin. This is acceptable in the case of normal bioavailability studies, but the possibility of a different mechanism of transport should be borne in mind when considering topical corticosteroids as therapeutic agents used on diseased skin, although it seems highly probable that the basic principles will be similar (99). Scott and Kalz (105) found differences in the penetration rate of radiolabeled hydrocortisone through normal, lichenified and irradiated skin, but did not appear to have found differences in the mechanism of percutaneous penetration.

1.4 RESERVOIR

The existence of a reservoir was first suggested by Malkinson and Ferguson (129) after noting a levelling off of the excretion of hydrocortisone after topical application. Although the location of the reservoir was unknown it was suggested that it may be in the skin.

An extensive study by Vickers (130) concluded that some corticosteroid is retained in the stratum corneum after topical application. Blanching was observed, as expected, after topical application of corticosteroids and was observable for up to 15 days after repeated occlusion. Similar initial observations were made after intradermal injection, but occlusion after 2 days produced no blanching. Corticosteroids applied to the skin from which the stratum corneum had been removed by stripping produced the expected blanching response after 1 hour, but reocclusion of the sites had no

effect, thus indicating that the steroid is most likely stored in the stratum corneum. The reservoir has since been found to be located in the hair follicles, sebaceous glands and horny layer of the skin, with the main site being localized in the horny layer (66). In a further experiment by Vickers (130), sites of blanching after topical corticosteroid application were demarcated. The stratum corneum over one half of some of the sites was then removed by stripping. The other sites were either completely stripped or left unstripped. Reocclusion produced blanching in the unstripped sites and in the unstripped portion of the sites from which the stratum corneum had been removed from half the site. No blanching was observed at the stripped sites. The third experiment in this series employed partially stripped, completely stripped and unstripped sites. The blanching elicited after reocclusion in the unstripped and partially stripped sites excluded the possibility that the blanching after reocclusion was due to a surface residue of corticosteroid. This series of experiments served to prove that a reservoir for topical corticosteroids does exist, can only be established in unstripped skin and is destroyed by removal of the stratum corneum.

The amount of corticosteroid stored in the reservoir has been found to depend on the amount absorbed through the skin. In one series of experiments between 82% and 99,9% of the steroids applied was recovered from the horny layer after five hours (95). The intensity of blanching in the initial stages of the experiment has, in most cases, been found to correlate with the observation of blanching after reocclusion at a later stage. This has been observed in the cases of increased corticosteroid-induced blanching due to occlusion (51,115,130), due to the vehicle (4,131-134) and due to the inherent potency of the corticosteroid or the ability of the preparation to elicit blanching (4,51,132,134-138). Similar results have been found in the case of studies utilizing radiolabeled steroids (115,139).

Although more intensive studies need to be performed to elucidate the mechanism of the reservoir effect, it has been suggested that it is due to the binding of the corticosteroid to dermal protein (95).

Another suggestion is that one of the main causes of the formation of the reservoir is the slow removal of the drug by capillaries, which is further affected by the vasoconstrictive properties of topical corticosteroids (140). A favourable oil/water partition coefficient is also thought to increase the reservoir effect (140).

1.5 MULTIPLE APPLICATION AND DOSAGE REGIMEN

Treatment schedules with topical corticosteroids have, until recently, been arbitrary because of the lack of information regarding the efficacy of differing regimens (13,141), the 2 to 4 times a day application having been accepted as the norm (142,143).

Du Vivier and Stoughton (141) and Du Vivier (144) showed, by measuring inhibition of DNA synthesis and mitosis in animals, that tolerance to topical corticosteroids can be induced by repeated application of the corticosteroid. Du Vivier *et al.* (143) further found that neither a twice daily nor an alternate day regimen is able to maintain a continuous suppression of DNA synthesis. Webster *et al.* (145,146) have studied urinary excretion rates of hydrocortisone in monkeys. Greater absorption was found after a single application of corticosteroid when compared to the same amount of corticosteroid applied in divided doses over a period of 12 hours (145). Enhanced penetration of hydrocortisone was also found after one week of daily applications of corticosteroid (146). Although comparisons of this kind should be made with caution it would appear that frequent application leads to an increase in absorption, but a partial decrease in effects.

Tolerance to the blanching effect of topical corticosteroids has also been shown by numerous workers. Several workers (141,147,148) have demonstrated the depression of the blanching response after the multiple application of corticosteroid, followed by recovery after a rest period with subsequent tachyphylaxis after a further multiple application (147-149). The blanching response during the period after the resting period was, however, found to be smaller than the original response (147), although similar area under the curve values

have been reported in some cases (149). The degree of tolerance after the resting period has been found to be smaller when employing once daily, twice daily and once every alternate day, as opposed to 3 times a day dosage regimens (149). Tachyphylaxis has also been reported to be induced more rapidly by corticosteroid preparations inducing a more intense initial blanching response (141,148), although this has not been found to be true in all cases (147,150). The onset of tolerance seems to be related to the onset of blanching of the corticosteroid as seen in the cases of Synalar cream and Betnovate cream (51,147).

The fact that patients observe that they become resistant to a topically applied corticosteroid after constant use (148) may imply the occurrence of tachyphylaxis in the clinical situation. The above experiments have made it possible to suggest possible improved dosage regimens for topical corticosteroid therapy, with obvious clinical advantages and the reduction of side effects which can arise with continuous therapy (142,147,149), in addition to reducing the cost of therapy and improving patient compliance (149). Suggestions have included daily applications for 5 day courses separated by 2 day resting periods, as opposed to continuous therapy (147) and once daily applications as opposed to twice daily (149). It is interesting to note that in some cases the blanching response has been found to be greater after daily as opposed to twice daily applications (149), while the reverse of this was noted in other cases (149,151). A clinical study (142) has shown that although once daily and 3 times daily regimens are both effective, the 3 times daily regimen is superior overall, whereas another study (41) has shown that once a day treatment appeared to be as effective as twice daily applications. A study using the *Rhus* dermatitis assay has indicated that a 3 times daily application was superior to the once daily application (152).

The lack of research into dosage regimens and the often contradictory results from existing reports allow only suggestions to be made about dosage regimens at this stage.

1.6 FORMULATION ASPECTS

The importance of the vehicle into which a topical corticosteroid is incorporated is becoming increasingly recognized. The ideal vehicle should be cosmetically acceptable, physically stable, physiologically inert and provide an environment in which the drug is stable and from which the drug is readily released (126,153). Release of the drug from the vehicle and stability of the drug in the vehicle may, however, be in conflict with each other. Allen and Gupta (154) have reported a case where the stability of hydrocortisone is unsatisfactory in the vehicle which allows the most efficient release of the drug. A general goal in topical vehicle design should be to maximize the release and penetrability of the drug without unfavourably altering other relevant vehicle properties (100,126).

The percutaneous absorption of a drug is frequently an extremely inefficient process (82). The galenical preparation should therefore facilitate, as much as possible, the migration of the drug to the target site (95) so as to allow the drug to exert its pharmacological effect (155). The way in which the vehicle is involved in the therapeutic efficacy of the topical corticosteroid is illustrated in a diagram by Katz and Poulsen (82).

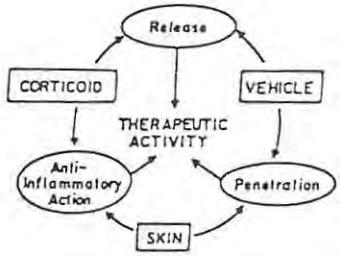


Figure 1.6.1 Interactions of release, penetration, and anti-inflammatory activity

The diagram illustrates that therapeutic activity is dependent on release, penetration and anti-inflammatory action which in turn are dependent on the corticosteroid, the skin and the vehicle. The arrival of the drug at the site of action involves the release of the

drug from the vehicle and the subsequent penetration through the skin barrier. In the event of the skin barrier being damaged (which is often the case in a diseased state) the rate-limiting step is the release of the drug from the vehicle (156).

The most common pharmaceutical vehicles for topical corticosteroids used in clinical practice are ointments, creams, lotions and gels. The extent to which the corticosteroid is released from these vehicles is closely related to the solubility of the drug in the vehicle, the particle size of the drug, the occlusivity of the vehicle and the presence of accelerants.

1.6.1 Ointments

Reports on the effect of the base on percutaneous absorption and therapeutic activity are plentiful, but often contradictory. Although numerous experiments have shown that the constitution of the vehicle plays a definite role in bioavailability and therapeutic efficacy, a clear explanation of how this happens has not yet been found (121).

Section 1.3.1.2 described the effect of hydration of the stratum corneum on the percutaneous absorption of corticosteroids after topical application. It has been found that the vehicle has a profound effect on the hydration of the skin barrier. Greases and oils have been found to be the most occlusive types of vehicles and consequently induce the greatest degree of hydration (94). The inclusion of a surfactant in these results in a water-in-oil emulsion which has lower hydrating ability than the greases and oils. Oil-in-water emulsions, formed by an increase in the water/oil ratio, cause the least hydration of the stratum corneum and have further been found to show the most variation in permeability to water vapour and the hydration of the stratum corneum (94). It should be noted that greases and oils cause hydration by preventing evaporation of moisture from the skin whereas the emulsions both wet the skin and have varying degrees of occlusivity. The onset of hydration due to the former will therefore not be as rapid.

An early experiment in which hydrocortisone was incorporated into four different ointment bases led to the conclusion that the different therapeutic effects found with varying bases containing hydrocortisone was due to the reaction of the skin to the particular base and not to a difference in the extent or rate of release of the corticosteroid from the ointment (105). An experiment using fluclorolone acetonide in petrolatum ointment, hydrophilic petrolatum and propylene glycol ointment showed no difference in the blanching activity of the corticosteroid in the first two vehicles, but a significantly superior response from the propylene glycol ointment (121).

The importance of not drawing hard and fast conclusions regarding the release of a corticosteroid from ointment bases after an *in vitro* assay is shown in a series of experiments by Ostrenga *et al.* (126). Release of fluocinonide into isopropyl myristate from five different ointments was found to be poor whereas *in vivo* blanching results were found to be good. The authors offer the possible explanations that in the former case poor release is associated with the small diffusion coefficients of the steroid in the external and internal phases of the emulsions whereas in the latter the whole vehicle may be absorbed, thereby carrying the corticosteroid into the skin, or that the phases separate after application to the skin. An *in vivo* blanching trial using betamethasone benzoate in seven different ointment bases gave similar results (157). The inclusion of propylene glycol or isopropyl myristate into the formulations was found to increase the blanching repsonse of the corticosteroid. White soft paraffin containing 5% of either propylene glycol or isopropyl myristate elicited superior blanching responses to a white soft paraffin - white beeswax (95%:5%) vehicle. The corticosteroid in a base containing 5% propylene glycol in Macrogol ointment B.P.C. produced a slightly higher blanching response than the corticosteroid in a pure Macrogol ointment B.P.C. base (although this was not statistically significant), with both of these, however, eliciting a significantly inferior response to the white soft paraffin - propylene glycol base. This again emphasizes the fact that although

one constituent in a base can significantly alter the release of a corticosteroid, the formulation of the vehicle should be considered as an entity.

Fluocinolone acetonide in FAPG has been found to exhibit a superior ability to suppress skin thickening caused by the base in guinea pigs when compared to the same concentration of the same drug in propylene glycol ointment (158). It is interesting to note in this series of experiments that the percentage suppression of skin thickening caused by a 0,3 g/100 g concentration of betamethasone 17-valerate in propylene glycol ointment was above 90% as compared to a 70% suppression caused by the same concentration in FAPG base, while the percentage suppression at 0,03 g/100 g was similar in both cases (approximately 30%). Below this concentration the corticosteroid in propylene glycol base did not inhibit skin thickening and although the results indicated the expected decrease in the inhibition of skin thickening at lower concentrations of corticosteroid in FAPG, the points on the graph appear to be somewhat scattered.

1.6.2 Creams

Differences in the clinical efficacy and blanching ability of corticosteroids in different cream bases have also shown the importance of the vehicle on corticosteroid release and activity. These experiments have revolved mainly around the solubility of the drug (especially in propylene glycol) and are discussed in the relevant sections (sections 1.6.6.9 and 1.6.7).

In a clinical study using hydrocortisone in two cream bases the improvement of disease after 3 weeks was equal for both preparations; one of the preparations however caused relief significantly sooner than the other (159). This difference in onset of action is presumed to be a factor of the base employed.

1.6.3 Gels

Gels are aqueous vehicles containing only a small proportion of

material not soluble in water and often showing physical characteristics similar to the oil-in-water emulsions (94). These vehicles maintain (after equilibrium has been reached) the lowest level of hydration of the stratum corneum. Gels, being single phase systems, do not give rise to the problem of the partitioning of the corticosteroid between the phases of the two phase emulsion systems (4). The reservoir effect has also been shown to be greater with gels than with creams (4). As is the case with ointment and cream formulations, the proportion of propylene glycol in a gel has an effect on the availability of the corticosteroid from the vehicle (160).

1.6.4 Lotions

Although numerous workers use alcoholic solutions of corticosteroids in preliminary investigations of new compounds, little has been reported on the effects that the formulation of lotions has on the bioavailability of corticosteroids. Slightly gelled alcoholic solutions of corticosteroids have been found to be cosmetically more acceptable than creams and ointments to patients requiring corticosteroid treatment of the scalp (35).

1.6.5 Comparison of Vehicles

Blanching assays using the same corticosteroid in equal concentrations have shown a statistically significant difference in the corticosteroid-induced blanching response from different vehicles, although there are exceptions to this (161). Various investigations have provided the following ranking for different vehicles in terms of intensity of corticosteroid-induced blanching (from highest to lowest): foams, ointments, gels, creams, lotions (134,162-164). The superior blanching obtained from ointment bases is more than likely due to an increase in the hydration of the stratum corneum. White soft paraffin was found to depress transepidermal water loss for up to 16 hours when left on the skin (165), the high occlusivity of white soft paraffin (165) being due to its inability to absorb water (166). The alteration of a component

within a formulation has also been found to significantly alter the blanching response of a topical corticosteroid. Fluocinolone acetonide in white soft paraffin was found to elicit an inferior response to the corticosteroid in aqueous cream (167), whereas the inclusion of 5% propylene glycol to the white soft paraffin led to a superior blanching response (168).

1.6.6 Penetration Enhancers

The penetration of corticosteroids into the skin is known to be an inefficient process and has led to the search for non-irritating substances of low toxicity to enhance penetration (169). Many compounds have been screened and the most common and/or promising ones are discussed below.

1.6.6.1 Dimethylsulfoxide (DMSO)

DMSO has been known for some time to be a good solvent and to enhance the percutaneous absorption of corticosteroids in man (170).

A blanching study using various concentrations of both fluocinolone acetonide and DMSO showed that DMSO in concentrations of 10% and 25% decreased, by a factor of 5, the minimum concentration of corticosteroid required to cause blanching in the unoccluded mode with no significant changes observed in the occluded mode (170). The effect of concentration of DMSO was further elucidated in a study using varying concentrations of DMSO and triamcinolone acetonide in alcoholic solution (171). A 10 times greater concentration (from 0,01% to 0,1%) of corticosteroid was required to elicit similar blanching responses when the concentration of DMSO was reduced from 100% or 80% to 60% or 40%. The amount of radiolabeled hydrocortisone measured in urine over a 5 day period was found to be greater by a factor of about 3,5 when applied in a vehicle containing DMSO (172,173), with the greatest increase having been observed in the first 2 days (173). *In vivo* and *in vitro* experiments using excised human skin also showed increased penetration of the corticosteroid (170). It is interesting to note that if the solvent

and corticosteroid were left in contact with the skin for a long period of time (24 hours), greater penetration was achieved with water than with DMSO as opposed to greater penetration (by a factor of about 7) from DMSO with transient exposure (25 minutes). The authors suggest that this may be due to the facilitation of a rapid initial penetration of the corticosteroid from DMSO through the stratum corneum. The fact that skin from different parts of the body (leg and breast) was used for the different studies may have had an effect on the results. The rapid penetration of fluocinolone acetonide from a solution containing DMSO was further shown by the blanching induced by the corticosteroid after having been left on the skin for only 5 minutes (139). No blanching was observed when 95% ethanol was used as the control. The presence of radiolabeled fluocinolone acetonide and hydrocortisone retained in the horny layer of the skin over a 16 day period after application in a DMSO-containing vehicle has been established (139). No radiolabeled corticosteroid was, however, observed on day 2 in the case of hydrocortisone and day 4 in the case of fluocinolone when applied in a 95% ethanol solution. The amount of corticosteroid retained in the reservoir of the stratum corneum is therefore also increased by the presence of DMSO. DMSO has been investigated in the clinical situation where triamcinolone acetonide applied to psoriatic lesions in 90% DMSO was found to be superior than when applied in a cream base (173).

Problems with the use of DMSO as an accelerant are its odour, possible toxicity and irritant effect on the skin (97,167,171,174), although some workers have reported no irritation (172) and others have claimed that it is not toxic (171).

A suggested mechanism for the increased penetration of corticosteroids through the stratum corneum in the presence of DMSO and other aprotic solvents (for example dimethylformamide and dimethylacetamide) is the substitution of the bound water in the stratum corneum for a looser structure (108). The hydrogen bonding between water and DMSO has been found to be 1,3 times greater than between water molecules (175). *In vitro* experiments using human

abdominal skin specimens have further shown that DMSO itself penetrates the skin (176). DMSO has been found not to destroy the dense intercellular cement substances which bind the horny cells into a membranous fabric and therefore not to affect the integrity of the membrane even after exposure for twenty days (171). It seems to exert its effect on skin permeability by producing structural changes, such as swelling of the stratum corneum (82) because of its hygroscopicity (171) and by possible replacement of water as the continuous membrane phase of the skin barrier (82), although another report claims that it does not alter the structure of the skin (100).

1.6.6.2 Dimethylacetamide (DMA)

Similar observations to those discussed above have been made in the case of DMA. Hydrocortisone alcohol (0,1%) produced blanching in ointment and cream vehicles containing DMA whereas 0,1% of the corticosteroid in the standard B.P.and B.P.C. bases produced no visible blanching (167). Control studies indicated that the DMA vehicles themselves produced no blanching. The observation by Feldman and Maibach (172) of urinary excretion of hydrocortisone over a 5 day period yielded the interesting result that the extent of percutaneous absorption of the corticosteroid from DMA was less than that applied in pure acetone. *In vitro* and *in vivo* results (measuring radioactivity of corticosteroid on the skin) have indicated that hydrocortisone penetrates the skin to a larger degree and is retained in the stratum corneum for a longer period when applied in DMA as compared to control vehicles (a cream base and 95% ethanol) (177). An increase in percutaneous absorption of three corticosteroids (assessed by the degree of blanching) was also established after examination of four semi-solid dosage forms (178). The absorption of all three corticosteroids was greatest from a base containing DMA and the increase in absorption was found to be greater for hydrocortisone than fluocinolone acetonide and triamcinolone acetonide (controls were performed as above). It should be remembered that the differences obtained in the case of formulated products may be due to the alteration of constituents other than the one being studied.

1.6.6.3 Tetrahydrofurfuryl Alcohol (THFA)

THFA has been shown to increase the percutaneous absorption of various topically applied corticosteroids. In a vehicle containing THFA and hydrocortisone, blanching was demonstrated with as little as 0,05% of the corticosteroid, whereas in the DMA formulations used, 0,1% of hydrocortisone was required to produce blanching (167). Enhancement of blanching activity has also been observed with triamcinolone acetonide and fluocinolone acetonide in the presence of THFA (178). It should again be noted that other constituents of the vehicles were different.

THFA, however, has the disadvantage of causing skin irritation and is in fact used as such in bioassays to assess topical corticosteroid activity (179-181).

1.6.6.4 Dimethylformamide (DMF)

In an *in vivo* study using radiolabeled testosterone and hydrocortisone the urinary excretion over a 5 day period was found to double when the steroid was applied in DMF (172), while an *in vitro* study of radiolabeled hydrocortisone showed an approximately 6-fold increase in penetration from DMF compared to 95% ethanol (177). An assay comparing the blanching produced by betamethasone 17-benzoate when dissolved in DMF and dimethylisosorbide (a substance known to be poorly absorbed) indicated no signifcant differences in the blanching response (133).

1.6.6.5 Urea

In vitro studies of the absorption of radiolabeled hydrocortisone through excised human and guinea pig skin after the addition of 10% urea to the vehicle showed a minimal increase in percutaneous absorption through human skin and a decrease in absorption through guinea pig skin in the case of a Tween 20/water vehicle and a similar decrease in the penetration through guinea pig skin from a cream base

(182). Contrary to this, urinary excretion of radiolabeled hydrocortisone acetate was found to be significantly greater by a factor of approximately 2 and was detectable for twice as long after topical application of the corticosteroid in a cream base containing 10% urea when compared to a cream base without urea (183). A urea-induced increase in corticosteroid penetration has further been reported in an *in vitro* study using excised hairless mouse skin (184).

In a clinical trial using patients with atopic dermatitis 1% hydrocortisone cream containing 10% and 20% urea was found to be signficantly superior in alleviating symptoms when compared to the cream containing no urea (185). The authors noted that no statistically significant clinical differences were observed in a comparison of a hydrocortisone cream containing 10% urea with a cream containing 20%, but that the incidence of stinging after application was smaller with the former. Other clinical trials have verified the improved efficacy of hydrocortisone when applied in combination with urea (186-188). It should be noted, when studying the effects of preparations containing urea in combination with a corticosteroid, that urea itself has been found to alleviate the symptoms of skin diseases like ichthyosis and other hyperkeratotic disorders (189-191).

In vivo blanching assays using a triamcinolone acetonide cream containing 30% urea indicated a significant increase in bioavailability of the corticosteroid, with the placebo base (containing urea but no corticosteroid) showing minimal blanching (192). A similar study using betamethasone 17-valerate in a cream containing 10% and 15% urea, however, showed a significant decrease in the blanching activity of the corticosteroid in the urea-containing creams as opposed to the steroid-containing cream with no urea (193). Barry and Woodford (135) found no increase in the blanching activity of hydrocortisone in a urea-containing preparation in a 6 hour single application experiment, while superior blanching activity from the urea-containing base was shown in the case of a 5 day multiple application (147). This apparent disparity in the

above results is most likely related to the period of skin/vehicle contact (118). The mechanism by which urea increases absorption of some corticosteroids is unknown, although it is probably a combination of its keratolytic activity and hydration of the stratum corneum due to its ability to increase the water binding capacity of the stratum corneum (118,189). The keratolytic and hydration effects of urea obviously require a contact time of more than 6 hours which may explain the lack of increased blanching in two of these experiments (135,193) and the observed increase in the 16 hour (192) and 5 day (147) applications. This, however, does not explain the decrease in blanching in the case of betamethasone 17-valerate.

1.6.6.6 Salicylic Acid

A marked increase in the penetration of triamcinolone acetonide through excised human skin has been noted after the addition of 10% salicylic acid to the formulation (194). Peak penetration was reached at the same time with and without salicylic acid and although the authors were not able to offer an explanation for the enhanced penetration of the corticosteroid, damage of the membrane by salicylic acid was excluded. *In vivo* blanching studies using triamcinolone acetonide and 5% salicylic acid, however, showed a marked decrease in the blanching activity of the corticosteroid in conjunction with salicylic acid (192), whereas 3% salicylic acid with flumethasone pivalate showed a significant increase in blanching (195).

Using skin thickness in guinea pigs as an indicator of anti-inflammatory action, Sarkany and Gaylarde (196) found that the anti-inflammatory action of hydrocortisone alcohol was completely suppressed by the addition of salicylic acid and acetylsalicylic acid. Similar results have been observed in urinary excretion studies of radiolabeled hydrocortisone in monkeys where a statistically non-significant decrease in percutaneous penetration was reported after the addition of 1% and 10% salicylic acid (197).

1.6.6.7 Azone (AZ)

Azone (1-dodecylazacycloheptan-2-one or AZ) is a new chemical agent that has been found to enhance the percutaneous absorption of topically applied corticosteroids (198). *In vitro* penetration of triamcinolone acetonide was enhanced by the addition of AZ to the vehicle, the largest increase having occured with 10% of AZ. Blanching elicited by triamcinolone acetonide, desonide and amcinonide was enhanced with the addition of AZ, the increase in blanching activity apparently not having been effected by the concentration of AZ (2% - 100%).

AZ has the virtues of being colourless, relatively odourless and producing minimal irritation to human skin.

1.6.6.8 N,N-Diethyl-*m*-toluamide

Another substance that has been studied, but about which little has been reported with respect to its penetration enhancing ability, is N,N-diethyl-*m*-toluamide. It has been found to facilitate the penetration of several corticosteroids through hairless mouse skin (169,184) and to increase the blanching induced by creams and ointments containing hydrocortisone (169). It has the advantages of being effective in low concentrations (5%) and of having stood the test of time with respect to safety.

1.6.6.9 Propylene Glycol

Water loss from the skin has been found to increase with as little as 10% propylene glycol when applied to the skin at low relative humidity (199). In a report by Baker (165) the addition of 5% propylene glycol to soft white paraffin decreased the occlusivity of the vehicle and 5% propylene glycol in water was found to produce minor but constant depression of the water barrier of the skin. *In vitro* penetration of hydrocortisone butyrate was found to increase by 25% from a cream containing 40% propylene glycol in dry atmospheric conditions when compared with moist conditions (194). In creams

without propylene glycol more corticosteroid penetrated under moist than under dry conditions. A similar experiment using Plastibase/propylene glycol, however, showed no effect due to the differing relative humidity (200).

Blanching assays using 0,025% and 0,1% of fluocinolone acetonide (168) and betamethasone benzoate (157) showed enhanced blanching with the addition of 5% propylene glycol to white soft paraffin base, the corticosteroid being dissolved in the propylene glycol. Increased blanching in a formulation containing propylene glycol has further been seen in the case of fluclorolone acetonide in FAPG base (153).

Percutaneous penetration of radiolabeled hydrocortisone when measured by urinary excretion was found to decrease when applied in 25% propylene glycol in acetone when compared to the control in pure acetone (172). The release of fluocinolone acetonide, flucocinonide and betamethasone 17-valerate from propylene glycol-water gels into isopropyl myristate indicates that release is greatest when the amount of propylene glycol is equal to that required to dissolve the corticosteroid (96,125,201), findings which correlate well with *in vivo* blanching studies (96,125). The solubility of betamethasone 17-valerate and fluclorolone acetonide in a propylene glycol-water mixture was found to increase with an increase in propylene glycol concentration, with a corresponding decrease in the diffusion coefficient of the corticosteroid between this mixture and isopropyl myristate (125,202).

An excess and an insufficiency of propylene glycol both lead to a decrease in the amount of corticosteroid released into the receptor phase, the former being due to increased affinity of the vehicle for the corticosteroid and the latter being due to dissolution of the corticosteroid which becomes the rate-limiting step (125,201,202). It is important to notice that different corticosteroid esters have different solubilities in propylene glycol thus making it impossible to determine a general optimum concentration of propylene glycol in all vehicles for all topical corticosteroids, larger quantities of propylene glycol being required for the less soluble compounds

(96,201). The diffusion of a drug from the vehicle into the skin is dependent on the thermodynamic activity of the drug in the vehicle and is greatest when the concentration of the drug equals the saturation solubility in that vehicle (174). An *in vitro* study of hydrocortisone butyrate indicated an enhancement of percutaneous penetration of the corticosteroid from a formulated product containing propylene glycol (194,200). Increasing the concentration of propylene glycol in Plastibase from 6% to 18% however did not diminish the penetration, 9% having been required to dissolve the corticosteroid. The authors explain this by assuming that the propylene glycol and hydrocortisone butyrate penetrate the skin together. The effect of propylene glycol concentration on fluocinonide has further been established in a series of experiments by Haleblian *et al.* (160) where an *in vivo* and two *in vitro* methods were used in the assessment. Although a correlation was found between the penetration or release and propylene glycol concentration, it is interesting to note that the peaks of the curves did not coincide, maxima ranging from about 70% to 80%.

1.6.7 Solubility and Particle Size

It has been found that the maximum diffusion of a drug out of a vehicle or across a barrier is obtained by the inclusion into the vehicle of the minimum amount of solvent required to completely dissolve the drug without adversely affecting the partition coefficient (124,126,155,section 1.6.6.9). Good correlation has been found between the percentages of fluocinonide and dexamethasone alcohol dissolved in cream and ointment vehicles and the release of the drug in both *in vitro* and *in vivo* studies (82,126,203). Mathematical equations describing the effect of drug solubility in the vehicle have been elucidated by Higuchi (204) and Ostrenga *et al.* (96).

The effect of particle size on the activity of topical corticosteroids has been investigated. The blanching response and clinical efficacy of a dispersion of micronized fluocinolone acetonide has been found to be superior to a dispersion of the coarse particle, but inferior to the response of dissolved drug (168,205).

1.6.8 Dilution

The preceding sections illustrate the importance of careful, scientific considerations during the design of a vehicle for topical corticosteroids. The undesirable practice of dilution is, however, still requested by prescribers (9,206) and was fairly recently suggested in a journal (207), even though the very large range of proprietary topical corticosteroids available today makes dilution unnecessary (9,28). Dilution may also lure the prescriber into a false sense of safety if he is under the impression that the potency of the diluted preparation is reduced by the same magnitude as the dilution factor (68), a fact that has recently been disproved by several workers (77,208-212). The degree of blanching elicited by a 1 in 10 dilution of Dermovate ointment was found to be similar to that elicited by undiluted Betnovate ointment, a potent topical corticosteroid (208). Similar findings have been reported for numerous corticosteroid preparations where dilutions ranging from 4 - fold to 32-fold have shown similar blanching responses (77,211,212).

Another potential problem associated with extemporaneous dilution is microbial contamination. The risk of contamination, especially with *Ps aeruginosa* in hospital dispensaries, is real (213) and the antibacterial properties of preservatives may be inhibited by incompatibilities with the diluent or by dilution to below the minimum inhibitory concentration (214). *Pseudomonas* has also been found to use glucocorticoid esters as a metabolic substrate (215). Microbiological studies into the usefulness of Unguentum Merck as a diluent for Betnovate and Dermovate creams have, however, shown that no viable micro-organisms were detected in an extemporaneously diluted preparation for 3 months after preparation (216).

The blanching activities of these and other steroid-containing creams diluted with Unguentum Merck have further been found to be equipotent to those diluted with cetomacrogol cream, thus suggesting the suitability of Unguentum Merck as an alternative to cetomacrogol cream as a diluent for the test corticosteroid creams (77,216). High performance liquid chromatographic (HPLC) investigations of

several proprietary topical corticosteroid creams indicated chemical stability of the corticosteroid for 32 weeks after dilution with Unguentum Merck. Unguentum Merck has also been found by Ryatt *et al.* (211) not to cause degradation of betamethasone 17-valerate.

Reports of the stability of the corticosteroid after dilution of proprietary preparations are, however, contradictory. Magnus *et al.* (210) found the blanching activity and concentration of betamethasone 17-valerate to be constant for up to 14 months after dilution of Betnovate cream with various diluents (one exception was noted). Ray-Johnson (217) found the concentrations of various corticosteroids to remain constant for up to 32 weeks after dilution with Unguentum Merck, but found a 60% reduction in the concentration of betamethasone 17-valerate (due to conversion to the 21 isomer) 2 weeks after dilution of Betnovate cream with emulsifying ointment. Remarkably rapid degradation of betamethasone 17-valerate after dilution of Betnovate ointment with emulsifying ointment and other diluents has been reported by several workers (212,218,219). When a betamethasone 17-valerate containing ointment was diluted with emulsifying ointment and stored at room temperature, the half-life of the corticosteroid was found to be less than 1 hour for a 50% dilution (218) and approximately 4 hours for a 1 in 4 dilution (212,219). The rate of corticosteroid decomposition was further found to increase with an increase in the dilution factor (218,219).

1.7 MECHANISM OF BLANCHING

The mechanism of corticosteroid-induced blanching has not yet been fully elucidated, although available literature seems to favour vasoconstrictor activity of the corticosteroid (220).

Several corticosteroids tested have been found to increase the sensitivity of ocular blood vessels to topical norepinephrine (221). In an experiment using fluocinolone acetonide, Juhlin (222) found no potentiation of epinephrine or norepinephrine by the corticosteroid and concluded that blanching is probably due to a vasoconstrictor effect of the corticosteroid *per se*. Mahajani *et al.* (223) have, on

the other hand, found that certain corticosteroid-containing preparations significantly increased the vasoconstrictor response of epinephrine. Tests using topically applied triamcinolone acetonide and systemically administered guanethidine (a norepinephrine blocking agent) showed that no blanching occurred in most of the guanethidine treated patients, thus indicating that norepinephrine is probably implicated in the blanching phenomenon (224).

Clearance of Xenon 133 has indicated a statistically significant decrease in peripheral blood flow after topical treatment with corticosteroids (225), and plethysmographic recordings of skin pulses have indicated that corticosteroid-induced blanching in normal skin is most likely due to decongestion of the capillaries as opposed to vasoconstriction (226), an idea previously suggested by Baker and Sattar (227).

The influence of prostaglandins (which are known to prevent vasodilation during the inflammatory reaction and which are implicated in the anti-inflammatory activity of topical corticosteroids (section 1.1.1)) on blanching has been suggested (24), but does not appear to have been studied.

Marks *et al.* (228) have studied the effect of various glucocorticoid antagonists on the corticosteroid-induced blanching response and have found that blanching was reduced by the glucocorticoid antagonists, but not by steroids that are inactive in glucocorticoid systems; glucocorticoid antagonists were further found not to affect epinephrine-induced vasoconstriction. The results of these studies led the authors to conclude that the vasoconstrictor effects of topical corticosteroids are mediated by occupancy of classical glucocorticoid receptors, rather than by non-specific pharmacological mechanisms.

1.8 THE BLANCHING ASSAY

The blanching effect that topical corticosteroids have on the skin was first used experimentally by McKenzie and Stoughton (111) to

study the influence of occlusion of the site of application of a corticosteroid. The comparatively crude assay described by them in 1962 is the basis of the sophisticated assay used by many workers today in bioavailability studies and has been described as the method of choice for the primary evaluation of topically applied corticosteroids (229). Although various aspects of the blanching assay have been investigated, discussed and altered by numerous workers, there seems to be a distinct lack of uniformity and concensus as to the best assay procedure.

1.8.1 Grading the Intensity of Pallor

In their original study, McKenzie and Stoughton (111) made no attempt to grade the blanching response which was recorded as present or absent. Sarkany *et al.* (167) and Barrett *et al.* (168) appear to be the first group of workers that attempted to grade the blanching response using a 0 - 3 scale, a step that drew criticism from McKenzie (230). Various other workers have suggested that anything more than a 0 - 1 scoring scale provides no advantages (86,231,232). More recently investigators have used 0 - 4 ratings, some using half-point ratings within these limits (4,51,77,133,134,136-138,147,149,157,209,233-237) and some using both the area and degree of pallor to determine the rating (178,238). Engel *et al.* (238) found good correlation between results using degree of blanching and spot diameter as a means of assessing blanching ability. A comparison of the 0 - 1 and 0 - 3 scoring systems has shown that the more refined system showed a distinction between corticosteroids that was not apparent with the ungraded 0 - 1 scoring system (5). The ungraded recording of responses may therefore be useful in experiments on corticosteroids eliciting only a weak blanching response, but is of dubious value in the case of more potent topical corticosteroids (5,231). Whether to grade the blanching response or not should therefore depend on the expected intensity of the response and the aim of the experiment; experiments aimed at ascertaining the minimum concentration of corticosteroid required to induce blanching will not benefit from the complicated grading procedure.

The subjectivity of the visual assessment of pallor has been recognized (195,239,240) and several attempts have been made to develop a more accurate means of assessment. Reid and Brookes (178) described a reflectance spectrophotometric technique of measuring differences between the test area and the area immediately adjacent to it. Zaun and Altmeyer (239) have described a system whereby corticosteroid-induced blanching is assessed reflexphotometrically by measuring the difference between sites treated with corticosteroid and control sites treated only with vehicle. The placebo effect of the vehicle and experimental conditions are thus taken into consideration. The instrumental method of assessing pallor has been used to investigate the influence of solvents on the blanching ability of corticosteroids (241), the blanching ability of desoxycorticosterone (a mineralocorticoid) (120), the duration and peak of activity (120,242), the influence of corticosteroid concentration (120), tachyphylaxis (150,240), the influence of the vehicle on blanching (192), the influence of dilution of proprietary preparations on corticosteroid-induced blanching (211,212) and the corticosteroid-induced suppression of UV irradiation (243).

Dawson *et al.* (244) developed a reflectance spectrophotometric method of assessing skin colour and found good correlation between the visual and instrumental measurement of corticosteroid-induced blanching, as did Feather *et al.* (245) on comparing their results to those obtained visually by Barry and Woodford (51). Kiraly and Soos (246) attempted to ascertain whether there was a difference between the results of a trial obtained by visual and instrumental means of assessing blanching. The results obtained by measuring the differences in reflection of the site using a tristimulus colourimeter before and after application of the corticosteroid, indicated that the instrumental method offered no advantage over the visual determination of blanching.

1.8.2 Time of Observation and Number of Observations

Two other aspects of the blanching assay that vary greatly between workers are the time interval between removal of the residual

corticosteroid and observation of the blanching areas and the number of observations made over an extended period of time. McKenzie and Stoughton (111) in their original paper made no mention of how long after removal of the occlusive dressing and residual corticosteroid they made their observations, although they do mention that the keratin takes approximately 30 minutes to return to normal after removal of the Saran wrap. In a later experiment McKenzie and Atkinson (86) specified that readings were taken 1 hour after removal of the occlusive dressing by which time the white macerated epidermis had dried, whereas Barry and Brace (235) have claimed that a 10 minute interval between removal of the occlusive dressing and assessment of pallor is sufficient not to affect the assessment. This single observation of the blanching response has been used by many workers (83,85,86,162,168,178,208,211,212,246-250), one of whom (248) made his observation 1 to 2 minutes after removal of the occlusive wrap and residual corticosteroid and others not stating how long after removal their observations were made (91,167,178, 211,238,251,252), although it would appear as though the observations were made immediately after removal of the corticosteroid. The time between removal of the dressing and residual corticosteroid and observation has varied from 1 - 2 minutes (248), 10 minutes (4,51,133), 15 minutes (75,249), 30 minutes (168,246), 1 hour (5,10,86,195,208,210,250,253), 1½ hours (254), 2 hours (85,116,121,126,157,232,255-257), 3 - 4 hours (162,220) to 5 hours (212). Weirich and Lutz (257) have commented that the single observation of blanching sites too soon after removal of the occlusive dressing may be a possible source of error in the blanching assay.

There appears to be no indication of the utilization of multiple reading times before 1970 when Burdick *et al.* (258) observed the degree of blanching at 8, 24 and 32 hours and Christie and Moore-Robinson (121,256) made their observations at 8 and 24 hours after application of the corticosteroid preparation. The significance of the multiple reading times becomes evident when noting that hydrocortisone showed significantly greater blanching activity than the placebo at the 8 hour, but not at the 24 and 32 hour readings,

whereas fluocinolone acetonide showed enhancement of blanching activity over the 32 hour duration of the trial (258). Similar differences in comparative blanching responses have been noted by other workers at different reading times (4,51,77,116, 121,132,135,157,163,220,231,253,257,259). Multiple readings would, in the same way, be significant when attempting to assess the relative rate of onset of action of the blanching response as well as the time at which maximum blanching is attained. These differences in the intensity of blanching at different times have not been explained but could be due to a difference in the reservoir of corticosteroid in the stratum corneum (121), to the duration of action of the corticosteroid as prescribed by its half-life (20) or to the onset of action due to the inherent properties of the drug or its release from the vehicle. Reading times have been extended to 48 hours (116,259) and 96 hours (4,133) after application, with the inclusion of additional readings between the first and last observations (4,51,77,134,136-138,210,231,234,235,237,253,254,257,260).

The extension of the reading time and the increased number of readings results in a response-time profile and allows the determination of the area under the curve value, as used in pharmacokinetic measurements in bioavailability studies for other pharmaceutical dosage forms (51,255,259). A further factor in favour of extended and multiple reading times is the ability of the vehicle itself to cause blanching. The blanching elicited by the unmedicated vehicle has been found to diminish more rapidly than that of the corticosteroid itself (253,261), a factor that would be relevant when assessing corticosteroid-containing semi-solid formulations by means of the blanching assay. Burdick (232) has claimed that multiple reading times are essential to obtain a correct interpretation of this shortened assay, particularly if two different corticosteroids are being compared. This statement, made specifically for the comparison of two different corticosteroids, could be extended so as to encompass all applications of the human blanching assay.

1.8.3 Occluded versus Unoccluded

The effects of occlusion have been discussed in section 1.3.1.2. This section will deal with aspects of the effect of occlusion on the blanching assay.

The original blanching assay performed by McKenzie and Stoughton (111) and McKenzie (91) were performed in both the occluded and the unoccluded modes of application. This aspect of the assay has also varied greatly and deserves further comment. The use of the occlusive dressing in the blanching assay has been discussed by several workers who have noted the following potential problems.

a) Formulations have been found to spread unevenly under the wrap (162).
b) The hydration induced by the occlusion tended to obscure any difference which the formulation might have made to penetration without the use of occlusion (51,133,162,253). Occlusion has been suggested to favour poorly designed vehicles by masking the more subtle effects of base and corticosteroid interaction (232). It also does not reflect the usual clinical use of these preparations (116,232,253,255). Use of occlusion may further provoke a maximal response of the corticosteroid preparation, thus not allowing discrimination between formulations (231).
c) The variability in sweating between subjects has led to vast variations in responses when occlusion was used with formulated products (162).
d) The migration of corticosteroid, between the skin and the demarcating tape, from its site of application to an adjacent site has been found to occur when too many sites in parallel are used without division of the tape (121). The use of individual pieces of tape with fewer sites punched out of them has overcome this problem.

It has been found that occlusion not only enhances the blanching response, but also changes the profile of the blanching curve and the relative areas under the curve (51,116,255). Significant

differences of the same product have in some cases been found to occur in either the occluded or the unoccluded mode of application, but not in both (231). Significant differences would of course be expected in both modes in products with great differences in blanching activities (231). It has been noted that the occlusion of ointments produces less of a difference than the occlusion of creams when compared to their relative nonoccluded blanching responses (132). Further occlusion of the already occlusive ointment base thus apparently has less of an effect on the blanching ability than in the case of creams (132). Occlusion has further been found to mask the inherent differences between ointment and cream bases where the significant differences between a cream and an ointment containing the same corticosteroid in the unoccluded mode was not observed in the occluded mode (163).

1.8.4 Period of Steroid/Skin Contact

The time that the corticosteroid is in contact with the skin has also varied greatly, with no real concensus of opinion as to what this should be (5).

The early blanching assays utilized a 16 hour contact time (91,111,167,168). McKenzie and Atkinson (86) left the corticosteroid on the skin for 15 hours but made no comment as to why the time of contact was altered. Other periods of application have been 2 hours (5,220), 3 hours (5,260), 3½ hours (169), 4 hours (220), 6 hours (4,51,77,116,121,126,132-138,157,163,210,232,234,235,237,246,253,255, 256,260,262), 6½ hours (254), 8 hours (75),11 hours (195), 15 hours (85), 16 hours (167,192,227,247,257), 18 hours (5,116,252), 16 - 18 hours (83), 20 hours (162,220), 21 hours (178), 21 - 24 hours (263) and 24 hours (249). Coldman and Lockerbie (261) reduced the application time used by Coldman *et al.* (259) from 8 hours to 5 hours so as to allow an additional reading to be made at 6½ hours. Others have reduced the application time still more for the same reason (5). It has been suggested that the dosage of corticosteroid applied should be sufficiently large to allow the reduction in application time (5). Ishihara (220) found no significant differences in the

comparison of the blanching activities of hydrocortisone 17-butyrate and triamcinolone acetonide after 2, 4 and 20 hours of contact with occlusion. Szadurski *et al.* (10) found that very few sites showed blanching after occluded application for 1 or 2 hours as opposed to contact of between 3 and 7 hours, while Magnus *et al.* (114) found an increase in the degree of blanching from 2 to 8 hours with no increase thereafter up to 12 hours of contact under occlusion.

The 6 hour application time has the advantages of showing the rate of onset of action and rate of release of the corticosteroids being studied (116), approximating the contact time between corticosteroid and skin in the 3 times daily application in the clinical situation (255), allowing the construction of a blanching profile (255), causing discomfort to the volunteers for a shorter period of time (256) and providing a severe and discriminatory test for assessing the effect of penetration enhancers (133) and vehicles.

1.8.5 Vehicle

The vehicle into which the corticosteroid is incorporated is also an important factor in the blanching assay. The original studies by McKenzie and Stoughton (111) and McKenzie (91) utilized alcoholic solutions of the corticosteroid to ascertain the effect of occlusion on the percutaneous absorption of corticosteroids. McKenzie and Atkinson (86) utilized the assay as a screening procedure for a large number of corticosteroid compounds. Sarkany *et al.* (167) then used the assay to assess the role of vehicles in the percutaneous absorption of corticosteroids.

The alcoholic blanching assay has since been used by many workers as a screening procedure to ascertain the blanching ability of corticosteroids and to determine ED_{50} values of various corticosteroids (83,85,153,220,227,235,252,256,257,262). The assay has been used with formulated products to determine the effect of formulation on the bioavailabilities of the corticosteroids (4,77,121,126, 134-136,153,157,163,168,193,210,220,253,254,258,264), to compare the blanching abilities of products containing different

corticosteroids (4,51,132,137,138,153,220,232,254) and to compare the blanching effect and clinical efficacy of the corticosteroid in the formulated products (see section 1.9.6). Increasing the accuracy of extrapolation of the clinical effect from the blanching assay in most cases requires the use of formulated products as opposed to alcoholic solutions of the corticosteroid under test (220).

1.8.6 Site of Application

The most commonly used site of application of the corticosteroid has, since the original experiment by McKenzie and Stoughton (111), been the flexor aspect of the forearm (5,83,114,162,230,232,237,256). Some observations have been made with respect to this site.

a) The left arm has been reported to be more sensitive than the right (86) although it should be noted that the corticosteroids were applied to the left and right arms by different workers and no mention is made as to whether the volunteers were left or right handed. Kirsch *et al.* (265) found no differences between right and left arms while Pepler *et al.* (157) found differences, but did not state what the differences were. The differences were further only found during the early part of the trial.
b) A greater response has been reported on the upper forearm than at the wrist (230,256) while some workers (232) have reported poor blanching within about 4 cm of both the wrist and the elbow, or inconsistent blanching in this area (237).
c) Burdick (232) has further reported difficulty in reading sites located on the lateral and medial surfaces of the arm and has suggested avoiding volunteers with very narrow forearms, although no differences in blanching response have been found between the medial and lateral sides of the forearm (265). Barry and Woodford (237) have reported that blanching was inconsistent for people with narrow forearms.
d) Inconsistent blanching has also been reported for people using their forearm muscles consistently throughout the duration of corticosteroid application, e.g. typists (237).

e) Barry and Woodford (237) have reported difficulty in assessing the blanching in volunteers whose blood vessels became enlarged when their arms hang downwards.

The back has been suggested as an alternative site for blanching assays (120,169,192,239,241-243,263,266-269). Sutton *et al.* (266) have found that the arm is more sensitive and reactive to corticosteroids, but the blanching response is more difficult to interpret on the arm. The arm is, however, undoubtedly more convenient when performing the assay on healthy, ambulatory volunteers.

1.9 OTHER BIOASSAYS USED TO ASSESS TOPICAL CORTICOSTEROID ACTIVITY

Although the human blanching assay has become the most popular assay among many workers studying the activity of topical corticosteroids, several other bioassays are available to assess corticosteroid activity. These assays tend to be more tedious and more painful and although some of them are more closely related to the clinical uses of topical steroids than the blanching assay they have, in the main, not gained popularity over the years.

1.9.1 Suppression of Experimentally Induced Inflammation

Since topical corticosteroids are largely used for the treatment of inflammatory skin disorders, these bioassays mimic the clinical use of these agents more closely than the other bioassays (118).

1.9.1.1 Croton Oil

The anti-inflammatory activity of a test compound can be ascertained by measuring the extent to which the corticosteroid inhibits inflammation elicited by croton oil. The croton oil is applied to the skin and the corticosteroid is injected intradermally (270) or applied topically before (267), at the same time as (270-274) or after (21,116,275) the application of the croton oil. The

experiment can be conducted on humans (21,116,267,270) and on animals (271,272,274,275). Glen *et al.* (273) have modified the assay by the application of a mixture of croton oil and sulphuric acid, thereby causing more severe inflammation than croton oil alone and allowing tests to be performed on more intense inflammation.

1.9.1.2 Tetrahydrofurfuryl Alcohol (THFA)

In this assay the THFA acts both as the irritant and the solvent for the corticosteroid (179-181). The activity of the corticosteroid is indicated by its ability to inhibit the contact dermatitis induced by THFA. Although the corticosteroid being tested has to penetrate intact skin to exert its anti-inflammatory action, the test cannot be used as such for formulated products (180).

1.9.1.3 Kerosene

Corticosteroid formulations are applied to the skin for 6 hours after which time kerosone is applied and left in place for 18 - 20 hours (267,268). Anti-inflammatory activity is assessed by grading the kerosene-induced reaction at the various sites.

1.9.1.4 Histamine

This assay is based on the ability of the corticosteroid to suppress the reaction of histamine (276). The corticosteroid is applied in solution before treatment with histamine, although the assay could conceivably be used with formulated products.

1.9.1.5 *Rhus* Dermatitis

A modification of the experimentally induced inflammation assay is the suppression of experimentally induced dermatitis. *Rhus* oleoresin is applied to the skin for 72 hours after which the corticosteroid preparation is applied over a period of 4 days (152). The authors warn that only highly sensitized subjects are usable and that a severe allergic reaction is required for assessment of the suppresive effects of the drug.

1.9.1.6 Mustard Oil and Nitric Acid

The ability of the corticosteroid to inhibit the inflammation induced by either mustard oil or nitric acid is measured and used as an indication of the efficacy of the corticosteroid (277).

1.9.1.7 Ultraviolet Irradiation

The ability of the corticosteroid to suppress UV-induced inflammation is ascertained by exposing the test site to UV radiation before the application of the corticosteroid and measuring the degree of erythema inhibition (116,243,255). Kanof (278), who first used the assay procedure in 1955 in the assessment of hydrocortisone, reported a diminution in erythema only if the corticosteroid was applied within 24 hours before exposure to UV rays. Scott and Kalz (277) found maximum inhibition if the corticosteroid was applied 6 - 8 hours before exposure, with a rapid decrease in inhibition up to 16 hours and no affect after that. Unfavourable responses were obtained if the interval between corticosteroid application and UV exposure was too short or if the corticosteroid was applied after UV exposure. Similar results with respect to the time interval between irradiation and corticosteroid application have been found by Altmeyer and Krumrey (243). Kalz and Scott (279) have described a similar assay utilizing Grenz rays as opposed to UV rays.

UV irradiation studies have also been performed in *in vitro* (280) and in *in vivo* animal studies (281). The *in vitro* study (280) involved the assessment of the effect of hydrocortisone in irradiated foetal rat skin grown in organ culture. The corticosteroid was found to reduce and retard the cellular breakdown of irradiated skin. The animal studies (281), which were performed on rats, involved the application of an ethanolic solution of the corticosteroid immediately after irradiation or the subcutaneous injection of the corticosteroid 1 hour prior to irradiation. Both the topically applied and systemically administered corticosteroids were found to demonstrate a dose-related inhibition of erythema. The erythema inhibition was, however, found to be more effective after topical administration.

1.9.1.8 Stripping

Wells (282) described a method of assessing the activity of hydrocortisone by applying the corticosteroid to areas of skin that had been traumatized by stripping with cellophane tape. This method has since been used to assess the effect of vehicles (283), to compare different concentrations of triamcinolone acetonide to hydrocortisone (284) and to screen new corticosteroid compounds (247).

1.9.2 Thymus Involution

The percutaneous absorption and anti-inflammatory activities of topically applied corticosteroids are ascertained by measuring the inhibition of thymus activity in young rats after application of the corticosteroid and irritant (271,274,275,285,286). Lerner *et al.* (287) have described a method where thymus involution is measured after subcutaneous implantation of cotton pellets followed by parenteral administration of the test steroid. It should be noted that the correlation of potencies between rat and man has been found to become less reliable with an increase in corticosteroid potency (272).

1.9.3 Antigranuloma

This assay involves the subcutaneous insertion of cotton pellets that have been impregnated with the test steroid into the experimental animals (272,288). Control pellets (containing no steroid) are similarly inserted. The potency and activity of the corticosteroids are indicated by their ability to inhibit the formation of scar tissue. It is important to note that although this assay gives an indication of the anti-inflammatory activity of a local corticosteroid, it provides no information with respect to the ability of the steroid to penetrate the skin. This is a similar disadvantage to that of the assay described by Lerner *et al.* (287) who administered the steroid parenterally after implantation of the cotton pellets. DiPasquale *et al.* (285) described a modified

granuloma pouch procedure in which percutaneous absorption and vehicle effects are considered by applying the corticosteroid topically. The granuloma pouch technique as first described by Seyle (289) required an injection of an irritant (croton oil) into the air pouch and the corticosteroid into the wall of the pouch.

1.9.4 Rat Ear Assay

Use is made in this assay of the fact that rat ears increase in size after the application of an irritant. The irritant (croton oil) and corticosteroid are applied to one of the ears of the rat, the other being left as a control (271,275). Banfi *et al.* (274) in addition applied only the irritant to the ears of a control group. Boris and Hurley (286) have described a similar method where one ear of each of the experimental group of rats is treated with the irritant (cantharidin) and corticosteroid and one ear of each of the control group is treated only with the irritant. The corticosteroid is applied simultaneously with (271,274,286) or shortly after the irritant (275). Corticosteroid activity is ascertained by noting the suppression of increase in the weight of the test ear. This assay can be used for corticosteroid-containing solutions (271,274,275,286) and for formulated products (275).

1.9.5 Cytological Studies

These assays are based on the pharmacological activities of the corticosteroids with respect to either their mechanism of action or side effects at a cellular level.

1.9.5.1 Fibroblast Inhibition

This *in vitro* assay gives an indication of the anti-inflammatory activity of corticosteroids by observation of the corticosteroid-induced inhibition of fibroblasts (290-293), which are known to be involved in the inflammatory process.

1.9.5.2 Epidermal Thinning

Spearman and Jarrett (294) have described an assay whereby the activity of corticosteroids is assessed by measuring the extent to which the corticosteroid produces thinning of the epidermis in rat tails. Barnes *et al.* (158) have described a modification of this assay where the corticosteroid activity is measured by its ability to suppress the epidermal thickening caused by the vehicle.

1.9.5.3 Mitotic Rate

The antimitotic effect of corticosteroids has been well established and well documented (29-31). Fisher and Maibach (29) suggested that this reduction in mitotic rate be used as a bioassay for corticosteroids. The mitotic index of the epidermis after oral administration or topical application of the corticosteroid is ascertained and used as an indication of corticosteroid potency. Marks *et al.* (295) have described a similar assay using hairless mouse skin instead of human skin.

1.9.6 Clinical Trials

The ultimate test for efficacy of a topical corticosteroid formulation is the clinical trial. The remainder of this discussion will deal with cases where investigators have reported the results of clinical trials and blanching assays, thereby illustrating the good correlation between these two bioassays and confirming the appropriateness of the blanching assay (247).

Bagatell and Augustine (247) compared the blanching abilities of five corticosteroids in alcoholic solution to the clinical efficacy in psoriasis and various other corticosteroid-responsive dermatoses, to the same corticosteroids formulated in an identical cream base. The rank order of these corticosteroids was the same in blanching and clinical trials with one exception which the authors feel was due to the low solubility of the corticosteroid in the cream base. Burdick (116), in studying the effect of the vehicle on fluocinonide and

fluocinolone acetonide found that the activity of the more potent fluocinonide was reduced to that shown by fluocinolone acetonide in a blanching assay, a double-blind paired comparison Scholtz and Dumas psoriasis assay , as well as an intensive clinical trial using a number of different dermatoses. Further evidence of correlation can be found in the superiority of fluocinonide in FAPG cream over betamethasone 17-valerate cream in the unoccluded blanching assay and in the treatment of various skin disorders (296). These two preparations were found to elicit approximately equal blanching under occlusion. Several other reports have substantiated the claim that a good correlation exists between the results obtained from blanching assays and clinical trials *viz:* fluocinonide 0,05% gel was superior to betamethasone benzoate 0,025% gel (160); 0,05% diflorasone diacetate in a cream containing 15% propylene glycol was similar to 0,05% fluocinonide in FAPG base (251); 0,05% betamethasone 17,21-dipropionate scalp application was more active than 0,1% betamethasone 17-valerate scalp application (297); a new glycol ointment containing 0,05% betamethasone dipropionate was found to be superior to both the marketed cream and ointment containing this corticosteroid in the same concentration (39); hydrocortisone cream 0,1% (Dioderm) was found to be superior to hydrocortisone cream B.P.C. 1% (298); no significant differences were found in comparisons of 0,05% desonide cream with 0,01% triamcinolone acetonide cream (75). Stoughton (104) further cites five separate assays where a correlation was found between the blanching assay and the response of psoriatic plaques.

It is interesting to note that the prevailing disease being treated during the clinical trial can affect the overall correlation; psoriasis has been said to provide a more discriminating test than eczema (299). Bagatell (300) found that a preparation containing halcinonide was superior to a preparation containing betamethasone 17-valerate in the treatment of psoriasis, although the preparations elicited similar responses in various other skin disorders and the blanching assay. Allenby and Sparkes (72) conversely found no differences between Locoid ointment and Eumovate ointment in the treatment of eczema, whereas Eumovate ointment was more effective than Locoid ointment in the treatment of psoriasis.

CHAPTER 2

METHODOLOGY AND EVALUATION OF RESULTS

2.1 THE BLANCHING ASSAY

The general method of experimentation remained the same throughout this study with a few minor changes which are noted in the discussion of the relevant trials.

Twelve healthy male or female Caucasian subjects were selected for each trial from a panel of volunteers known to show a response to a standard preparation (Betnovate cream) applied under occlusion for 6 hours. The volunteers were requested not to take part in a particular trial if they were using any form of systemic medication or had received topical corticosteroids for at least 6 weeks prior to the investigation.

Only proprietary preparations were used and these were purchased from a local pharmacy shortly before each trial. The first gram of each tube of cream or ointment was discarded in case of any interaction between the closure and the formulation. The preparations were applied to the flexor aspect of the forearm by means of a small disposable syringe which had been filled immediately prior to use in order to minimize any possible interaction between the corticosteroid and the plastic matrix of the syringe barrel. The needles used were of constant size (25 g 5/8) and had been cut to 5 mm to facilitate extrusion of the preparations. Four stripes (7 mm) of the preparations (equivalent to approximately 3,2 mg) were applied to twelve 7 mm x 7 mm sites on each forearm of the volunteers (figure 2.1.1). In the case of lotions, 5µl of the lotion (equivalent to approximately 4 mg) were applied by means of a micropipette.

Four different application patterns were used in each trial. The creams were not applied in the same pattern on both arms of a volunteer and the random allocation of the application charts

(containing the pattern) and coding of the syringes were performed by two different persons involved with the trial. The 12 sites were demarcated by applying 6 self-adhesive labels, from which 2 holes had been punched, onto each arm (figure 2.1.1). The areas adjacent to the wrists and elbows were avoided, unless the subject had short forearms (232,237). The preparations were evenly spread with a glass rod (or the tip of the micropipette in the case of lotions) prior to occlusion or protection of the sites. All the sites on one arm of each volunteer were occluded while the sites on the other arm were left unoccluded. Occlusion was attained by means of 6 individual strips of non-porous plastic tape (Blenderm surgical tape) placed over the self-adhesive labels (figure 2.1.2) and the sites that were left unoccluded were protected by cardboard or plastic coverings designed so as to allow a free flow of air and held in place by Micropore surgical tape (figure 2.1.3).

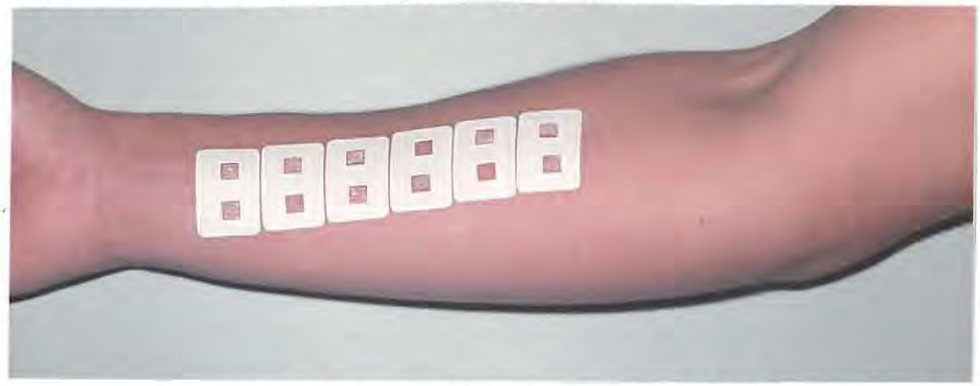


Figure 2.1.1 Arm with demarcating labels and 4 stripes of preparation

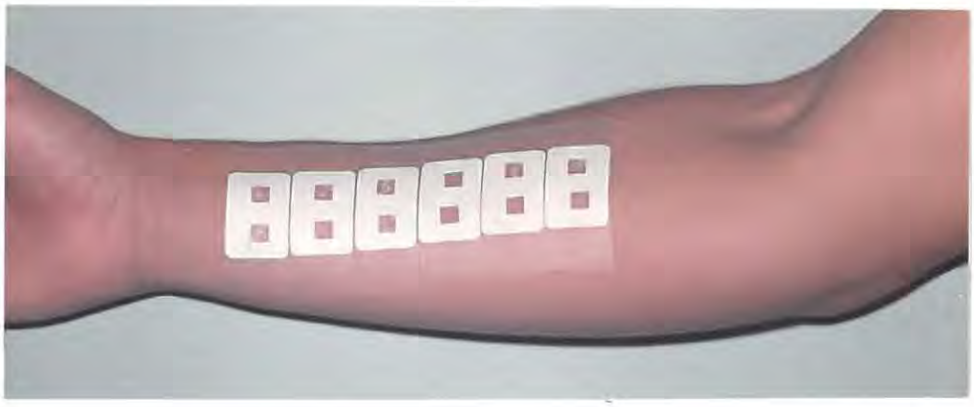


Figure 2.1.2 Occluding tape applied over demarcating labels

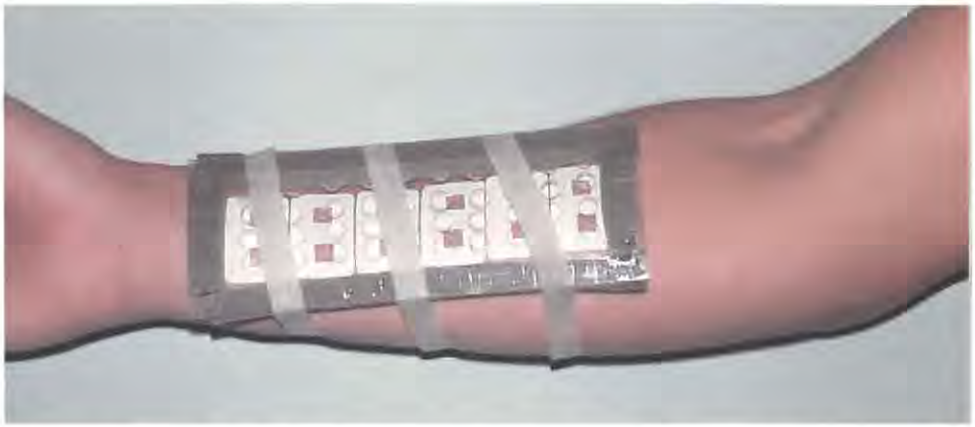


Figure 2.1.3 Plastic covering used to protect the unoccluded application sites

The various procedures were carried out by different persons, but each procedure was carried out by the same person for any one trial. All volunteers partaking in a particular trial were processed sequentially at 5 minute intervals in order to minimize any possible effects of environmental variables such as ambient temperature and humidity. The total application procedure therefore took 1 hour, with the average of that having been taken as the start of the trial for purposes of observation of the blanching sites. The formulations were allowed to remain in contact with the skin for 6 hours, after which the protective coverings, occluding tape and demarcating labels were removed. The volunteers removed the occluding tape and demarcating lables slowly so as to avoid stripping of the skin and to minimize erythema. The residual steroid was then removed by gentle washing with toilet soap and warm water and the arms patted dry with a towel. The puckering observed at the occluded sites and occasional mild erythema were found to disappear after about 30 minutes.

Assessment of the blanching responses was done independently by 3 experienced observers at several predetermined times which allowed the construction of blanching profiles and statistical analysis at a number of times over an extended period. The arms were held horizontally on a desk directly in front of the observers for assessment of the blanching responses. Scoring was done on a 0 - 4 scale where 0 = normal skin, 1 = slight blanching, 2 = more intense

blanching, 3 = general even and distinct blanching and 4 = marked and very intense blanching. Figures 2.1.4 and 2.1.5 depict blanching responses observed at 10 hours and 12 hours after application. Standard lighting by overhead fluorescent lamps was used throughout each investigation.

Volunteers could continue with their normal daily activities, but were required not to partake in any heavy exercise, were requested not to bath or shower for the duration of the trial and were not allowed to consume alcohol at any time during the trial.

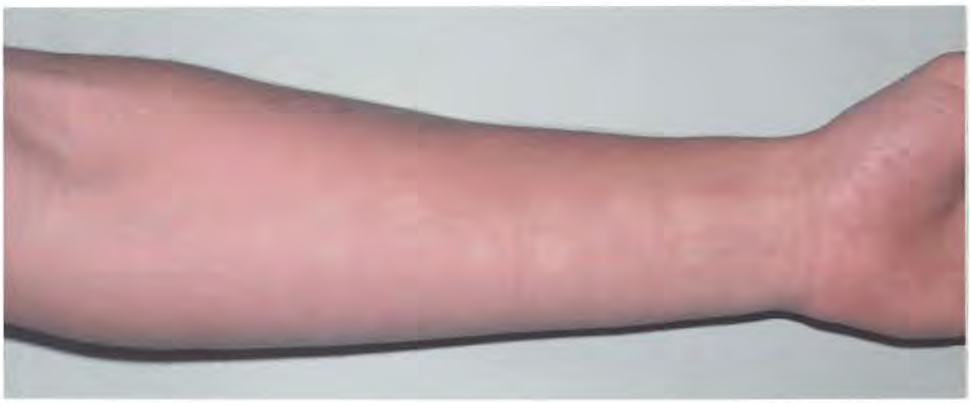


Figure 2.1.4 Blanching responses 10 hours after application

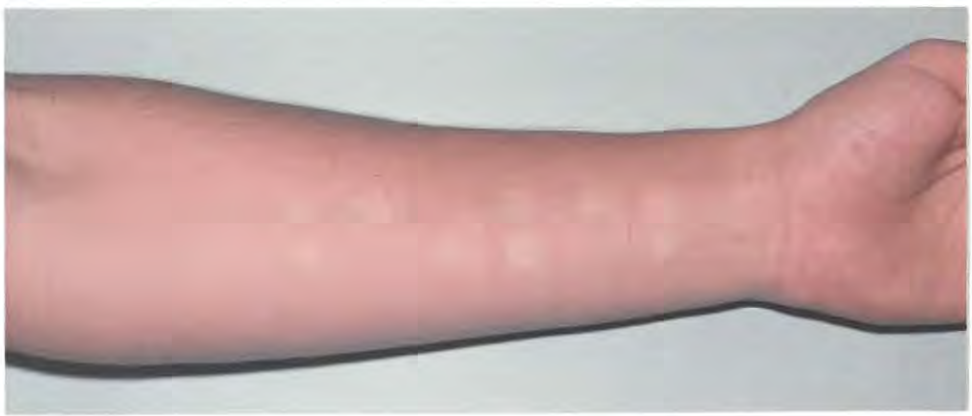


Figure 2.1.5 Blanching responses 12 hours after application

2.2 EVALUATION OF RESULTS

The results obtained by the 3 observers were pooled for the purpose of evaluation and the data processed as described in section 2.3. The statistical methods used in this study have been previously described (231,301-303).

2.2.1 Calculation of Percent Total Possible Score (%TPS)

The %TPS was calculated as follows :

Total possible score (TPS) = B x O x S x V

where B = the maximum blanching score attainable for any one site
O = the number of observers
S = the number of sites per arm
V = the number of volunteers

Note that the number of sites per arm (S) is not the total number of sites on the arm, but the number of sites that the particular preparation occupied on the arm.

$$\%TPS = \frac{\text{actual score}}{TPS} \times 100$$

2.2.2 Determination of the Area under the Curve (AUC)

The trapezoidal rule was used to calculate the AUC values. The parameters used to obtain this value were the %TPS values and the time in hours after application of the corticosteroids. The AUC is therefore reported in terms of %TPS and time in hours. The calculation for the AUC is represented by the formula

$$\text{Area} = \tfrac{1}{2} (R_1 \times R_2) \, .t$$

where R_1 = response at $time_1$
R_2 = response at $time_2$
t = time between R_1 and R_2.

Magnus (301) discussed the use of a corrected AUC value by determining the elimination constant from the slope of the curve between the last two readings. It was however decided that, due to the very small blanching response at the final stages of the trials, the corrected AUC determination would not be employed in this study.

2.2.3 Statistical Evaluation of Results

Three methods were used to evaluate the results (231).

2.2.3.1 Number of Sites Exhibiting Blanching (Yes/No Analysis)

This method involved a chi-squared analysis of the number of sites that exhibited blanching (Yes) and the number of sites at which no blanching could be seen (No). It is in effect an analysis utilizing a simple 2 point (0 - 1) scoring system and is therefore the least sensitive of the three methods as it gives no indication of the degree of observed blanching (303). The ungraded recording of responses may be useful in experiments on corticosteroids eliciting only a weak blanching response, but is of dubious value in the case of more potent topical corticosteroids (5,231). For this method a 2 x 2 contingency table was examined using Yates' Correction Factor for continuity (304). Chi-squared values greater than 3,84 signified real differences based on the 95% level of significance and assuming 1 degree of freedom (304).

2.2.3.2 Intensity of Blanching (Graded Response Analysis)

In this method of statistical analysis chi-squared values were obtained by comparing the intensity of observed blanching for two preparations by using the 0 - 4 scoring system described in section 2.1. Since a 5 point (0 - 4) scoring system was used a 2 x 5 contingency table was obtained for each of the product comparisons.

Chi-squared values greater than 9,49 signified real differences based on the 95% level of significance and assuming 4 degrees of freedom (304).

2.2.3.3 Paired Comparison of Adjacent Application Sites

This method involved a direct comparison of the observed blanching (or absence thereof) at different application sites with the observer recording that one site exhibited more blanching than the other, both sites exhibited equal blanching or that no blanching was observed at either site. The procedure of McNemar (304) was employed to obtain chi-squared values. Values of greater than 3,84 signified real differences based on the 95% level of significance and assuming 1 degree of freedom (304).

2.3 COMPUTER ANALYSIS OF RAW DATA

The graded blanching responses and comparisons of adjacent sites for trials performed in our laboratories were originally processed by manually counting the responses and comparisons to obtain data for statistical analysis and the construction of blanching profiles. The collation of the raw data was time consuming and took approximately five days per trial.

A computer programme (reproduced in Appendix 3) was then compiled to generate the data required for statistical analysis and the construction of blanching profiles from the information obtained at each observation time. It now takes a competant operator approximately 12 hours to feed in the information obtained from a trial and generate the data required for statistical analysis and the construction of blanching profiles.

The original programme (Appendix 3) was compiled for a Wang 2200 Basic Desk Top Mini-Computer and has been converted for use on an Apple II or Apple II+ Mini-Computer. The programme for the Apple II is reproduced in table 2.3.1. The programme has been designed for use by operators with minimal knowledge of computers and prompts the

operator for information when it is required, e.g., "Were the sites occluded or unoccluded?"

The programme as reproduced in the table allows certain experimental variables to have a maximum value which, if exceeded in a particular trial, will require alteration of the programme.

The maximum number of different randomized application patterns (see section 2.1) usable in a trial is 4. These randomized patterns are referred to as "charts" in the programme. The use of more than 4 charts requires the alteration of lines 140 and 2420, as well as the addition of extra chart "names" between lines 220 and 230.

The programme also does not allow the use of more than 14 sites of application in any one chart. Exceeding 14 sites of application per chart will therefore require the alteration of lines 170 and 2440.

The number of different preparations used in a trial is limited to 8. The use of more than 8 preparations will therefore require the alteration of the relevant dimensions in lines 10, 20 and 30. The figure "8" will have to be replaced by the number of preparations in the following dimensions in these lines : O$(8), S$8, T1(8,5), T2(12,8), P1(8,12), R1(8,12) and R2(8,12). Lines 260, 400 and 2460 will also have to be altered accordingly.

The maximum number of pairs of sites that may be compared in any chart (see above) has been limited to 12. An excess of 12 comparisons requires similar alterations to the dimensions in lines 10, 20 and 30 as described in the preceding paragraph. The limit of 12 will require alteration of the following dimensions : T2,(12,8), E(12), P1(8,12), Y(12), R1(8,12) and R2(8,12). Alterations will further have to be made to lines 590, 910, 2480 and 2540.

As previously mentioned (section 2.1) use is made in our laboratories of a 5 point scoring system *(i.e.* 0-4) for the assessment of the blanching responses. The alterations to the programme discussed above are only required if certain values exceed the limits included

in the programme, whereas any changes in the scoring system will necessitate the alterations discussed below. The figure "5" in the dimension T1(8,5) in line 20 will have to be altered. The figure will be altered to the highest number in the scoring system plus 1, e.g., if a 0-8 system is used this figure will be 9. This dimension only requires alteration if the maximum blanching score is greater than 4. Alterations will also have to be made to lines 1080, 1340, 1960, 2000 and 2040. The figure "5" in lines 1080 and 1960 will be replaced by the figure as calculated for the dimension T1(8,5) described above; the figure "4" in lines 1340 and 2000 will be replaced by the figure equal to the maximum blanching score attainable and line 2040 will be altered accordingly depending on this maximum blanching score.

Certain modifications to the programme discussed above are currently being undertaken in our laboratories. A facility to correct data that has been fed into the computer is being investigated. At present an error in entering the recorded blanching score or the result of the comparison of two sites will cause the operator to have to re-enter all the data for that particular time interval. A further useful inclusion into the programme is a facility for performing the statistical analyses described in section 2.2.3.

TABLE 2.3.1 COMPUTER PROGRAMME USED ON THE APPLE II MINI-COMPUTER

```
 10  DIM C$(8),O$(8),S$(8)
 20  DIM T1(8,5),T2(12,8),E(12),P1(8,12),Y(12)
 30  DIM R1(8,12),R2(8,12)
 40  HOME
 50  PRINT "WERE THE SITES OCCLUDED OR UNOCCLUDED?"
 60  INPUT Z$
 70  PRINT : PRINT : PRINT
 80  PRINT "TIME OF DATA COLLECTION"
 90  PRINT "ENTER THE TIME AS A NUMBER"
100  INPUT T
110  PRINT : PRINT
120  PRINT "NUMBER OF CHARTS USED"
130  INPUT N1
140  IF N1 > 4 THEN 2420
150  PRINT "NUMBER OF SITES PER CHART"
160  INPUT N
170  IF N > 14 THEN 2440
180  REM  ASSIGN CHART NAMES
190  LET C$(1) = "A"
200  LET C$(2) = "B"
```

```
210  LET C$(3) = "C"
220  LET C$(4) = "D"
230  PRINT : PRINT
240  PRINT "NUMBER OF PREPARATIONS USED"
250  INPUT N
260  IF N > 8 THEN 2460
270  REM  READ IN PREPARATION NAMES
280  FOR I = 1 TO N
290  PRINT "PREPARATION ";I;"?"
300  INPUT O$(I)
310  NEXT I
320  PRINT : PRINT
330  PRINT "READ IN STRINGS OF NUMBERS REPRESENTING"
340  PRINT "THE PREPARATION LAYOUTS IN THE CHARTS"
350  PRINT
360  PRINT "NUMBER OF STRINGS = NUMBER OF CHARTS"
370  PRINT
380  PRINT "NUMBER OF DIGITS PER STRING = NUMBER OF SITES"
390  PRINT
400  PRINT "DIGITS ARE 1-8 CORRESPONDING TO"
410  PRINT "PREPARATION TYPE AS ENTERED ABOVE"
420  FOR I = 1 TO N1
430  PRINT : PRINT : PRINT
440  PRINT "CHART",C$(I);"?"
450  INPUT S$(I)
460  NEXT I
470  PRINT : PRINT
480  PRINT "READ IN PAIRS OF SITE NUMBERS FOR SITES"
490  PRINT "WHICH ARE TO BE COMPARED"
500  PRINT
510  PRINT "ENTER ONE PAIR PER LINE;NUMBERS"
520  PRINT "SEPARATED BY COMMAS"
530  FOR I = 1 TO N1
540  PRINT : PRINT
550  PRINT "CHART",C$(I)
560  PRINT
570  PRINT "NUMBER OF PAIRS TO BE COMPARED"
580  INPUT Y(I)
590  IF Y(I) > 12 THEN 2480
600  REM  CREATE TABLES FOR THE COMPARISONS OF
610  REM  SITE/PREPARATION PAIRS
620  FOR J = 1 TO Y(I)
630  INPUT R1(I,J),R2(I,J)
640  NEXT J
650  NEXT I
660  REM  INITIALIZE
670  LET K = 0
680  REM  FOR EACH CHART
690  FOR I = 1 TO N1
700  REM  FOR EACH SITE PAIR
710  FOR J = 1 TO Y(I)
720  LET R = R1(I,J)
730  REM  FIND PREP. OF FIRST SITE OF PAIR
740 S1$ =  MID$ (S$(I),R,1)
750 S1 =  VAL (S1$)
760  LET R = R2(I,J)
770  REM  FIND PREP. OF SECOND SITE OF PAIR
780 S1$ =  MID$ (S$(I),R,1)
790 S2 =  VAL (S1$)
800  REM  FORM A NUMERIC CODE FOR THE PREP. PAIR
810  LET S = S1 * 10 + S2
820  REM  CHECK IF THIS PREP. PAIR IS IN THE LIST ALREADY
830  REM  ADD IT IF IT IS NOT. ASSIGN ITS POSITION IN THE
840  REM  LIST TO A POINTER BETWEEN SITE PAIR IN CURRENT
850  REM  CHART AND PREP. PAIR IN THE LIST.
860  IF K = 0 THEN 900
870  FOR R = 1 TO K
```

```
880  IF E(R) = S THEN 940
890  NEXT R
900  LET K = K + 1
910  IF K > 12 THEN 2530
920  LET E(K) = S
930  LET R = K
940  LET P1(I,J) = R
950  NEXT J
960  NEXT I
970  REM  NOW K IS THE NO. OF PREP. PAIRS
980  REM  TO BE COMPARED
990  LET N2 = K
1000  PR# 1
1010  PRINT "**********************************************************
     ***********"
1020  PRINT "* PROCESSED DATA FROM BLANCHING TRIALS ON TOPICAL STEROID PRE
     PARATIONS *"
1030  PRINT "**********************************************************
     ***********"
1040  PR# 0
1050  REM  INITIALIZE TO 0 A TABLE FOR COUNTING NO.
1060  REM  OF 0'S,1'S,2'S,ETC FOR EACH PREP.,1-M
1070  FOR I = 1 TO M
1080  FOR J = 1 TO 5
1090  LET T1(I,J) = 0
1100  NEXT J
1110  NEXT I
1120  REM  INITIALIZE TO 0 A TABLE FOR COUNTING THE
1130  REM  NO. OF 0'S,='S,>'S AND <'S.
1140  FOR J = 1 TO N2
1150  FOR K = 1 TO 4
1160  LET T2(J,K) = 0
1170  NEXT K
1180  NEXT J
1190  LET C = 0
1200  HOME
1210  REM  CARD-READING AND DATA SORT
1220  PRINT "READ CARD NUMBER"
1230  INPUT C0
1240  PRINT : PRINT : PRINT
1250  PRINT "READ CHART NAME"
1260  INPUT X$
1270  LET C = C + 1
1280  FOR I = 1 TO N1
1290  IF X$ = C$(I) THEN 1320
1300  NEXT I
1310  GOTO 2510
1320  LET L = I
1330  PRINT : PRINT : PRINT
1340  PRINT "READ IN A STRING OF NUMBERS 0-4"
1350  PRINT "CORRESPONDING TO THE APPROPRIATE STRING"
1360  PRINT "OF THE CHART AND REPRESENTING THE"
1370  PRINT "DEGREE OF BLANCHING AT EACH SITE."
1380  INPUT D$
1390  REM  CORRELATE FOR EACH SITE A PREP.  NUMBER
1400  FOR I = 1 TO N
1410  LET D = 0
1420  LET D1$ =  MID$ (D$,I,1)
1430 D =  VAL (D1$)
1440  LET D = D + 1
1450  REM  D WILL NOW HAVE A VALUE EQUAL TO 1 GREATER
1460  REM  THAN THE BLANCHING SCORE
1470  LET S = 0
1480  LET S1$ =  MID$ (S$(L),I,1)
1490 S =  VAL (S1$)
1500  REM  NOW S IS A POINTER TO PREP. TYPE
1510  REM  THEN ADD THE BLANCHING SCORE TO THE
```

```
1520  REM  APPROPRIATE ELEMENT OF T1
1530  LET T1(S,D) = T1(S,D) + 1
1540  NEXT I
1550  PRINT : PRINT
1560  PRINT "INPUT IN CORRECT ORDER RESULTS OF
1570  PRINT "COMPARISONS FOR APPROPRIATE CHART."
1580  PRINT "1:0  2:=   3:>   4:<"
1590  INPUT P$
1600  REM  STORE NO. OF 0'S,='S,>'S,<'S PER
1610  REM  PREP. PAIR FOR EVERY SITE PAIR
1620  FOR J = 1 TO Y(L)
1630  REM  LOCATE CORRECT COLUMN OF PREP. PAIR
1640  LET P = P1(L,J)
1650  REM  DECODE FROM "COMPARISON STRING" THE
1660  REM  INFORMATION FROM SITE PAIR COMPARISON
1670  LET S1$ =  MID$ (P$,J,1)
1680 S =  VAL (S1$)
1690  REM  NOW STORE THIS
1700  LET T2(P,S) = T2(P,S) + 1
1710  NEXT J
1720  PRINT : PRINT
1730  PRINT "IS THERE A NEXT CARD? (Y/N)"
1740  INPUT B$
1750  IF B$ = "Y" THEN 1300
1760  IF B$ = "N" THEN 1790
1770  PRINT "INVALID ANSWER. RETYPE"
1780  GOTO 1730
1790  PR# 1
1800  PRINT : PRINT
1810  PRINT "RESULTS OF BLANCHING"
1820  PRINT "--------------------"
1830  PRINT
1840  PRINT "THE SITES WERE ";Z$
1850  PRINT "TIME OF COLLECTION WAS ";T;" HOURS AFTER APPLICATION"
1860  PRINT N1;" CHARTS WERE USED"
1870  FOR I = 1 TO N1
1880  PRINT "CHART",I,S$(I)
1890  NEXT I
1900  PRINT
1910  FOR I = 1 TO M
1920  PRINT
1930  PRINT "PREPARATION ";I; TAB( 4);O$(I)
1940  LET A0 = 0
1950  LET A1 = 0
1960  FOR J = 1 TO 5
1970  LET K = J - 1
1980  PRINT "NUMBER OF ";K;"'S:    ";T1(I,J)
1990  LET A = K * T1(I,J)
2000  LET A1 = A1 + 4 * T1(I,J)
2010  PRINT "TOTAL OF ALL ";K;"'S:      ";A
2020  LET A0 = A0 + A
2030  NEXT J
2040  PRINT "SUM TOTAL OVER 1'S, 2'S, 3'S, 4'S:",A0
2050  LET A2 = (A0 / A1) * 80
2060  PRINT "PERCENTAGE OF TOTAL POSSIBLE SCORE  ";A2
2070  NEXT I
2080  PRINT
2090  PRINT "RESULTS OF COMPARISONS"
2100  FOR J = 1 TO N2
2110  LET A = E(J)
2120  LET A0 =  INT (A / 10)
2130  LET A1 = A - (A0 * 10)
2140  PRINT
2150  PRINT "COMPARING PREPARATION PAIR:";A
2160  PRINT "I.E., ";O$(A0);" WITH ";O$(A1)
2170  LET I = 1
2180  PRINT "NUMBER OF 0",T2(J,I)
```

```
2190  LET I = 2
2200  PRINT "NUMBER OF =",T2(J,I)
2210  LET I = 3
2220  PRINT "NUMBER OF >",T2(J,I)
2230  LET I = 4
2240  PRINT "NUMBER OF <",T2(J,I)
2250  NEXT J
2260  PR# 0
2270  PRINT "IS THERE ANOTHER TIME INTERVAL"
2280  PRINT "USING THE SAME CHARTS? (Y/N)"
2290  INPUT B$
2300  IF B$ = "Y" THEN 2330
2310  IF B$ = "N" THEN 2380
2320  PRINT "INVALID ANSWER.RETYPE"
2330  PRINT "TIME OF DATA COLLECTION"
2340  INPUT T
2350  PRINT "OCCLUDED OR UNOCCLUDED"
2360  INPUT Z$
2370  GOTO 1000
2380  PRINT "PROGRAMME TERMINATING"
2390  STOP
2400  REM  ERROR MESSAGES
2410  REM  --------------
2420  PRINT "ERROR. NUMBER OF CHARTS EXCEEDS 4"
2430  GOTO 120
2440  PRINT "ERROR. MAXIMUM 14 SITES ALLOWED"
2450  GOTO 150
2460  PRINT "ERROR. MAXIMUM 8 PREPARATIONS ALLOWED"
2470  GOTO 240
2480  PRINT "ERROR. MAXIMUM 12 PAIRS OF SITE NUMBERS"
2490  PRINT "CAN BE COMPARED"
2500  GOTO 570
2510  PRINT "ERROR. INVALID CHART NAME FOR CARD",CO
2520  GOTO 1220
2530  PRINT "ERROR. NUMBER OF PREPARATION PAIRS MAY NOT"
2540  PRINT "NOT EXCEED 12"
2550  STOP
2560  END
```

CHAPTER 3

RESULTS AND DISCUSSION

3.1 COMPARISON OF THREE BETAMETHASONE 17-VALERATE-CONTAINING PREPARATIONS

The 17-valerate ester of betamethasone has been shown by McKenzie and Atkinson (86) to exhibit significantly greater blanching activity than the parent alcohol in the human blanching assay. The clinical usefulness of topically applied betamethasone 17-valerate has been reported since 1964 (34,35,40,73,86,186,247,297,305-314) and Smith (206) has recently reported its high incidence of inclusion in prescriptions for topical medication in England.

Blanching studies comparing the activities of commercially available preparations containing equal concentrations of the same corticosteroids have shown differences in the intensity of pallor in some cases and equivalence in others. No statistically significant differences in blanching activities were found in commercially available preparations containing equal quantities of corticosteroid in the cases of hydrocortisone (135,147), diflucortolone valerate (163), the pivalate and hexanoate esters of fluocortolone (132), fluocinolone acetonide (253) and triamcinolone acetonide (134,137,260), while in some studies statistically significant differences were found in preparations containing fluocinolone acetonide (253) and triamcinolone acetonide (56,137,259,260). It is interesting to note the apparent disparities in the cases of fluocinolone acetonide and triamcinolone acetonide. The comparison of fluocinolone acetonide-containing preparations showed no statistically significant differences for both creams and ointments (creams compared to each other and ointments compared to each other) in the occluded mode of application, whereas statistically significant differences were found in the unoccluded mode (253). This emphasizes the importance of utilizing both modes of application in blanching trials, especially as the unoccluded mode is most

commonly used in therapy. Barry and Woodford (51,132,233) found no statistically significant differences in the blanching abilities of ointments containing triamcinolone acetonide, whereas statistically significant differences were found between certain creams containing this corticosteroid. It is worthwhile noting that experiments performed at different times on similar products have produced similar results (51,137). Coldman *et al.* (259) found statistically significant differences between two proprietary creams and two proprietary ointments containing triamcinolone acetonide, whereas Poulsen and Rorsman (260) found statistically significant differences between two creams containing triamcinolone acetonide but not between the corresponding ointments. It is therefore important not to assume bioequivalence between creams once this has been found in ointments and *vice versa*. There does not appear to be any literature on the comparative blanching activities of proprietary betamethasone 17-valerate-containing preparations other than on products including additional pharmacologically active ingredients (51,132), as well as a report by Poulsen and Rorsman (260) on two of the products discussed in the present experiment and a report of the present experiment (315).

The present experiment was performed to compare the blanching activities of three proprietary betamethasone 17-valerate-containing creams and ointments, *viz.* Betnovate, Persivate and Celestoderm-V. The manufacturers of Persivate do not market a lotion; investigation of Betnovate lotion and Celestoderm-V lotion is discussed in section 3.2. The differences in the blanching responses elicited by corticosteroid-containing creams and ointments has been discussed in section 1.6.5.

Two separate trials were therefore mounted, one to investigate the three creams and one to investigate the three ointments. The preparations were applied to the forearms of twelve volunteers in both the occluded and unoccluded modes of application. The degree of blanching was observed independently by three experienced observers at 7,8,9,10,12,14,16,18,28 and 32 hours after application in both trials, with an additional reading at 52 hours after application in the trial on ointments.

The non-availability of raw materials and convenient assay techniques have generally rendered the instrumental analysis of some corticosteroid-containing formulations impossible in our laboratories, concentrations of corticosteroid stated on the commercial packages being taken as correct. The accessibility of a convenient high performance liquid chromatographic technique (302) for the analysis of betamethasone 17-valerate-containing creams and ointments however made it possible to analyse the preparations used in this experiment. The analyses were performed using a methanol:water (70:30) mobile phase and a reverse phase μBondapak C_{18} column (Waters Associates) and indicated that the concentration of corticosteroid in all the preparations studied in this experiment were within the limits as specified in the United States Pharmacopoeia (316).

Figures 3.1.1 - 3.1.4 depict the blanching profiles obtained using the %TPS and the time in hours after application. All the preparations tested elicited maximum blanching between 12 and 16 hours after application, with the occluded creams showing maximum blanching slightly before the unoccluded creams. Occlusion could be expected to produce a more rapid onset of action which should be less noticeable in the case of ointments due to their inherent occlusive properties. Occlusion of the creams was, as expected, found to cause a relatively greater increase in blanching than occlusion of the ointments. Such intercomparisons should, however, be made with care as the two trials were mounted at different times utilizing different volunteers.

The results of the comparison of AUC values and chi-squared analyses for creams in the occluded mode are summarized in table 3.1.1.

TABLE 3.1.1

SUMMARY OF THE COMPARISONS OF CREAMS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Celestoderm-V > Persivate	Celestoderm-V > Persivate	Celestoderm-V = Persivate	Celestoderm-V = Persivate
Celestoderm-V > Betnovate	Celestoderm-V > Betnovate	Celestoderm-V > Betnovate	Celestoderm-V = Betnovate
Persivate > Betnovate	Persivate > Betnovate	Persivate = Betnovate	Persivate = Betnovate

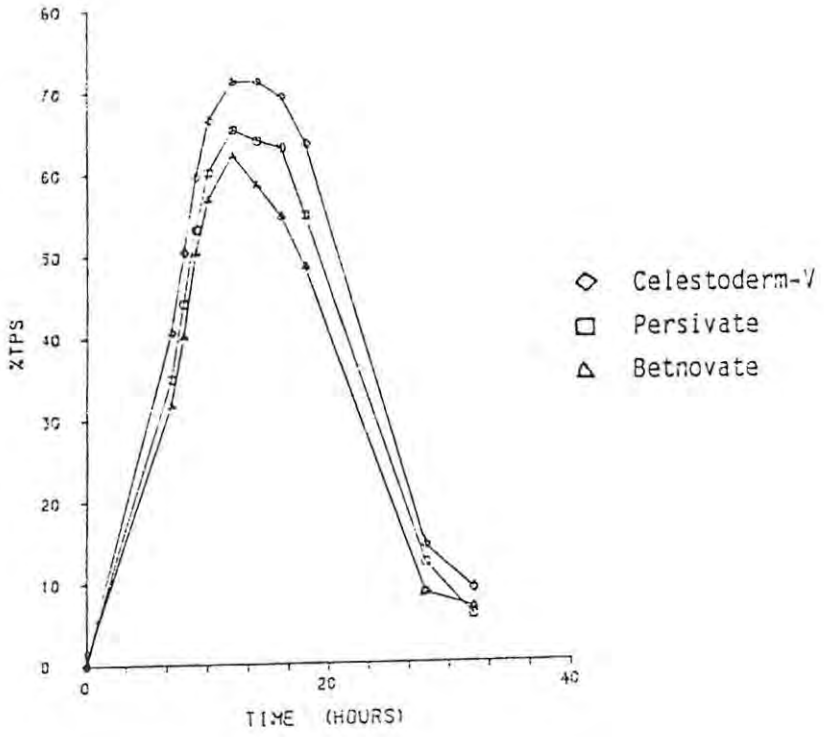


Figure 3.1.1 Blanching profiles of creams (occluded mode)

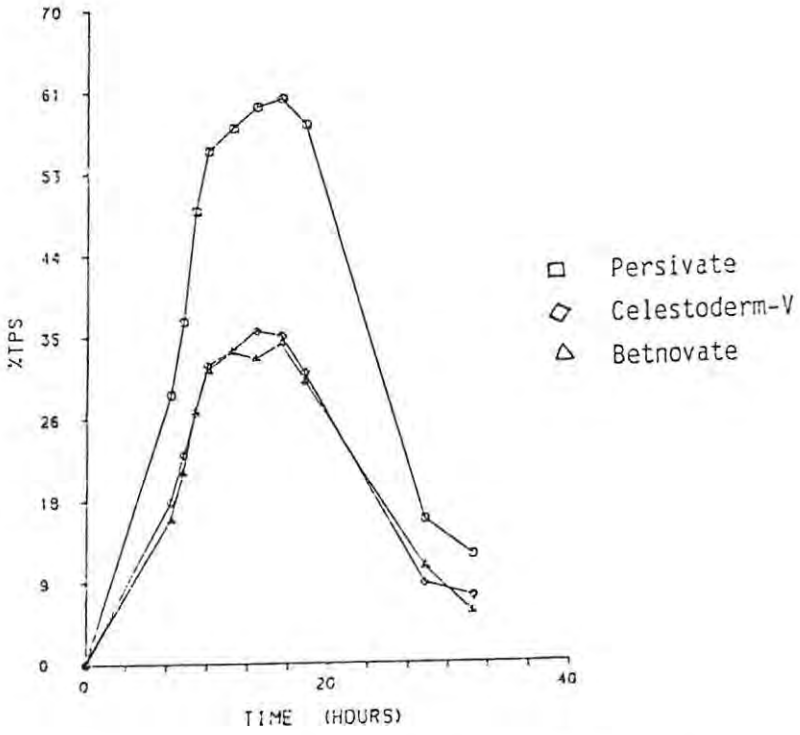


Figure 3.1.2 Blanching profiles of creams (unoccluded mode)

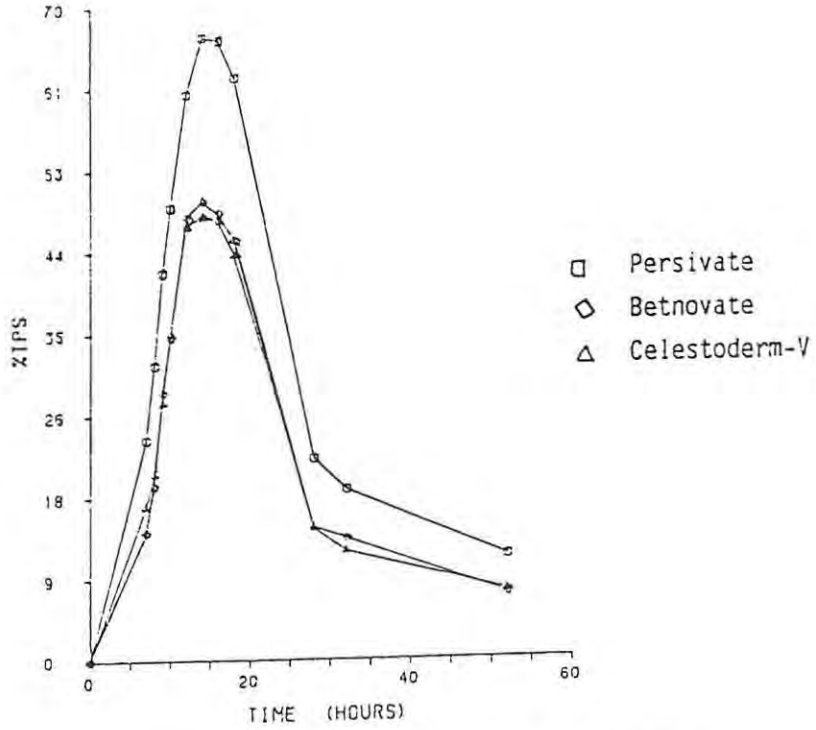


Figure 3.1.3 Blanching profiles of ointments (occluded mode)

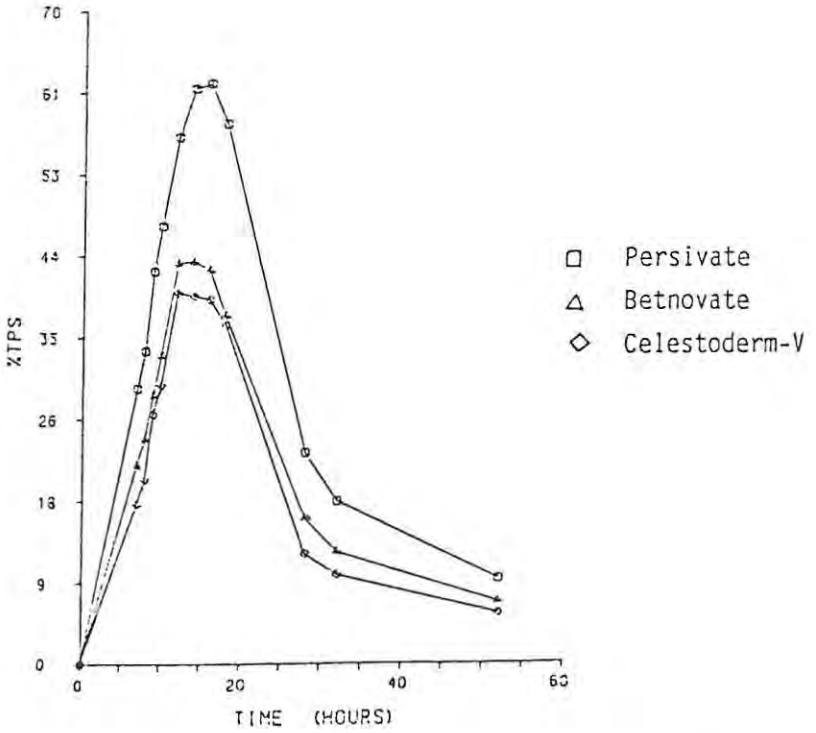


Figure 3.1.4 Blanching profiles of ointments (unoccluded mode)

For the comparison of adjacent sites, statistically significant differences were found at all time intervals in all cases with the exception of the comparisons of Persivate and Celestoderm-V at the 28 and 32 hour readings and Betnovate and Persivate at the 32 hour reading. Cognizance should, however, be taken of the fact that assessment at the 28 and 32 hour readings was being made on weak blanching responses; the average %TPS for all three of the occluded creams at these readings was only 9,41.

No statistically significant differences were noted in the graded response analysis of Celestoderm-V versus Betnovate at the 7, 8, 9, 12 and 32 hour readings. The chi-squared values that indicated that the differences between the blanching elicited by these two preparations was not statistically significantly different, with the exception of the 12 hour reading, however occurred at the beginning and end of the trial. Although chi-squared analysis at these readings indicated non-significance, the actual blanching scores favoured Celestoderm-V over Betnovate which coincides with the %TPS values and analysis of the comparison of adjacent sites. Statistically non-significant differences in the graded response analysis were noted at all times for the comparison of Betnovate and Persivate except the 16 hour reading which favoured Persivate. Similar findings were noted in the comparison of Persivate and Celestoderm-V with a statistically significant difference in favour of Celestoderm-V at the 18 hour reading.

The Yes/No analysis showed only three statistically significant differences, all of which favoured the same products as the other methods of analysis. The low sensitivity of this method of statistical analysis is discussed in section 2.2.3.1.

Table 3.1.2 contains a summary of the results of the comparisons of the AUC values and statistical analyses of the comparisons of the creams in the unoccluded mode of application.

TABLE 3.1.2

SUMMARY OF THE COMPARISONS OF CREAMS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V
Celestoderm-V = Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate
Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate

The only deviations from the summary in table 3.1.2 were the statistically non-significant differences obtained for the comparisons of Persivate and Betnovate at the 7 hour, and Persivate and Celestoderm-V at the 32 hour graded response analysis and at the 32 hour Yes/No analysis of Persivate and Celestoderm-V. The scores in all these cases favoured the products as indicated in the table.

The results of the comparisons between the ointments in the occluded mode of application are summarized in table 3.1.3.

TABLE 3.1.3

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V
Celestoderm-V > Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate
Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate

Deviations from the results in table 3.1.3 were noted at the 52 hour graded response analysis of Persivate and Betnovate and at the 32 hour and 52 hour analysis of Persivate and Celestoderm-V. Deviations were also noted at the 52 hour Yes/No analysis of Persivate and Celestoderm-V and at the 12 hour and 52 hour Yes/No analysis of Betnovate and Persivate. Although statistical analyses at these readings showed no significant differences, the results favoured the products as indicated in the table.

A summary of the results of the comparisons of ointments in the unoccluded mode is presented in table 3.1.4.

TABLE 3.1.4

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V	Persivate > Celestoderm-V
Betnovate > Celestoderm-V	Betnovate = Celestoderm-V	Betnovate = Celestoderm-V	Betnovate = Celestoderm-V
Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate	Persivate > Betnovate

Chi-squared analysis of the comparison of adjacent sites of Betnovate and Celestoderm-V indicated statistically significant differences in favour of Betnovate at 7, 8, 10, 14 and 28 hours after application. It cannot, however, be concluded that Betnovate elicited an overall superior blanching response to Celestoderm-V as these statistically significant differences were scattered throughout the trial. The other methods of analysis also did not indicate statistically significant superiority and the AUC value for Betnovate was only marginally greater than that for Celestoderm-V. Further deviations from the summary in table 3.1.4 were the comparison of adjacent sites of Betnovate and Persivate at the 52 hour reading, the graded response analyses of Persivate and Celestoderm-V at the 52 hour reading, the graded response analyses of Persivate and Celestoderm-V at the 52 hour reading, Betnovate and Celestoderm-V at the 8 hour reading and Persivate and Betnovate at the 32 hour and 52 hour readings. Deviations from the summary were also noted in the Yes/No analyses of the comparison of Betnovate and Celestoderm-V at the 7, 8 and 28 hour readings and of the comparison of Persivate and Betnovate at the 7, 8, 12 and 52 hour readings. The actual scores were in favour of the product as indicated in the summary in table 3.1.4.

A summary of the overall conclusions of the experiment is presented in table 3.1.5.

TABLE 3.1.5

CONCLUSIONS OF THE COMPARISON OF BETAMETHASONE 17-VALERATE-CONTAINING PREPARATIONS

Creams (occluded)	Celestoderm-V > Persivate > Betnovate
Creams (unoccluded)	Persivate > Celestoderm-V = Betnovate
Ointments (occluded)	Persivate > Celestoderm-V = Betnovate
Ointments (unoccluded)	Persivate > Celestoderm-V = Betnovate

It can be seen from table 3.1.5 that :

a) the corticosteroid was released more effectively from Persivate cream and ointment than from Betnovate cream and ointment in both modes of application;
b) the bioavailability of the corticosteroid was superior from Persivate ointment than from Celestoderm-V ointment in both modes of application and from Persivate cream in the unoccluded mode. It is interesting to note the reversal of ranking in the occluded and unoccluded creams; and
c) Celestoderm-V cream elicited superior blanching activity over Betnovate cream in the occluded mode, whereas bioequivalence was evident in the unoccluded mode and in both modes of application in the case of ointments.

The only results that have been reported on the comparison of the blanching abilities of betamethasone 17-valerate-containing preparations and that can be used as a comparison to this experiment appear in a report by Poulsen and Rorsman (260). They utilized commercially available Swedish preparations and found that *Betnovat* elicited a more intense blanching response than *Celestona valerat* in both modes of application in the case of creams and in the unoccluded ointments. This was reversed in the case of ointments applied under occlusion. Although it is probable that these preparations are the same as the ones available in South Africa, it cannot automatically be assumed that the formulations are the same. The results of the present study do not correlate with the study by Poulsen and Rorsman (260).

The present study therefore served to reinforce the findings that the formulation of a topical vehicle can have a significant effect on the release of corticosteroids from the vehicle, which could in turn influence the clinical efficacy of the product.

3.2 COMPARISON OF LOTIONS CONTAINING BETAMETHASONE ESTERS

This study served the purpose of comparing the blanching activities of two lotions containing betamethasone 17-valerate (Betnovate lotion and Celestoderm-V lotion) with each other and with a lotion containing betamethasone 17,21-dipropionate (Diprosone lotion).

Differences in the activities of the 17-propionate and 17-valerate esters of betamethasone in the alcoholic blanching assay have been reported by McKenzie and Atkinson (86). The ratios of the blanching abilities of betamethasone 17-propionate and betamethasone 17-valerate relative to fluocinolone acetonide were found to be 1,9 and 3,6 respectively, the 17-valerate ester thus eliciting almost twice the blanching response than the 17-propionate ester. Fredriksson *et al.* (297) have found that the blanching activity of betamethasone 17,21-dipropionate was superior to that of betamethasone 17-valerate in alcoholic solution. A 0,001% solution of the dipropionate elicited a superior response to a 0,005% solution of the valerate and was further found to elicit an approximately 3 times greater response to the valerate when applied in equal concentrations. The ED_{50} of the dipropionate has been found to be half that of the valerate (76). Semi-solid formulations containing the dipropionate ester have been found to elicit superior blanching responses to those containing twice the concentration of the valerate (76,161,260). Clinical dermatological studies have further shown the efficacy of betamethasone 17,21-dipropionate (36,38,39,41,317-321) and its superiority over betamethasone 17-valerate (297,322). Lofferer (309) has, however, reported that betamethasone 17-valerate was found to elicit a superior response to the dipropionate in the Scholz-Dumas psoriasis plaque test. The author offered no explanation for this apparent disparity although this may have been due to the vehicle. The clinical use of betamethasone 17-valerate has been discussed in section 3.1. Atrophogenicity and telangiectasia have also been found to be more pronounced in the case of the dipropionate as compared to the valerate ester (43). The significance of the 21-propionate moiety in the dipropionate ester therefore becomes evident from the preceding discussion. There appear to be no reports in the

literature on the comparison of proprietary betamethasone-containing lotions.

Five microliters of the lotions were applied to the forearms of twelve volunteers in a prearranged randomized pattern. The trial was performed in both the occluded and unoccluded modes of application, because in clinical practice the unoccluded mode is used most often, especially in the case of lotions which are mainly applied to the scalp and other hairy areas of the body. The degree of blanching was recorded independently by three experienced observers at 7,8,9,10,12,14,16,18,28 and 32 hours after application.

The blanching profiles of the corticosteroid lotions applied with and without occlusion are depicted in figures 3.2.1 and 3.2.2 respectively. Maximal blanching in the occluded mode occurred at 12 hours after application for Celestoderm-V and Betnovate, and 14 hours after application for Diprosone, although there was little difference between the 12 hour and the 14 hour responses of Diprosone. It is interesting to note that in the unoccluded mode of application Celestoderm-V and Betnovate both elicited the maximum blanching response at the 10 hour reading, whereas maximum blanching was observed at 16 hours after application for Diprosone. Mention was made in the previous experiment (section 3.1) that occlusion could be expected to produce a more rapid onset of action and that this effect should be less pronounced in the case of ointments due to the occlusive nature of the base. The interaction between the corticosteroid and the vehicle should be more significant in the cases of cream and ointments than in the case of lotions. This is due to the complexity of the semi-solid formulations and should support the conclusion that a more rapid onset of action could be expected in the case of an occluded lotion. This shorter time to reach maximal blanching was observed in the case of Diprosone lotion but was reversed for Celestoderm-V and Betnovate. More intense blanching responses were observed in all the preparations in the occluded mode of application. The irregular pattern of the profiles for the unoccluded applications could be due to the difficulty of accurately assessing a weak blanching response. This could possibly

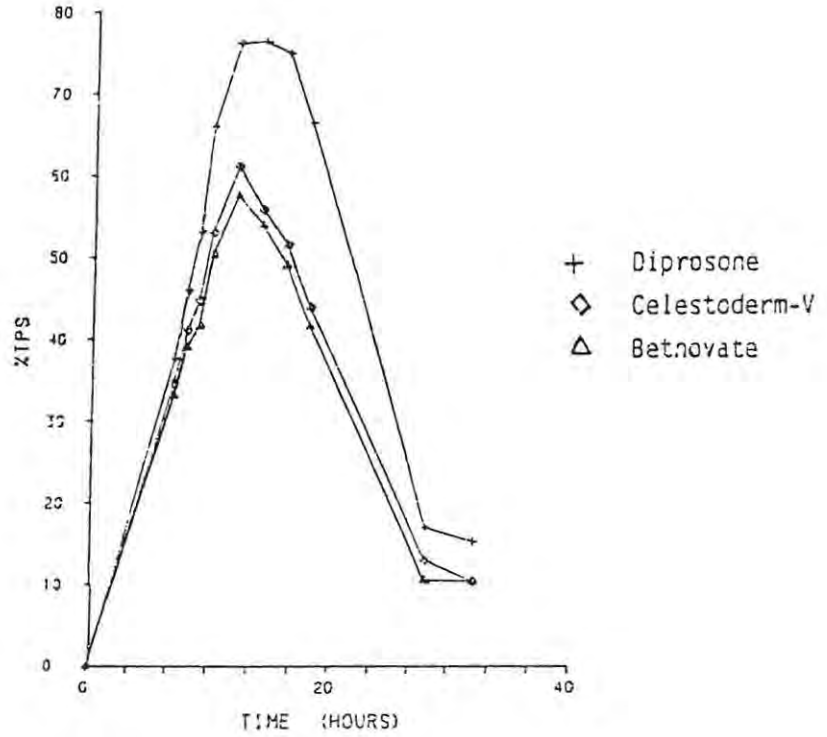


Figure 3.2.1 Blanching profiles of lotions (occluded mode)

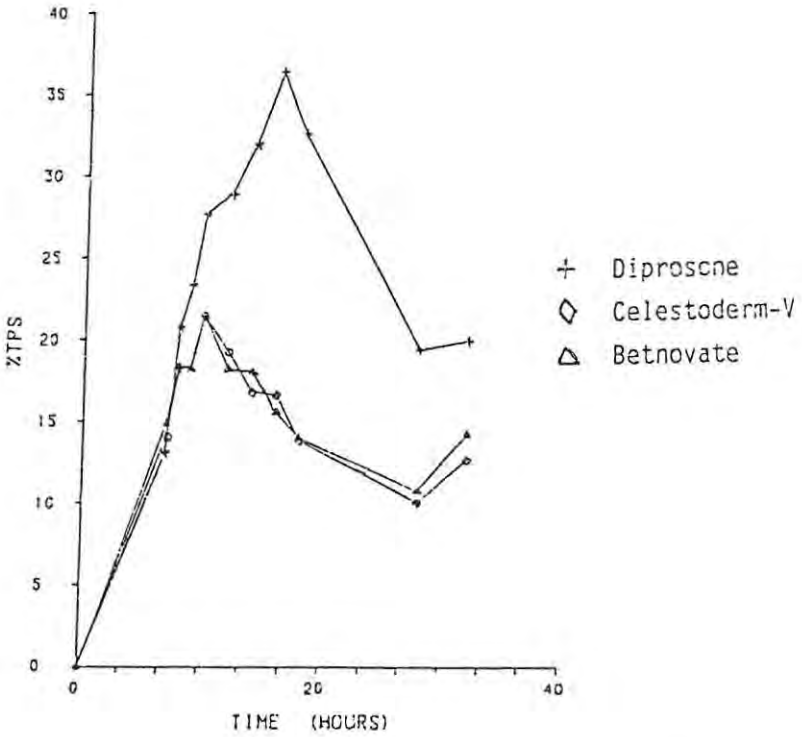


Figure 3.2.2 Blanching profiles of lotions (unoccluded mode)

also account for the apparent increase in blanching of the unoccluded lotions at the 32 hour reading, although this does not explain why this phenomenon occurs in one mode of application and not the other.

The conclusions drawn from the AUC values and the chi-squared analyses for the lotions applied under occlusion are summarized in table 3.2.1.

TABLE 3.2.1

SUMMARY OF THE COMPARISONS OF LOTIONS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Diprosone > Celestoderm-V	Diprosone > Celestoderm-V	Diprosone > Celestoderm-V	Diprosone = Celestoderm-V
Diprosone > Betnovate	Diprosone > Betnovate	Diprosone > Betnovate	Diprosone = Betnovate
Celestoderm-V > Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate

The AUC values indicated that Diprosone elicited a greater blanching response than Celestoderm-V which elicited a greater response than Betnovate, with the difference between Celestoderm-V and Betnovate being small. Analysis of the values obtained in the comparison of adjacent sites indicated statistically significant superiority of the blanching response of Diprosone over Celestoderm-V and Betnovate, with no statistically significant differences between the blanching responses of Celestoderm-V and Betnovate. The only deviations from this were noted in the statistically non-significant differences between Diprosone and Celestoderm-V at the readings taken at 7 and 8 hours after application. The number of responses recorded in favour of Diprosone however exceeded the number recorded in favour of Celestoderm-V. Chi-squared analysis of the graded responses similarly indicated the superior blanching ability of Diprosone to Celestoderm-V and Betnovate, and equivalence between the latter two. Deviations from the statistically significant results were noted at the 7, 28 and 32 hour readings for Diprosone and Celestoderm-V and the 7 hour reading for Diprosone and Betnovate. The actual blanching scores did, however, favour Diprosone in all cases. Analysis of the number of sites exhibiting blanching showed only four statistically significant differences, all of which favoured Diprosone over Celestoderm-V and Betnovate.

The results of the comparison of AUC values and chi-squared analyses for the lotions in the unoccluded mode of application are summarized in table 3.2.2.

TABLE 3.2.2

SUMMARY OF THE COMPARISONS OF LOTIONS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Diprosone > Celestoderm-V	Diprosone > Celestoderm-V	Diprosone > Celestoderm-V	Diprosone > Celestoderm-V
Diprosone > Betnovate	Diprosone > Betnovate	Diprosone > Betnovate	Diprosone > Betnovate
Celestoderm-V < Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate	Celestoderm-V = Betnovate

The AUC values of the blanching profiles indicated a similar superiority in the blanching response of Diprosone over Celestoderm-V and Betnovate to that noted in the occluded mode. The AUC of Betnovate was, however, greater than that of Celestoderm-V in the unoccluded mode, although the difference was small. Statistical analyses of the comparisons of adjacent sites and the graded responses indicated that Diprosone elicited more intense blanching than both Celestoderm-V and Betnovate. Statistically significant differences were observed in all cases in the graded response analyses between Diprosone and the other two lotions, with the exception of the 7 and 8 hour readings and the 9 hour comparison of adjacent sites of Diprosone and Celestoderm-V. The analysis of the number of sites at which blanching was exhibited indicated a statistically significantly superior blanching ability of Diprosone to Celestoderm-V from 14 hours after application and to Betnovate from 12 hours after application. No statistically significant differences were found between the blanching elicited by Betnovate and Celestoderm-V.

The overall conclusions of this experiment are presented in table 3.2.3.

TABLE 3.2.3

CONCLUSIONS OF THE COMPARISONS OF BETAMETHASONE-CONTAINING LOTIONS

OCCLUDED	UNOCCLUDED
Diprosone > Celestoderm-V	Diprosone > Celestoderm-V
Diprosone > Betnovate	Diprosone > Betnovate
Celestoderm-V = Betnovate	Celestoderm-V = Betnovate

The main conclusions that can be drawn from this experiment are that:

a) Diprosone lotion elicited statistically significantly superior blanching activity to Betnovate lotion and Celestoderm-V lotion, even though the concentration of the corticosteroid in Diprosone lotion was half that of the other two lotions. It would be expected that if these lotions were formulated to contain the same concentration of corticosteroid, the differences in the blanching activities would be even more marked. The correlation between the results obtained in blanching trials and clinical trials has been discussed in section 1.9.6 and mention was made earlier in the present discussion of the superiority of betamethasone 17,21-dipropionate over betamethasone 17-valerate in both of these bioassays. The observed superiority of betamethasone 17,21-dipropionate in the present study further serves to substantiate this widely accepted correlation.

b) There were no statistically significant differences between the blanching of Betnovate lotion and Celestoderm-V lotion. The effect of the vehicle on the release of the corticosteroid and consequent blanching response is well documented and has been discussed in section 1.6 and section 3.1. It could be expected, however, that due to the good surface coverage, skin contact and spreadability of lotions, different lotion bases should not significantly affect corticosteroid release.

3.3 COMPARISON OF DERMOVATE, BETNOVATE AND EUMOVATE CREAMS AND OINTMENTS

The three corticosteroids studied in this experiment were clobetasol propionate, betamethasone valerate and clobetasone butyrate. These corticosteroids have been extensively studied in both clinical and blanching trials. As previously discussed (section 3.1), betamethasone valerate has been used in therapy since 1964 and enjoys wide acceptance amongst physicians. Clobetasone butyrate (72,76,208, 209,245,323) and clobetasol propionate (51,76-78,90,132,138,208,209, 245,250,260,263,309,324,325) have proved to be effective dermatological agents in clinical trials (72,309,323-325) and have been shown to elicit a response in the blanching assay (51,76-78,90,132,138,208,209,245,250,260,263,324). Clobetasol pro- pionate has been found to elicit a more intense blanching response than betamethasone valerate (51,76,77,90,132,138,208,245,250,260) and clobetasone butyrate (76,208,245) and has been found to be superior to betamethasone valerate in clinical trials (324,325). The blanching response elicited by betamethasone valerate has further been found to be superior to that of clobetasone butyrate (76,208,245).

The aim of this experiment was to assess the blanching activities of locally manufactured proprietary preparations containing these corticosteroids. The preparations used were Betnovate (betamethasone valerate) cream and ointment, Dermovate (clobetasol propionate) cream and ointment and Eumovate (clobetasone butyrate) cream and ointment.

Two trials were mounted, one for creams and one for ointments. The preparations were applied to the forearms of twelve volunteers in both the occluded and unoccluded modes of application and the degree of blanching was recorded independently by three experienced observers at 7,8,9,10,12,14,16,18,28,32,52 and 56 hours after application.

Figures 3.3.1 - 3.3.4 represent the blanching profiles obtained using the %TPS versus the time in hours after application. Dermovate cream and ointment elicited maximum blanching responses at 14 hours after application in both the occluded and unoccluded modes of application, while the maximum responses elicited by Betnovate were observed at 12 hours for the cream in both modes of application and the ointment in the unoccluded mode, and at 14 hours for the ointment in the occluded mode. The maximum responses for Eumovate ointment in both modes of application were observed at 10 hours, while the occluded and unoccluded creams appeared to elicit extended maxima from 9 hours to 12 hours and from 10 hours to 14 hours respectively. Occlusion was found not to affect the time after application at which Dermovate cream and ointment, Betnovate cream and Eumovate ointment elicited maximum blanching responses. Maximal blanching was found to occur sooner in the case of Eumovate cream when applied under occlusion and occurred later when Betnovate cream was applied under occlusion. The expected increase in the blanching response of the occluded preparations was observed in all cases except Dermovate cream and Eumovate ointment. The AUC values for Dermovate cream in the occluded and unoccluded modes of application were 1765,69 and 1934,78 respectively. This similar blanching response in both modes of application could indicate a very well formulated vehicle which facilitates release of the corticosteroid without occlusion, although a good blanching response could be expected from all but poorly formulated bases containing a corticosteroid as potent as clobetasol propionate. In cases where occlusion does not markedly improve the blanching response elicited by a preparation, it should be possible to attain an optimal clinical effect without the use of the cumbersome occlusive dressing, thus rendering therapy more acceptable to the patient (296). The diminished response of Eumovate ointment under occlusion is discussed below.

In this experiment Dermovate was compared to Betnovate in all cases and Betnovate was compared to Eumovate in all cases. Dermovate was not compared to Eumovate. The results of the comparison of AUC values and chi-squared analyses for creams in the occluded mode are summarized in table 3.3.1.

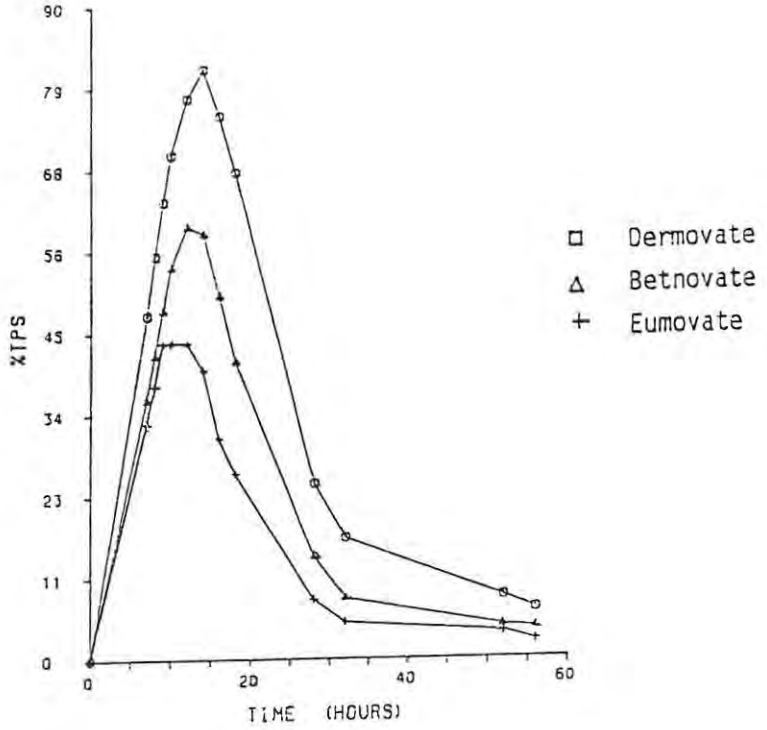


Figure 3.3.1 Blanching profiles of creams (occluded mode)

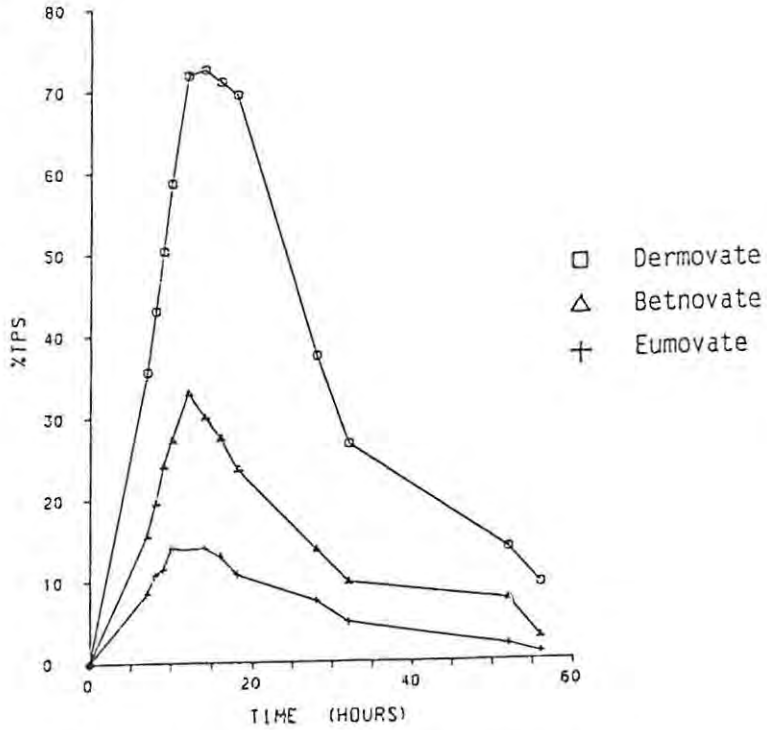


Figure 3.3.2 Blanching profiles of creams (unoccluded mode)

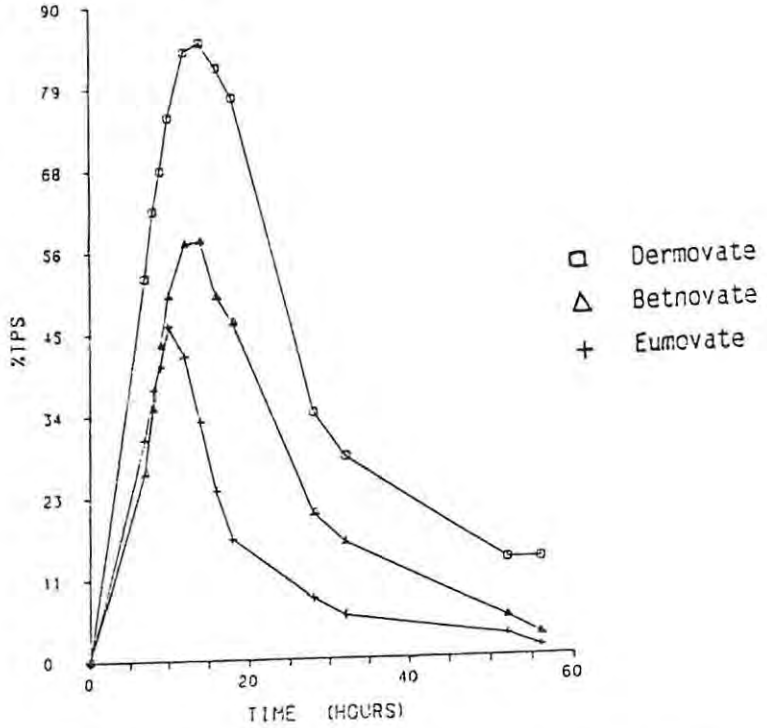


Figure 3.3.3 Blanching profiles of ointments (occluded mode)

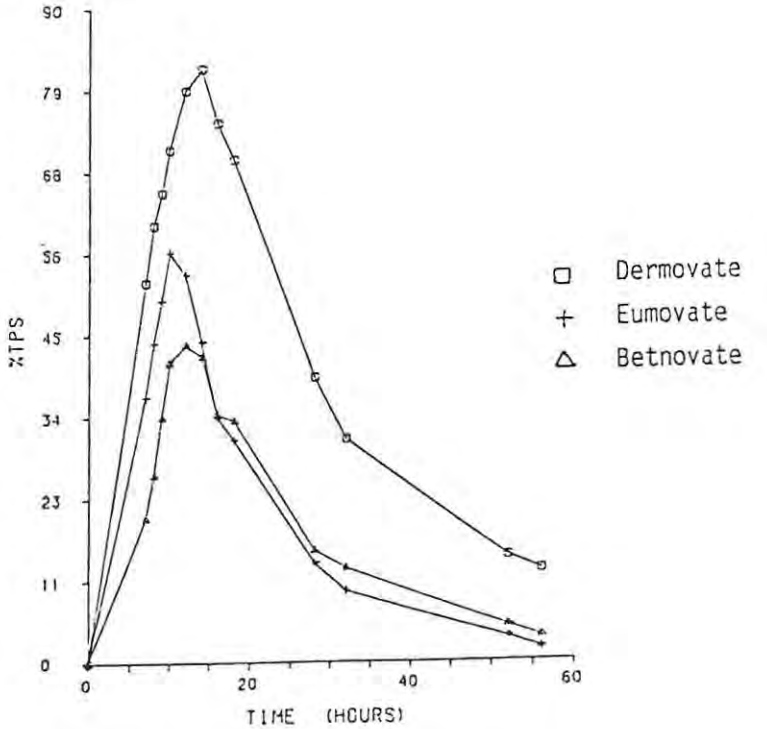


Figure 3.3.4 Blanching profiles of ointments (unoccluded mode)

TABLE 3.3.1

SUMMARY OF THE COMPARISONS OF CREAMS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate
Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate

It is clear from the summary in table 3.3.1 and from the data from which these conclusions were drawn that Dermovate cream elicited a superior blanching response to Betnovate cream in the occluded mode of application. The only deviations from the summary were the chi-squared values obtained at 10, 12 and 14 hours after application in the Yes/No analyses and the 56 hour graded response analysis. In the comparison of Betnovate with Eumovate, statistically non-significant chi-squared values were obtained at the beginning and end of the trial for the comparison of adjacent sites and graded response analyses and up to the 12 hour reading in the case of the Yes/No analysis. Overall observation of the results therefore indicated that Betnovate cream elicited a blanching response superior to that of Eumovate cream in the occluded mode of application.

Table 3.3.2 contains a summary of the results of the comparisons of AUC values and statistical analyses of the comparisons of the creams in the unoccluded mode of application.

TABLE 3.3.2

SUMMARY OF THE COMPARISONS OF CREAMS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate
Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate

A study of the AUC values and chi-squared analyses for these preparations in this mode of application indicated very clear cut superiority of the blanching responses of Dermovate cream over Betnovate cream and Betnovate cream over Eumovate cream. The only

statistically non-significant value was that of the analysis of the graded responses of Betnovate and Eumovate at the 56 hour reading. The expected increase in blanching of sites under occlusion was quite noticeable in the cases of Betnovate and Eumovate with vast differences in AUC values and %TPS values at peak times.

The results of the comparisons between the ointments in the occluded mode of application are summarized in table 3.3.3.

TABLE 3.3.3

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate
Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate	Betnovate > Eumovate

The superior blanching ability of Dermovate ointment to that of Betnovate ointment in the occluded mode of application can be seen from the AUC values and the chi-squared analyses. The only exception was the statistically non-significant value obtained for the Yes/No analysis at the 10 hour reading.

The comparison between Betnovate and Eumovate was somewhat less clear cut. Statistical analysis of the graded responses indicated no significant differences between the two preparations at the 7, 8, 9 and 10 hour readings, although the actual scores favoured Eumovate at the first two and Betnovate at the latter two readings, with all other readings favouring Betnovate. No statistically significant differences were found at the 52 hour and 56 hour readings. The comparison of adjacent sites at the 7 and 8 hour readings showed no statistically significant differences, with the 7 hour reading favouring Eumovate whereas the 8 hour reading favoured Betnovate. Statistically significant differences in favour of Betnovate were found at all the other reading times. The Yes/No analyses of the 7 and 8 hour readings favoured Eumovate, although the chi-squared values did not indicate statistically significant differences. The number of sites showing blanching at the 9, 10 and 12 hour reading

times were essentially equal for the two preparations with the remainder of the results favouring Betnovate. No statistically significant differences were found at the 14 and 56 hour readings. The %TPS values for Eumovate were greater than for Betnovate at the 7 and 8 hour readings with the values being greater for Betnovate from the 9 hour reading until the end of the trial. Eumovate ointment and Betnovate ointment could therefore be considered to have shown a similar onset of action with respect to their blanching responses until between 8 and 9 hours after application, with Betnovate eliciting a superior blanching response for the remainder of the 56 hours. Overall observation of the blanching profiles, the differences between the %TPS values obtained for the two preparations and the magnitude of the chi-squared values where significant differences were found, therefore led to the conclusion that Betnovate ointment elicited a superior blanching response to Eumovate ointment in the occluded mode of application.

A summary of the results of the comparisons of ointments in the unoccluded mode is presented in table 3.3.4.

TABLE 3.3.4

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate	Dermovate > Betnovate
Betnovate < Eumovate	Betnovate < Eumovate	Betnovate < Eumovate	Betnovate < Eumovate

The data obtained from the observation of blanching elicited by Dermovate ointment and Betnovate ointment in the unoccluded mode of application indicated an unequivocal superiority of Dermovate over Betnovate.

The comparison of Betnovate with Eumovate did, however, not give the expected results. Statistical analysis of the results indicated that Eumovate elicited a superior response to Betnovate up until the 12 hour reading in the graded response and Yes/No analyses and the 14 hour reading in the analysis of the comparison of adjacent sites.

The blanching profiles intersected at 16 hours after which no statistically significant differences were found. Eumovate ointment was therefore found to elicit a statistically significantly superior blanching response to Betnovate ointment until between 12 and 14 hours after application after which there were no statistically significant differences. It is interesting to compare the relative blanching responses of these two preparations in the occluded and unoccluded modes of application. Occlusion of the sites to which Betnovate had been applied produced the expected increase in blanching response whereas the unoccluded sites to which Eumovate had been applied elicited an overall blanching response 40% greater than the occluded sites. A reversal of rank order between these two preparations was therefore observed when applied in the two different modes. The same increase in the blanching response of Eumovate ointment when applied without occlusion has been observed in our laboratories on a previous occasion (326) and on subsequent occasions (327,section 3.5) with different volunteers, ointments from different batches and different observers having taken readings in the different trials. Some workers (76) have found Betnovate ointment to elicit a statistically significant superior blanching response to Molivate ointment (the original name for Eumovate ointment in the United Kingdom) in the unoccluded mode, and blanching elicited by Betnovate ointment was found to be superior to that of Eumovate ointment in the occluded mode of application using multiple reading times. Others (76,209) have, on the other hand, reported a lack of statistically significant differences in the blanching responses of Betnovate ointment and Eumovate ointment utilizing an occluded blanching assay with only one reading time. Wilson (299) has further reported the superior blanching ability of betamethasone 17-valerate to clobetasone butyrate in alcoholic solution. It is generally accepted that the alcoholic blanching assay should be used only as a screening procedure to give an indication of the activity of a corticosteroid and this trial and those cited above serve to reinforce the importance of performing assays on formulated products.

There do not appear to be any reports in the literature on trials assessing the blanching activity of clobetasone butyrate in both the

occluded and unoccluded modes of application during the same experiment or utilizing an internal standard which would allow inter-comparisons to be made. It is therefore not possible to compare the increased blanching response of Eumovate ointment when applied in both modes in this experiment to any other experiments. Barry and Woodford (51) have, however, found that Topilar cream applied without occlusion elicited a blanching response 35% greater than with occlusion, but offer no explanation for this superior blanching response.

The reason for the observed difference in the behaviour of Eumovate ointment when applied in the two modes of application is not obvious, but is open to speculation. There is a possibility that the corticosteroid in the formulation did not penetrate the hydrated stratum corneum to the same extent as the stratum corneum in its natural state. This is, however, an unlikely explanation as the expected increased blanching response was noted when Eumovate cream was applied under occlusion. Another possibility is that the corticosteroid was not well released from the ointment base. This may, however, have resulted in a slight difference in the blanching response from the occluded or unoccluded sites, or similar blanching with the two modes of application, but not a major increase in blanching from the unoccluded sites. Other possibilities that have been suggested (328) are that i) the act of occlusion may somehow have altered the nature of the formulation so that some substance was expelled from the bulk of the applied ointment and functioned as a barrier on the skin surface thus hindering the penetration of the corticosteroid molecules, ii) during occlusion an unknown substance penetrated from the applied ointment into the epidermis thus slowing down further passage of the corticosteroid molecules and iii) some substance with slight vasodilating properties accompanied the corticosteroid from the formulation into the dermis thus reducing the observed blanching. A combination of the above factors is naturally also possible. Another possibility is that hydration of the stratum corneum affected the partitioning of the corticosteroid between the vehicle and the skin. This would be specific for this combination of corticosteroid and base and the difference in the nature of the

cream and ointment bases would account for this effect not having been observed in the case of the occluded and unoccluded creams.

Although it is realized that every corticosteroid-containing formulation should be treated as a separate entity because of the unique interaction between the constituents of the vehicle and the corticosteroid molecule, the question arises as to why this increased blanching from unoccluded sites should have been observed for Eumovate ointment and has not been reported for any other corticosteroid-containing ointments. It is possible that one or all of these factors do occur in other formulations, but not to a large enough extent to reverse the observed effects of occlusion on the blanching response. It is worthwhile noting that the cream and ointment bases of locally manufactured Dermovate, Betnovate and Eumovate are all different (329). If any of the possible explanations in the preceding discussion caused this anomalous behaviour of Eumovate ointment, they would appear to be specific for clobetasone butyrate incorporated into this particular ointment base. Further research into the affect of occluding different ointment bases containing clobetasone butyrate is required to fully explain these results.

The superior blanching response of locally manufactured Dermovate cream and ointment to Betnovate cream and ointment correlated with a survey of international literature. Similar results have been found for alcoholic solutions (299,263) and formulated products (76,77,101, 138,145,208,209,245,250,260) containing clobetasol propionate or betamethasone valerate. Results of clinical trials in which both of these corticosteroids were used in the same trial have favoured clobetasol propionate although the differences were not statistically significant (309,324).

With respect to blanching abilities, the overall conclusions that can be drawn from this experiment are that :

a) Dermovate cream and ointment elicited a superior blanching response to Betnovate cream and ointment in both modes of application;

b) Betnovate cream elicited a greater response than Eumovate cream in both modes of application;
c) Betnovate ointment produced more blanching than Eumovate ointment in the occluded mode; and
d) Eumovate ointment elicited a superior response to Betnovate ointment in the unoccluded mode.

Direct comparisons between Dermovate and Eumovate were not made, but it is obvious from the results that Dermovate cream elicited a superior response to Eumovate cream in both modes of application and that Dermovate ointment elicited a superior blanching response to Eumovate ointment in the occluded mode. It further appears from the blanching profiles and AUC values that the same would be true for the ointments in the unoccluded mode.

3.4 COMPARISON OF EUMOVATE, ULTRALANUM AND ULTRADIL CREAMS AND OINTMENTS

Florone cream and ointment (section 3.5) are relatively new preparations that were recently marketed in South Africa for a short while. It was decided to attempt to grade these according to the potency scale of the United Kingdom MIMS (330). The assumption was made that the locally manufactured preparations would elicit similar blanching responses to those manufactured in the United Kingdom, or at least that they would fall into the same potency category. The standard preparations that were originally going to be used for that experiment were Dermovate (very potent), Nerisone (potent) and Eumovate (moderately potent), as these fall into the different potency categories (330). It was however decided, after obtaining the unusual results with Eumovate ointment (section 3,3), to attempt to find a cream and an ointment that elicited significantly less blanching than Eumovate cream and ointment.

Two separate trials were therefore mounted, one to observe the blanching elicited by Ultradil cream and Ultralanum cream compared to Eumovate cream, and one to observe the blanching elicited by the corresponding ointments. The active ingredients of the preparations used were Eumovate cream and ointment (clobetasone butyrate), Ultradil Plain cream and ointment (fluocortolone hexanoate, 0,1% and fluocortolone pivalate, 0,1%), Ultralanum cream (fluocortolone hexanoate, 0,25 % and fluocortolone pivalate, 0,25%) and Ultralanum ointment (fluocortolone, 0,25%, fluocortolone hexanoate, 0,25% and clemizole hexachlorophenate, 2,5%). Although several reports are available on the activities of various esters of fluocortolone (21,36,37,260,331) there is a paucity of information on the combinations as described above.

The preparations were applied to the forearms of twelve volunteers in both the occluded and unoccluded modes of application. Four experienced observers recorded the blanching intensity at 7,8,9,12,14,16,18,28 and 32 hours after application in the trial on creams and three observers recorded the intensity of blanching at

7,8,10,12,14,16,18,28 and 32 hours after application in the trial on ointments.

The blanching profiles obtained from this study are presented in figures 3.4.1 - 3.4.4. It is interesting to note the relatively earlier time at which maximum blanching was attained than would normally be expected. All the creams elicited maximum blanching responses 9 hours after application. A similar early peak was noted for Eumovate cream in the experiment reported in section 3.3. The apparently rapid onset of action and early maximum blanching responses could either be because no observations of the blanching responses were made at 10 hours after application or because of the tendency for corticosteroids that elicit a weak blanching response to attain maximal blanching more rapidly than those that elicit a good response (4). Ultradil and Ultralanum ointments peaked at 8 hours and 14 hours respectively in the occluded and unoccluded modes of application while the maximum blanching response for Eumovate was elicited at 12 hours after application in the occluded mode and 14 hours after application in the unoccluded mode. With reference to Ultradil it should be noted that observations were not made at 9 hours after application in this trial. The expected increase in AUC values when the preparations were applied under occlusion was noted for both Ultradil and Ultralanum creams and ointments and Eumovate cream, although the difference in the case of Ultralanum was small. The AUC for Eumovate ointment was, however, again found to be greater in the unoccluded mode (see section 3.3) giving a value of 30% greater than when applied under occlusion.

The conclusions drawn from the AUC values and the chi-squared analyses for the creams applied under occlusion are summarized in table 3.4.1.

TABLE 3.4.1

SUMMARY OF THE COMPARISONS OF CREAMS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Eumovate > Ultralanum	Eumovate > Ultralanum	Eumovate > Ultralanum	Eumovate = Ultralanum
Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil
Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum = Ultradil

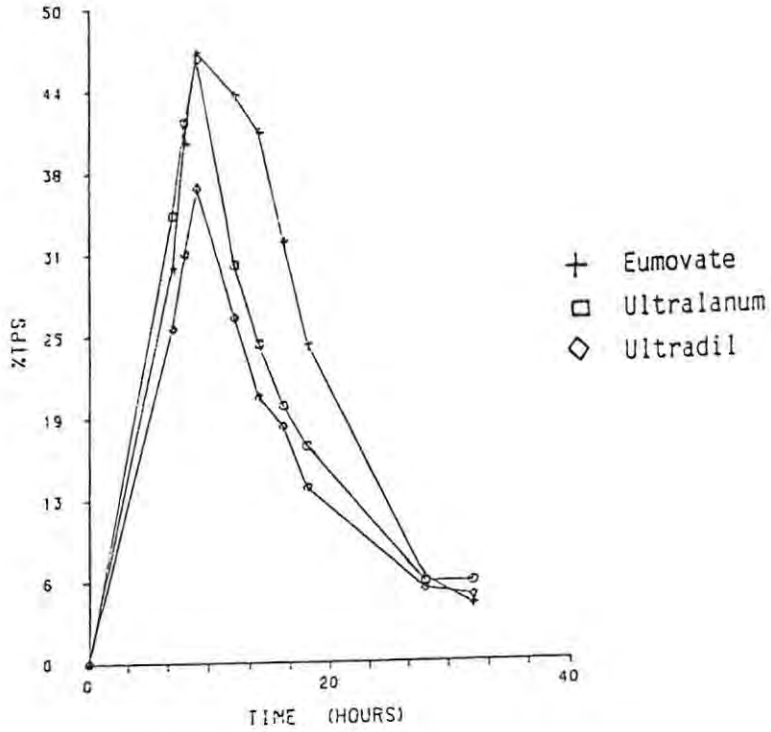


Figure 3.4.1 Blanching profiles of creams (occluded mode)

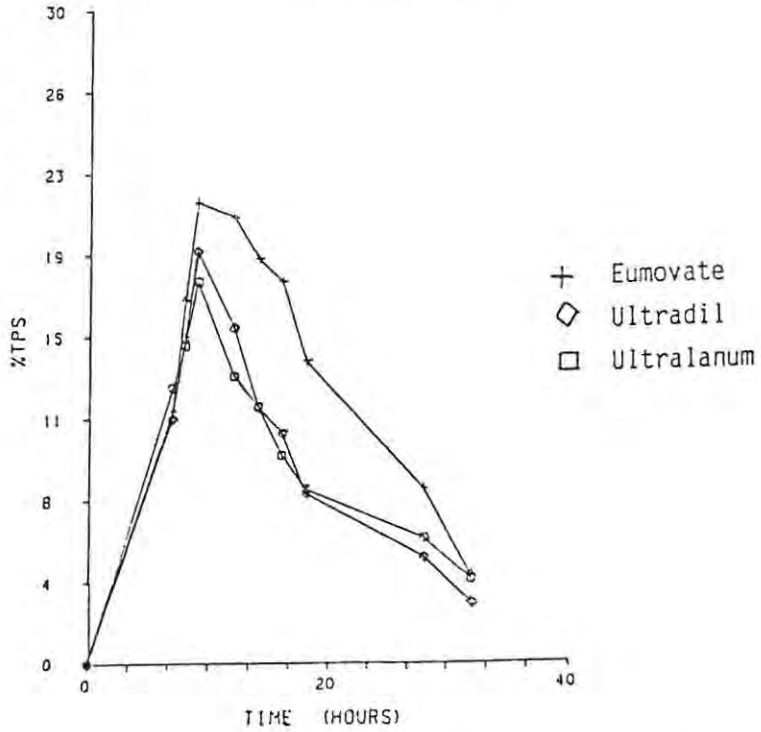


Figure 3.4.2 Blanching profiles of creams (unoccluded mode)

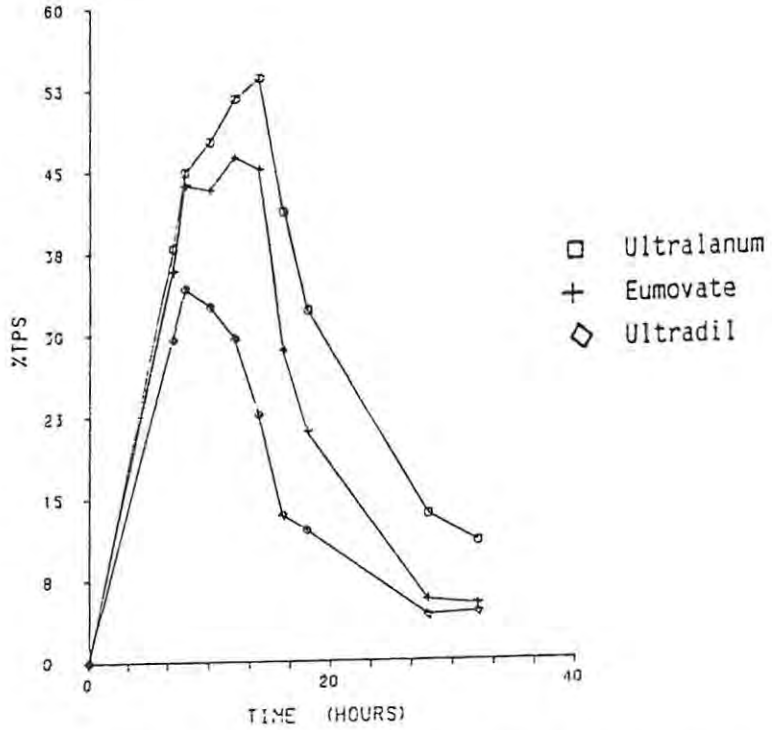


Figure 3.4.3 Blanching profiles of ointments (occluded mode)

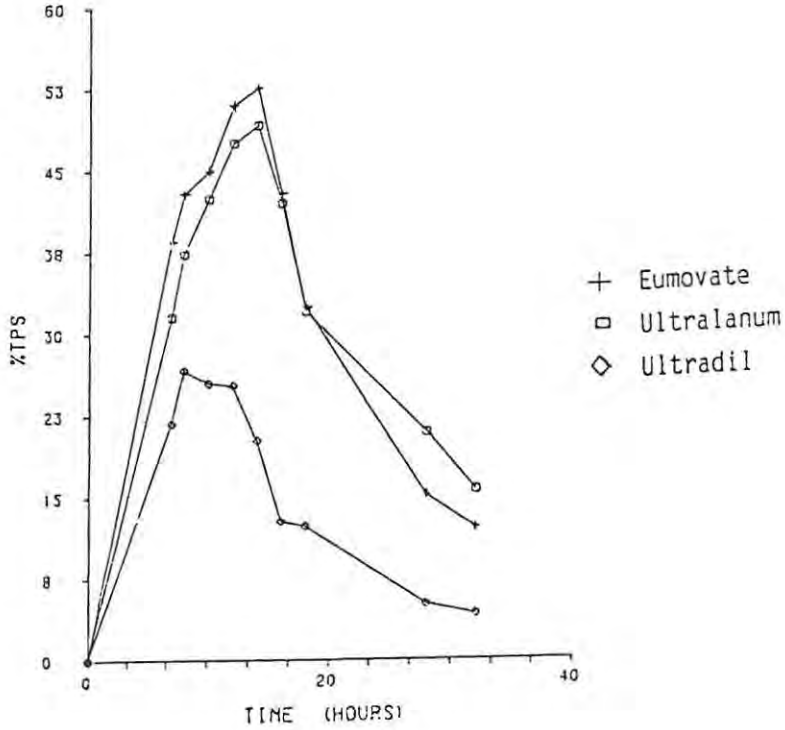


Figure 3.4.4 Blanching profiles of ointments (unoccluded mode)

The blanching profiles, AUC values and chi-squared analyses of the comparison of Eumovate and Ultralanum indicated that Eumovate elicited a superior blanching response to Ultralanum, although this was not statistically significant in all cases. Ultralanum appeared to have a slightly more rapid onset of action and to elicit a superior blanching response until the maximum %TPS was reached at 9 hours after application. It is worth noting that the maximum %TPS attained by these two products was almost identical. The blanching response of Ultralanum then diminished more rapidly than that of Eumovate, accounting for the noticeably larger AUC obtained for Eumovate. Statistically significant superiority was obtained for Eumovate between the 12 and 18 hour readings (inclusive) in the comparison of adjacent sites and graded response analyses and at 16 and 18 hours for the Yes/No analysis.

Statistically significant differences in favour of Eumovate over Ultradil were obtained at all reading times (except towards the end of the trial where blanching was minimal) in both the comparison of adjacent sites and graded response analyses. Significant differences were found at the 14, 16 and 18 hour readings for the Yes/No analysis. The onset of action of Ultradil was less rapid than for Eumovate and Ultralanum although maximum blanching occurred at the same time, *viz.* 9 hours after application. The maximum blanching for, and the AUC of Ultradil were, however, substantially lower than for the other two preparations. Chi-squared analyses of the results show that Ultradil elicited a significantly inferior blanching response to Ultralanum at the 7, 8, 9 and 12 hour readings for the comparison of adjacent sites, the 7, 8 and 9 hour readings for the analysis of the graded response and the 14 hour reading for the Yes/No analysis. All the other readings (with the exception of the 32 hour reading) indicated a slightly superior blanching response of Ultralanum to Ultradil. The probably spurious increase in blanching at the last time of observation has been observed in our laboratories on a small number of occasions and is most likely due to decreased accuracy in assessing the very small blanching responses. The average %TPS in this case was 5,15.

The results of the comparison of AUC values and chi-squared analyses for the creams in the unoccluded mode of application are summarized in table 3.4.2.

TABLE 3.4.2

SUMMARY OF THE COMPARISONS OF CREAMS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Eumovate > Ultralanum	Eumovate > Ultralanum	Eumovate > Ultralanum	Eumovate > Ultralanum
Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil
Ultralanum = Ultradil	Ultralanum = Ultradil	Ultralanum = Ultradil	Ultralanum = Ultradil

Observation of the blanching profiles shows that Eumovate cream induced a longer acting blanching response than the other two preparations in the unoccluded mode of application, a similar finding to that of the occluded mode. The %TPS attained for Ultralanum at the 7 hour reading was slightly higher than that for Eumovate after which Eumovate obtained a higher %TPS. All three methods of statistical analysis indicated that the blanching response of Eumovate was superior to that of Ultralanum from 12 hours after application to 18 hours after application. The comparison of adjacent sites at 28 hours was also statistically significantly greater in favour of Eumovate.

The comparison of Eumovate to Ultradil gave very similar results to that of Eumovate and Ultralanum, the only differences being that the blanching response of Ultradil was at no time superior to that of Eumovate and that the 28 hour chi-squared analysis of the comparison of adjacent sites did not indicate a significant difference in favour of Eumovate.

The blanching responses elicited by Ultradil and Ultralanum were essentially equal, as indicated by the shapes of the blanching curves, the almost identical AUC values and the absence of significant differences in the chi-squared analyses.

Table 3.4.3 contains a summary of the results of the comparisons of AUC values and statistical analyses of the comparisons of the ointments in the occluded mode of application.

TABLE 3.4.3

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Eumovate < Ultralanum	Eumovate < Ultralanum	Eumovate < Ultralanum	Eumovate < Ultralanum
Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil	Eumovate = Ultradil
Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum > Ultradil

Statistically significant superior blanching responses of Ultralanum to Eumovate were noted mainly in the latter hours of the trial, *i.e.* from 12 hours onwards for the comparison of adjacent sites and from 16 hours onwards for the graded response and Yes/No analyses.

Eumovate was found to elicit a statistically significantly superior response to Ultradil until the 28 hour reading. The number of sites exhibiting blanching at the reading times other than 14, 16 and 18 hours after application were not statistically different which accounts for the conclusion depicted in the summary in table 3.4.3. The low sensitivity of this particular test, however, prevents this from changing the conclusion that Eumovate elicited a statistically significantly superior blanching response to Ultradil.

A study of the blanching profiles, AUC values and results of the chi-squared analyses indicates very clearly that Ultralanum elicited a statistically significantly superior response to Ultradil, the only deviations from the statistically significant values obtained in the chi-squared analyses being those values obtained from the 7, 8, 10 and 12 hour readings of the Yes/No analysis.

The results of the comparisons between the ointments in the unoccluded mode of application are summarized in table 3.4.4.

TABLE 3.4.4

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Eumovate = Ultralanum	Eumovate = Ultralanum	Eumovate = Ultralanum	Eumovate = Ultralanum
Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil	Eumovate > Ultradil
Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum > Ultradil	Ultralanum > Ultradil

No statistically significant differences were found between the blanching elicited by Eumovate and Ultralanum apart from the superior result in favour of Ultralanum at the 28 hour reading of the graded response analysis and the 28 and 32 hour readings of the analysis of the comparison of adjacent sites. Although most of the chi-squared values are not statistically significant it is interesting to note that the blanching was superior in the case of Eumovate until 18 hours after application, after which Ultralanum elicited a superior response.

Chi-squared analyses of the comparisons between Ultradil and the other two preparations show that Ultradil elicited a statistically significant inferior blanching response at all times with the exception of the Yes/No comparison between Ultradil and Ultralanum at the 8 hour reading.

The overall conclusions of this experiment are presented in table 3.4.5.

TABLE 3.4.5

CONCLUSIONS OF THE COMPARISONS OF PREPARATIONS USED IN THIS EXPERIMENT

Creams (occluded)	Eumovate > Ultralanum > Ultradil
Creams (unoccluded)	Eumovate > Ultralanum = Ultradil
Ointments (occluded)	Ultralanum > Eumovate > Ultradil
Ointments (unoccluded)	Eumovate = Ultralanum > Ultradil

It can be seen from table 3.4.5 that there was no consistent rank order for these preparations in both the occluded and unoccluded modes of application. As previously mentioned, there is a paucity of information in the literature referring to blanching and clinical trials comparing the products used in this experiment. Reports of experiments in which blanching responses of creams containing fluocortolone pivalate and hexanoate 0,1% and 0,25% (equivalent to Ultradil and Ultralanum respectively as used in this experiment, but not manufactured in South Africa) have, contrary to the findings reported here, indicated no statistically significant differences between the blanching abilities of these preparations (4,51). It

has been suggested (see section 1.8.3) that occlusion of the sites may mask the effect of the vehicle, especially a vehicle that has not been designed to optimally release the corticosteroid. This may account for the fact that Ultralanum cream elicited a superior blanching response to Ultradil cream in the occluded but not the unoccluded mode of application. In the case of ointments, preparations containing 0,1% of the hexanoate and pivalate esters of fluocortolone (Ficoid-2 and Ultradil) have been found to elicit similar blanching responses (132), while a preparation equivalent to Ultralanum (South Africa) was found to elicit a statistically significant superior response to Ficoid-2 and Ultradil (132). This corresponds to the findings of the present experiment.

The aim of the present experiment was to find a preparation that elicited a significantly lower blanching response than Eumovate. Ultralanum met this requirement in all cases with the exception of the occluded ointment, which was probably due to a combination of the previously discussed anomalous behaviour of Eumovate ointment when applied without occlusion, and the small difference in the blanching responses of Ultralanum when applied with and without occlusion. The significantly inferior blanching response of Ultradil when compared to Eumovate is however unquestionable. It was therefore decided to include Ultradil as an example of a moderately potent preparation in the experiment performed to ascertain the potency category of Florone.

3.5 RANKING OF FLORONE CREAM AND OINTMENT

Florone (diflorasone diacetate) cream and ointment are relatively new preparations that were recently marketed in South Africa for a short while. This experiment served to rank Florone according to the potency scale in the United Kingdom MIMS (330). Available literature on diflorasone diacetate (251,305) indicated that Florone would not fall into the same category as hydrocortisone and hydrocortisone acetate (mildy potent) which led to the use of Dermovate cream and ointment (very potent), Nerisone cream and ointment (potent) and Ultradil cream and ointment (moderately potent) as standards for this experiment. The uses and efficacies of Dermovate (clobetasol propionate) and Ultradil (fluocortolone hexanoate and pivalate) have been discussed in sections 3.3 and 3.4 respectively. Nerisone contains diflucortolone valerate and is available as a cream, an ointment and a fatty ointment. The clinical efficacy (37,309,332,333) and blanching ability (10,78,138, 161,163,245,250) of this corticosteroid have been well documented. The active ingredient of Florone is diflorasone diacetate which has also been found to be effective clinically (251,305,334) and to elicit a good response in the blanching assay (251).

This experiment consisted of two trials, one on creams and one on ointments. Each trial was carried out in both the occluded and unoccluded modes of application on the forearms of twelve volunteers. The degree of blanching was assessed independently by three experienced observers at 7,8,9,10,12,14,16,18,28,32,52 and 56 hours after application.

The blanching profiles obtained are depicted in figures 3.5.1 - 3.5.4. It can be seen from these profiles that maximal blanching was elicited between 12 and 14 hours after application in all cases, except for Nerisone cream in the unoccluded mode of application which attained maximal blanching at 10 hours, and Ultradil cream and ointment which attained maximal blanching between 9 and 10 hours in both modes of application. The expected increase in the blanching response due to occlusion was observed in all the ointments and in

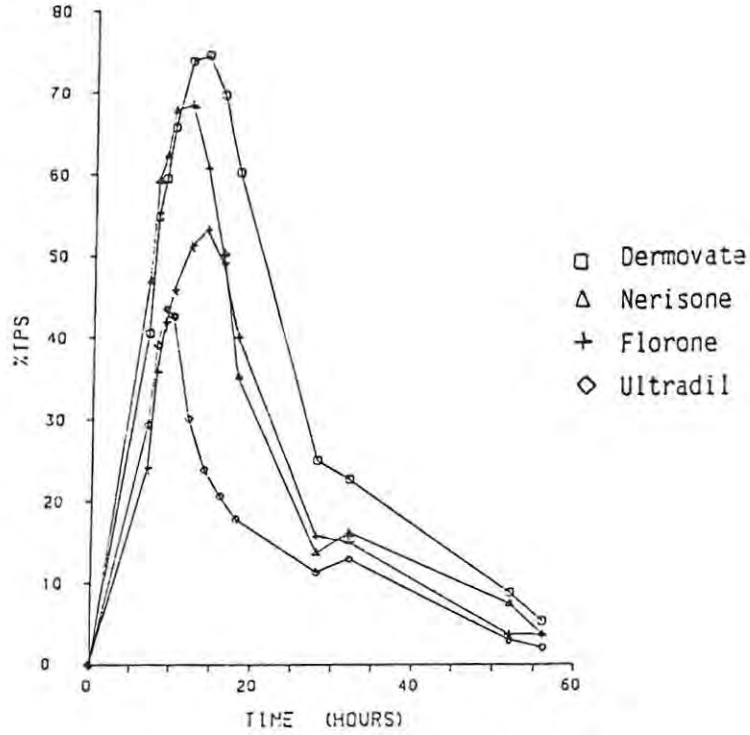


Figure 3.5.1 Blanching profiles of creams (occluded mode)

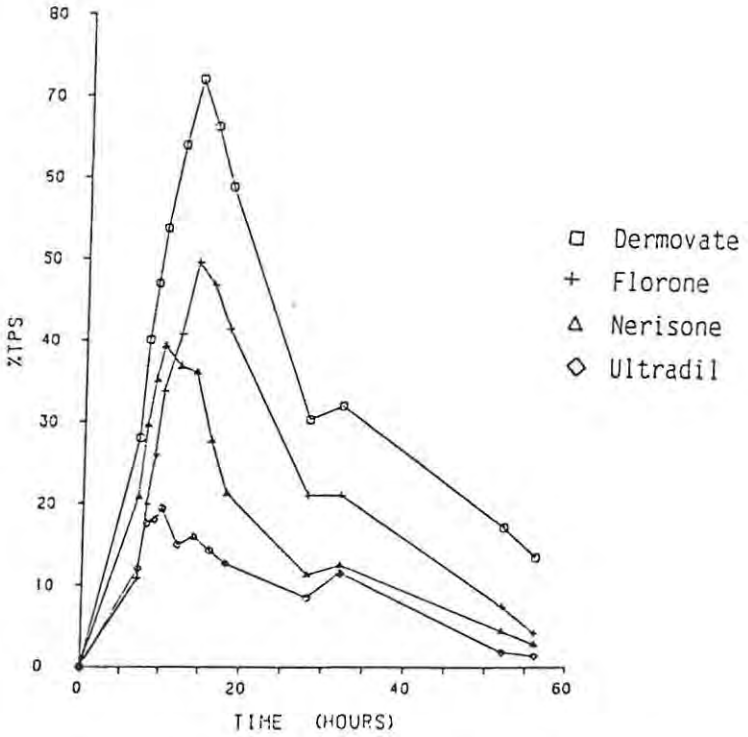


Figure 3.5.2 Blanching profiles of creams (unoccluded mode)

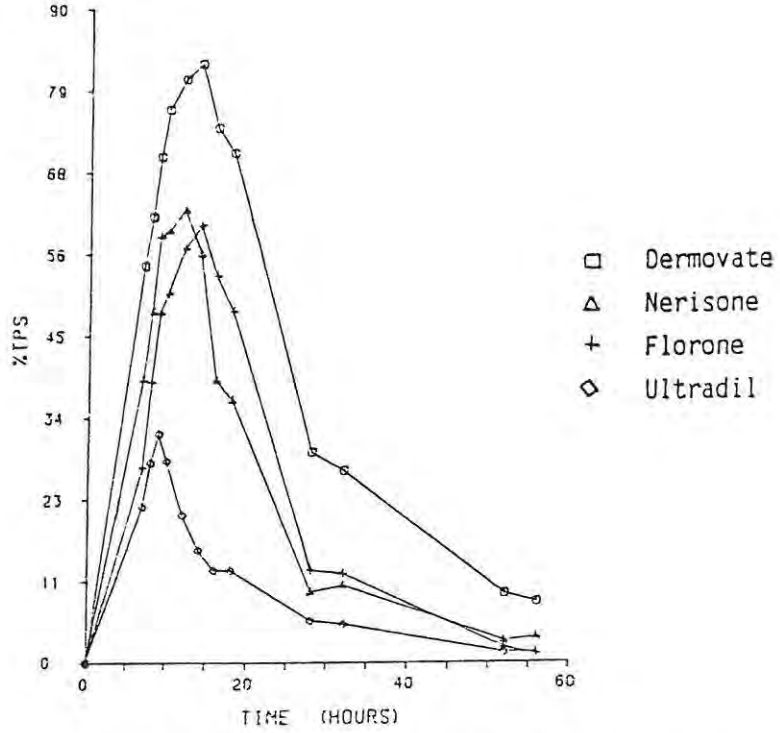


Figure 3.5.3 Blanching profiles of ointments (occluded mode)

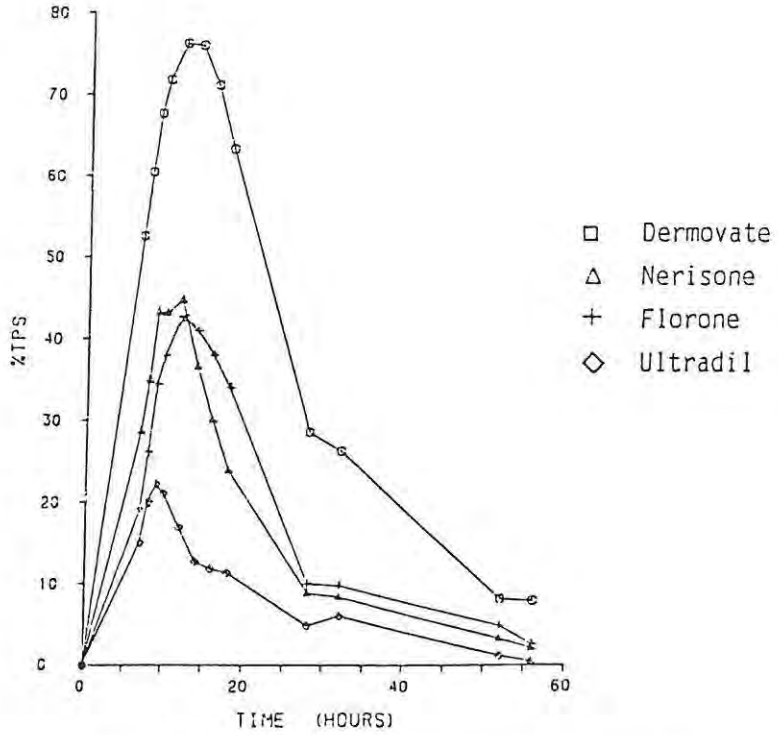


Figure 3.5.4 Blanching profiles of ointments (unoccluded mode)

Nerisone cream and Ultradil cream. The AUC values for Florone cream and Dermovate cream were essentially unaffected by occlusion of the application sites. The irregularities at the 28 and 32 hour readings in some of the blanching profiles (especially in the case of the creams in the present experiment) have been noted in our laboratories on a small number of occasions, but are hard to explain. Although a smooth curve at these points may have resulted in small changes of AUC and chi-squared values, it is felt that the overall conclusions with respect to differences in intensities of blanching elicited by the preparations would have been the same.

The results of the comparison of AUC values and chi-squared analyses for creams in the occluded mode are summarized in table 3.5.1.

TABLE 3.5.1

SUMMARY OF THE COMPARISONS OF CREAMS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Florone < Dermovate	Florone < Dermovate	Florone < Dermovate	Florone = Dermovate
Florone < Nerisone	Florone < Nerisone	Florone < Nerisone	Florone = Nerisone
Florone > Ultradil	Florone > Ultradil	Florone > Ultradil	Florone > Ultradil

A study of table 3.5.1 and the data from which these conclusions were drawn shows that Dermovate cream elicited a blanching response that is statistically significantly superior to that of Florone cream in the occluded mode of application. The only deviations from this were the chi-squared analyses of the graded response and comparison of adjacent sites at the 56 hour reading time and several readings in the Yes/No analysis. All the readings at which no statistically significant differences were found did, however, indicate that Dermovate elicited a superior response to Florone.

The comparison between Florone and Nerisone was, however, not as clear cut. In the Yes/No analysis statistically significant differences were only found at the 7, 8 and 52 hour readings, all in favour of Nerisone. The other two modes of analysis indicated statistically significant differences until the 14 hour reading (inclusive), also in favour of Nerisone. Nerisone was found to have

a more rapid onset of action than Florone and exhibited a maximum blanching response 2 hours earlier than Florone after which blanching decreased at a similar rate for the two preparations. The 28 and 32 hour readings of Nerisone have made comparison after the 14 hour reading difficult. A larger response at 28 hours and/or a smaller response at 32 hours would have resulted in a smooth curve. Had the smoothness of the curve been due to a greater response at 28 hours, the Nerisone curve would probably have indicated a superior blanching response throughout the trial, whereas a lower response at 32 hours would have resulted in the curve for Nerisone falling below that of Florone after the crossover point. The shapes and relative positions of the curves, however, indicated that this would not have resulted in statistically significant differences, thus not affecting the conclusion that Nerisone exhibited a superior blanching response to Florone.

Ultradil, in the experiment discussed in section 3.4, was found to exhibit an early maximum response with a gradual decrease of the blanching response. A similar pattern was noted in the present experiment resulting in no statistically significant differences between Ultradil and Florone until after the 10 hour reading. All chi-squared analyses then showed statistically significant differences in favour of Florone until (and including) 18 hours after application with the Yes/No and comparison of adjacent sites analyses further showing statistically significant differences at the 28 hour and 32 hour readings respectively. It can be seen from figure 3.5.1 that the curves were almost superimposable until the 9 hour reading after which Florone elicited a superior response.

Table 3.5.2 contains a summary of the results of the comparisons of the AUC values and statistical analyses of the comparisons of the creams in the unoccluded mode of application.

TABLE 3.5.2

SUMMARY OF THE COMPARISONS OF CREAMS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Florone < Dermovate	Florone < Dermovate	Florone < Dermovate	Florone < Dermovate
Florone > Nerisone	Florone > Nerisone	Florone > Nerisone	Florone > Nerisone
Florone > Ultradil	Florone > Ultradil	Florone > Ultradil	Florone > Ultradil

The superior blanching response of Dermovate to Florone is again obvious from a study of the AUC values, the blanching profiles and the chi-squared values of all three forms of comparison. Statistically significant differences in favour of Dermovate were noted at all the readings in the comparison of adjacent sites and graded response analyses. No statistically significant differences were noted at the 14, 16, 18 and 28 hour readings in the Yes/No analyses which could be expected due to the similar number of sites showing blanching at these times.

The comparison of Florone and Nerisone was however found to be somewhat more complicated. The AUC values for Florone cream in the occluded and unoccluded modes of application were found to be very similar (1132,63 and 1160,18 respectively). It was, however, noticed that the blanching response in the occluded mode was substantially greater than in the unoccluded mode until the time of maximum blanching with a gradual and similar decrease in blanching until 18 hours after application. The blanching response of the preparation applied under occlusion then appeared to decrease much more rapidly than that of the open application. The similar blanching response elicited with and without occlusion probably indicates a well formulated vehicle that releases the steroid equally well irrespective of the mode of application. Nerisone, on the other hand, showed the normal decrease in the blanching response when applied without occlusion. This has led to a different picture in the comparison of these two preparations in the unoccluded mode to that reported above in the occluded mode. Nerisone was found in the unoccluded mode to have a more rapid onset of action than Florone and to attain maximal blanching 10 hours after application. Statistically significant differences in favour of Nerisone were therefore found until 10 hours in the analysis of the comparison of adjacent sites, and until 9 hours in the graded response and Yes/No analyses. Statistically significant differences in favour of Florone were then found in the analysis of the comparison of adjacent sites from 14 to 52 hours, in the graded response analysis from 12 to 32 hours and in the Yes/No analysis from 16 to 32 hours after application. The difference in the AUC values, the larger number of

readings at which statistically significant differences were found and the magnitude of the chi-squared values at these readings have, however, led to the conclusion that Florone cream exhibited a superior blanching response to Nerisone cream in the unoccluded mode of application.

Florone cream was found to elicit a statistically significantly superior blanching reponse to Ultradil at most of the reading times. The chi-squared values indicate that no statistically significant differences were found in the comparison of adjacent sites at the 7 and 8 hour readings, the comparison of the blanching scores at the 7 and 56 hour readings and the Yes/No analysis at the 7, 8, 9 and 10 hour readings. The differences observed at the 8 hour graded response analysis deserves some comment. The %TPS values obtained for Florone and Ultradil at this reading time were very similar, *viz.* 19,91 and 17,59 respectively. The chi-squared analysis, however, indicated statistically significant differences because of the substantially larger number of 2's observed in the case of Florone although the influence that this had on the %TPS value was countered by the larger number of 1's observed in the case of Ultradil. The number of 1's for Florone and Ultradil were 53 and 74 respectively and the number of 2's were 15 and 1 respectively. The number of 0's, 3's and 4's were very similar for both preparations.

The results of the comparisons between the ointments in the occluded mode of application are summarized in table 3.5.3.

TABLE 3.5.3

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE OCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Florone < Dermovate	Florone < Dermovate	Florone < Dermovate	Florone < Dermovate
Florone = Nerisone	Florone = Nerisone	Florone = Nerisone	Florone = Nerisone
Florone > Ultradil	Florone > Ultradil	Florone >Ultradil	Florone > Ultradil

It can be seen from the blanching profiles, the AUC values and the chi-squared analyses that Dermovate ointment elicited a statistically significant superior blanching response to Florone ointment in the

occluded mode of application. The chi-squared values of the Yes/No analysis indicated that there were no statistically significant differences in the number of sites exhibiting blanching from 8 hours to 18 hours after application.

The AUC values obtained for Florone and Nerisone were found to be very similar. It can be seen from the blanching profiles that Nerisone exhibited a superior blanching response to Florone until the 12 hour reading and after the 32 hour reading. Chi-squared analyses of the various forms of comparison indicated several statistically significant differences which were in favour of Nerisone until the 12 hour reading and after the 32 hour reading and in favour of Florone between these times. Nerisone therefore elicited a superior blanching response in the initial stages of the trial with Florone exhibiting superior blanching in the latter stages. The similar intensity of the maximum blanching responses and the similar shapes of the blanching profiles have, however, led to the conclusion that the overall blanching responses elicited by Florone ointment and Nerisone ointment in the occluded mode of application were equivalent.

It is quite obvious from a study of the relevant data that Florone exhibited a superior blanching response to Ultradil in this mode of application. Deviations from the summary in Table 3.5.3 were only noticed at the 7, 52 and 56 hour graded response analysis, the 52 and 56 hour analysis of the comparison of adjacent sites and the 8, 52 and 56 hour comparisons of the number of sites that exhibited blanching.

A summary of the results of the comparisons of ointments in the unoccluded mode of application is presented in table 3.5.4.

TABLE 3.5.4

SUMMARY OF THE COMPARISONS OF OINTMENTS IN THE UNOCCLUDED MODE OF APPLICATION

AUC	COMPARISON OF ADJACENT SITES	GRADED RESPONSE	YES/NO
Florone < Dermovate	Florone < Dermovate	Florone < Dermovate	Florone < Dermovate
Florone = Nerisone	Florone = Nerisone	Florone = Nerisone	Florone = Nerisone
Florone > Ultradil	Florone > Ultradil	Florone > Ultradil	Florone > Ultradil

It is again obvious from the relevant data that Dermovate elicited a statistically significantly superior blanching response to Florone. Statistically significant differences were found at all reading times in all the methods of comparison with the exception of the 9 to 14 hour Yes/No analyses.

The comparison of Florone ointment and Nerisone ointment in the unoccluded mode was similar to that in the occluded mode of application. Statistically significant differences in favour of Nerisone were found until 10 hours after application in the comparison of adjacent sites and until 9 hours in the graded response analysis. Fewer statistically significant differences were found in favour of Florone in the unoccluded than in the occluded mode, although the data indicated that Florone elicited a superior blanching response to Nerisone after the crossover point at between 12 and 14 hours after application. Statistically significant differences in the comparison of the number of sites eliciting blanching were only found at the 7, 16 and 18 hour readings. It can, therefore, similarly be concluded that Nerisone ointment elicited a superior blanching response in the initial stages of the trial with Florone exhibiting superior blanching in the latter stages. The same reasons as mentioned in the case of the occluded ointments led to the conclusion that these two ointments elicited an equal blanching response in the unoccluded mode of application.

The blanching response elicited by Florone was found to be superior to that of Ultradil in all cases except at the beginning and end of the trial. Very few statistically non-significant differences were found.

A summary of the comparative blanching abilities of the preparations used in this experiment can be found in table 3.5.5.

TABLE 3.5.5

CONCLUSIONS OF THE COMPARISONS OF THE PREPARATIONS USED IN THIS EXPERIMENT

Creams (occluded)	Florone < Dermovate;	Florone < Nerisone;	Florone > Ultradil
Creams (unoccluded)	Florone < Dermovate;	Florone > Nerisone;	Florone > Ultradil
Ointments (occluded)	Florone < Dermovate;	Florone = Nerisone;	Florone > Ultradil
Ointments (unoccluded)	Florone < Dermovate;	Florone = Nerisone;	Florone > Ultradil

A study of the blanching profiles and the various methods of analysis indicated that Florone elicited an inferior response to that elicited by Dermovate and therefore did not fall into the same category as Dermovate, *viz.* very potent. Florone was conversely found to elicit a blanching response superior to that of Ultradil although the differences were not as great as between Dermovate and Florone. The results of the comparison of Florone cream and Nerisone cream were reversed for the two modes of application and equal in both modes of application for the ointments. It is therefore concluded that although Florone may fall into the upper limit of the moderately potent group of corticosteroid-containing preparations (Ultradil falls into this group) it is more likely that it falls, with Nerisone, into the potent group of corticosteroid-containing preparations.

3.6 SUMMARY, CONCLUSIONS AND FUTURE AREAS OF RESEARCH

The first experiment in this study (section 3.1) indicated that the blanching response elicited by betamethasone 17-valerate varied with different proprietary semi-solid formulations and that the rank order of blanching further depended on whether the application sites were occluded or unoccluded. In the second experiment (section 3.2) two proprietary lotions containing betamethasone 17-valerate were found to elicit similar blanching to each other and inferior blanching to a lotion containing betamethasone 17,21-dipropionate.

In the trials performed on Dermovate cream and ointment, Betnovate cream and ointment and Eumovate cream and ointment (section 3.3), the blanching responses were found to correlate with advertising claims of therapeutic efficacy in the medical media in the cases of creams applied in both modes of application and ointments applied in the occluded mode, but a reverse in rank order was found between Betnovate ointment and Eumovate ointment when applied in the unoccluded mode. Possible explanations for the anomalous behaviour of Eumovate ointment when applied with and without occlusion were discussed.

The results of the fourth experiment (section 3.4) indicated that Ultradil elicited an inferior blanching response to Eumovate and Ultralanum. Ultradil was therefore chosen as an example of a moderately potent preparation for use in the final experiment, which was performed to ascertain the potency category of Florone cream and ointment. Evaluation of the results of the final experiment (section 3.5) indicated that Florone cream and ointment most probably fall into the potent group of corticosteroid-containing preparations as classified in the United Kingdom MIMS.

In view of the literature studied and observations made during the period of this study, many future possible areas of research have become evident. The reasons for the multiple maxima and minima in the blanching profiles in the presentation of the results and discussions of individual trials are currently under investigation in

our laboratories. These irregularities could be a result of the inaccuracy of the visual assessment of the blanching responses or sudden substantial changes in ambient temperature. The former possibility could be overcome by the development of a convenient instrumental method of measuring the degree of blanching. An automated quantitative instrumental method of measuring blanching responses would, in addition, reduce the tedium of the many visual observations that are required at present.

Closely related to the above is an investigation of the consistency of standards used by observers to assign a score to a blanching response. This is also currently being studied in our laboratories together with an examination of the minimum number of observers and volunteers required to partake in a trial so as to ensure the statistical validity of the results of the human blanching assay.

Biological variability is generally well accepted in *in vivo* pharmacological and bioavailability studies, but does not appear to have been extensively studied with respect to corticosteroid-induced blanching. Some aspects of biological variability between volunteers and within volunteers, but over a number of trials, are currently under investigation in our laboratories.

Although many hypotheses prevail, the exact mechanism of percutaneous penetration of corticosteroids remains unresolved. Better understanding of this would possibly allow more accurate prediction of the extent of penetration of novel corticosteroid molecules. Related to this are investigations into the mechanisms by which the penetration of corticosteroids is affected by the various adjuvants and excipients used in semi-solid dosage forms.

Since the mechanism of blanching is not fully understood, more investigations into this area are required. Knowledge of the mechanism of blanching may reveal why this phenomenon is observable in some individuals and not in others, whether metabolism of the corticosteroid in the skin has any effect on blanching and whether the concomitant use of other drugs affects blanching. The answers

to these questions should allow more reproducible selection of volunteers for trials.

The introduction of highly potent topical corticosteroid preparations has given rise to concern relating to systemic absorption and consequent undesirable side effects. Research designed to determine whether a correlation exists between the blanching ability and serum/plasma concentration of applied corticosteroid could be useful in toxicity studies (303).

APPENDIX 1

PREPARATIONS USED IN THIS STUDY

Betnovate cream, ointment and lotion (0,1% betamethasone 17-valerate) Glaxo (Pty) Ltd., P O Box 485, Germiston, 1400.

Celestoderm-V cream, ointment and lotion (0,1% betamethasone 17-valerate) Scherag (Pty) Ltd., P O Box 46, Isando, 1600.

Dermovate cream and ointment (0,05% clobetasol propionate) Glaxo (Pty) Ltd., P O Box 485, Germiston, 1400.

Diprosone lotion (0,05% betamethasone dipropionate) Scherag (Pty) Ltd., P O Box 46, Isando, 1600.

Eumovate cream and ointment (0,05% clobetasone butyrate) Glaxo (Pty) Ltd., P O Box 485, Germiston, 1400.

Florone cream and ointment (0,05% diflorasone diacetate) Upjohn (Pty) Ltd., P O Box 246, Isando, 1600.

Nerisone cream and ointment (0,01% diflucortolone valerate) Schering A.G., P O Box 10259, Johannesburg, 2000.

Persivate cream and ointment (0,1% betamethasone 17-valerate) Lennon Ltd., P O Box 3371, Johannesburg, 2000.

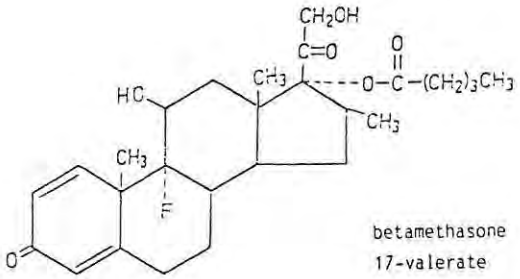
Ultradil Plain cream and ointment (0,1% fluocortolone hexanoate and 0,1% fluocortolone pivalate) Schering A.G., P O Box 10259, Johannesburg, 2000.

Ultralanum cream (0,25% fluocortolone hexanoate and 0,25% fluocortolone pivalate) Schering A.G., P O Box 10259, Johannesburg, 2000.

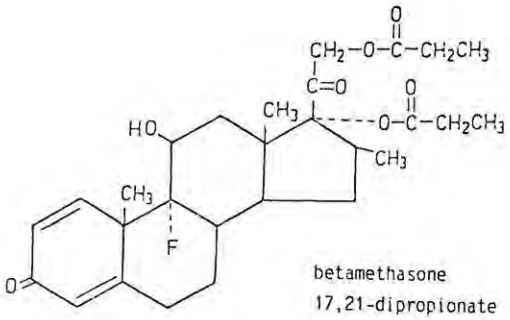
Ultralanum ointment (0,25% fluocortolone, 0,25% fluocortolone hexanoate and 2,5% clemizole hexachloropherate) Schering A.G., P O Box 10259, Johannesburg, 2000.

APPENDIX 2

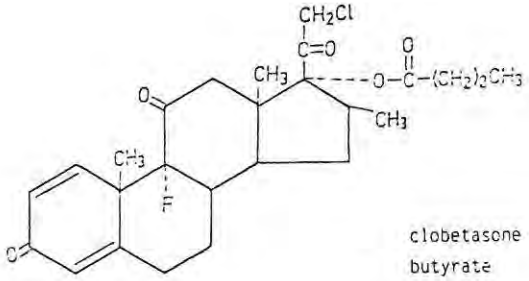
STRUCTURES OF CORTICOSTEROIDS STUDIED IN THIS WORK



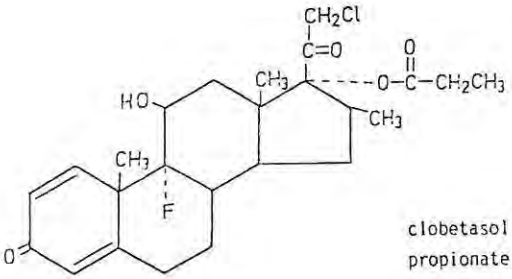
betamethasone
17-valerate



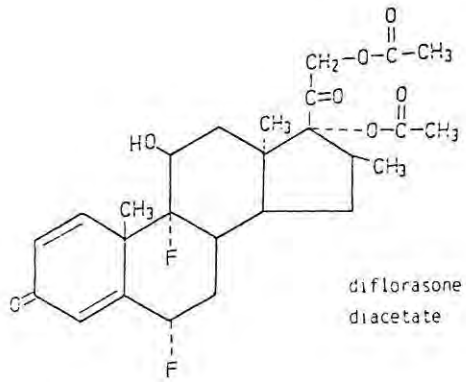
betamethasone
17,21-dipropionate



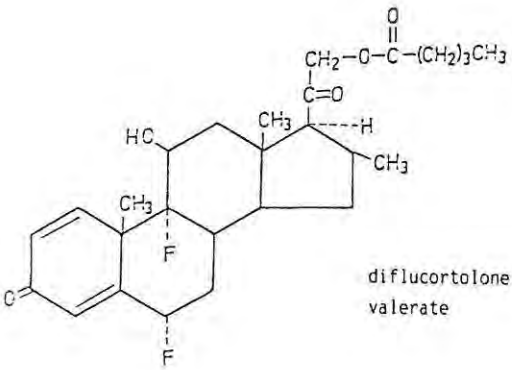
clobetasone
butyrate



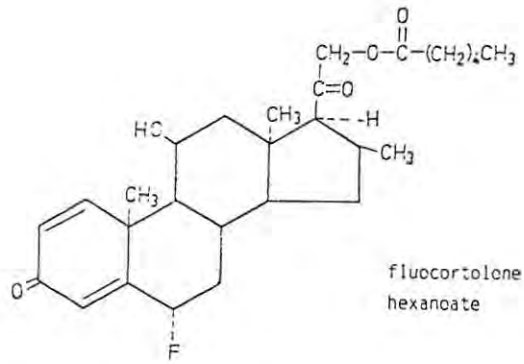
clobetasol
propionate



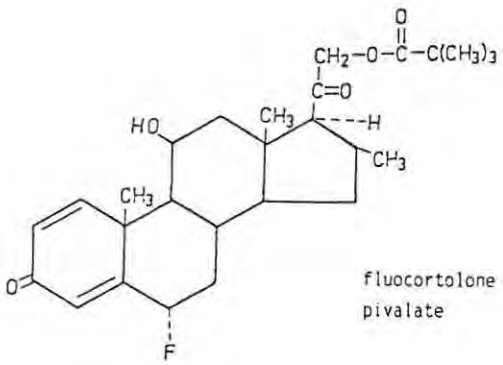
diflorasone
diacetate



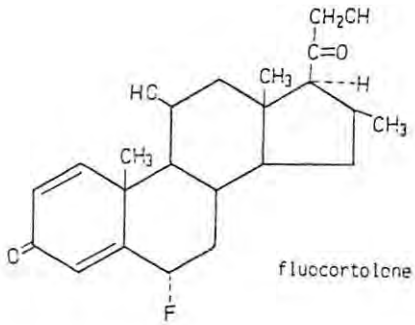
diflucortolone
valerate



fluocortolone hexanoate



fluocortolone pivalate



fluocortolone

APPENDIX 3

This appendix comprises the computer programme (referred to in section 2.3) compiled for use with the Wang 2200 Basic Desk Top Mini-Computer.

```
10 DIM C$(8),O$(8),S$(8),Z$12,D$14,S1$1,P$12,B$3
20 DIM T1(8,5),T2(12,4),E(12),P1(8,12),Y(12),D1$1
30 DIM R1(8,12),R2(8,12),X$1
40 SELECT PRINT 005
50 REM INITIALIZATION
60 REM --------------
70 INPUT "WERE THE SITES OCCLUDED OR UNOCCLUDED",Z$
80 INPUT "ENTER THE TIME OF DATA COLLECTION",T
90 INPUT "NUMBER OF CHARTS USED",N1
100 IF N1 ] 4 THEN 2520
110 INPUT "NUMBER OF SITES PER CHART",N
120 IF N ] 14 THEN 2540
130 REM ASSIGN CHART NAMES
140 LET C$(1) = "A"
150 LET C$(2) = "B"
160 LET C$(3) = "C"
170 LET C$(4) = "D"
180 REM
190 INPUT "NUMBER OF PREPARATIONS USED",M
200 IF M ] 8 THEN 2560
210 REM READ IN PREPARATION NAMES
220 FOR I =1 TO M
230 PRINT "PREPARATION";I;"?"
240 INPUT O$(I)
250 NEXT I
260 REM
270 PRINT "READ IN STRINGS OF NUMBERS REPRESENTING THE"
280 PRINT "PREPARATION LAYOUT IN THE CHARTS."
290 PRINT "NUMBER OF STRINGS = NUMBER OF CHARTS"
300 PRINT "NUMBER OF DIGITS PER STRING = NUMBER OF SITES"
310 PRINT "DIGITS ARE 1-8 CORRESPONDING TO PREPARATION TYPE"
320 PRINT "AS ENTERED IN ABOVE"
330 FOR I = 1 TO N1
340 PRINT "CHART",C$(I);"?"
350 INPUT S$(I)
360 NEXT I
370 REM
380 REM INITIALIZE TO 0 A TABLE FOR COUNTING NO. OF 0,1,2,3,4
390 REM FOR EACH PREPARATION, 1-M.
400 FOR I = 1 TO M
410 FOR J = 1 TO 5
420 LET T1(I,J)= 0
430 NEXT J
440 NEXT I
450 REM
460 PRINT "READ IN PAIRS OF SITE NUMBERS FOR SITES WHICH"
470 PRINT "ARE TO BE COMPARED"
480 PRINT
490 PRINT "ENTER ONE PAIR PER LINE, NUMBERS SEPARATED BY COMMAS"
```

```
500 FOR I = 1 TO N1
510 PRINT "CHART",C$(I)
520 INPUT "NUMBER OF PAIRS TO BE COMPARED",Y(I)
530 IF Y(I) ] 12 THEN 2580
540 FOR J = 1 TO Y(I)
550 INPUT R1(I,J),R2(I,J)
560 NEXT J
570 NEXT I
580 REM
590 REM CORRELATE SITE PAIR TO OINTMENT PAIR
600 REM MAKE A LIST E(K), K = 1-12, OF ALL PREPARATION
610 REM PAIRS TO BE COMPARED
620 REM
630 REM INITIALIZE
640 LET K=0
650 REM
660 REM FOR EVERY CHART-
670 FOR I=1 TO N1
680 REM FOR EVERY SITE PAIR-
690 FOR J=1 TO Y(I)
700 LET R=R1(I,J)
710 REM FIND PREPARATION OF FIRST SITE OF PAIR
720 LET S1$=STR(S$(I),R,1)
730 CONVERT S1$ TO S1
740 LET R=R2(I,J)
750 REM FIND PREPARATION OF SECOND SITE OF PAIR
760 LET S1$=STR(S$(I),R,1)
770 CONVERT S1$ TO S2
780 REM NOW FORM A NUMERIC CODE FOR THE PREPARATION PAIR
790 LET S=S1*10+S2
800 REM CHECK IF THIS PREPARATION PAIR IS IN THE LIST
810 REM ALREADY.  ADD IT IF IT IS NOT.  ASSIGN ITS
820 REM POSITION IN THE LIST TO A POINTER BETWEEN
830 REM SITE PAIR IN CURRENT CHART AND PREPARATION PAIR
840 REM IN THE LIST.
850 REM
860 IF K=0 THEN 900
870 FOR R=1 TO K
880 IF E(R)=S THEN 940
890 NEXT R
900 LET K=K+1
910 IF K ]12 THEN 2630
920 LET E(K)=S
930 LET R=K
940 LET P1(I,J)=R
950 NEXT J
960 NEXT I
970 REM NOW K IS THE NUMBER OF PREPARATION
980 REM PAIRS TO BE COMPARED
990 LET N2=K
1000 REM
1010 REM INITIALIZE TO 0 A TABLE FOR COUNTING NO.OF 0,=,],[,
1020 REM FOR EACH OINTMENT PAIR.
1030 FOR J=1 TO N2
1040 FOR K=1 TO 4
1050 LET T2(J,K)=0
1060 NEXT K
1070 NEXT J
1080 REM
1090 REM CARD-READING AND DATA SORT
1100 GOTO 1250
```

```
1110 REM NEW INFORMATION FOR NEXT TIME INTERVAL
1120 INPUT "TIME OF DATA COLLECTION",T
1130 INPUT "OCCLUDED OR UNOCCLUDED",Z$
1140 FOR I=1 TO M
1150 FOR J=1 TO 5
1160 LET T1(I,J)=0
1170 NEXT J
1180 NEXT I
1190 FOR J=1 TO N2
1200 FOR K=1 TO 4
1210 LET T2(J,K)=0
1220 NEXT K
1230 NEXT J
1240 REM
1250 LET C=0
1260 INPUT "READ CARD NUMBER",C0
1270 INPUT "READ CHART NAME",X$
1280 REM
1290 REM FIND POINTER TO CHART
1300 FOR I=1 TO N1
1310 IF X$=C$(I) THEN 1360
1320 NEXT I
1330 IF X$ = "END" THEN 1850
1340 GOTO 2610
1350 REM I IS A POINTER TO THE CHART.
1360 LET L=I
1370 LET C=C+1
1380 REM
1390 REM READ IN DATA FOR SITES.
1400 PRINT "READ IN A STRING OF NUMBERS 0-4 CORRESPONDING"
1410 PRINT "TO THE APPROPRIATE STRING OF THE CHART AND"
1420 PRINT "REPRESENTING DEGREE OF BLANCHING FOR EACH SITE"
1430 INPUT D$
1440 REM CORRELATE FOR EACH SITE AN PREPARATION NUMBER
1450 FOR I=1 TO N
1460 LET D=0
1470 LET D1$=STR(D$,I,1)
1480 CONVERT D1$ TO D
1490 LET D=D+1
1500 REM NOW D WILL HAVE A VALUE 1-5 CORRESPONDING TO THE
1510 REM 0-4 VALUE OF THE SITE BLANCHING
1520 REM
1530 LET S=0
1540 LET S1$=STR(S$(L),I,1)
1550 CONVERT S1$ TO S
1560 REM NOW S IS A POINTER TO PREPARATION TYPE
1570 REM
1580 REM ADD TO THE APPROPRIATE ELEMENT OF T1
1590 LET T1(S,D)=T1(S,D)+1
1600 NEXT I
1610 REM
1620 REM NOW READ IN COMPARISON INFORMATION
1630 PRINT "INPUT IN CORRECT ORDER RESULTS OF COMPARISONS"
1640 PRINT "FOR APPROPRIATE CHART"
1650 PRINT "1:0   2:=   3:]   4:["
1660 INPUT P$
1670 REM STORE NO. OF 0,=,],[ PER PREPARATION PAIR
1680 REM FOR EVERY SITE PAIR-
1690 FOR J=1 TO Y(L)
1700 REM LOCATE CORRECT COLUMN OF PREPARATION PAIR
1710 LET P=P1(L,J)
```

```
1720 REM DECODE FROM "COMPARISON STRING" THE INFORMATION
1730 REM FROM SITE PAIR COMPARISON
1740 LET S1$=STR(P$,J,1)
1750 CONVERT S1$ TO S
1760 REM NOW STORE
1770 LET T2(P,S)=T2(P,S)+1
1780 NEXT J
1790 REM
1800 REM READ NEXT CARD
1810 INPUT "IS THERE ANOTHER CARD ; YES/NO",B$
1820 IF B$="YES" THEN 1260
1830 IF B$="NO" THEN 1890
1840 GOTO 1260
1850 REM
1860 REM FORM SUM OVER ALL STORED INFORMATION
1870 REM ----------------------------------
1880 REM
1890 SELECT PRINT 211
1900 PRINT "PROCESSED DATA FROM BLANCHING TRIALS"
1910 PRINT "ON TOPICAL STEROID PREPARATIONS."
1920 PRINT "RESULTS OF BLANCHING"
1930 PRINT "--------------------"
1940 PRINT
1950 PRINT "SITES",Z$
1960 PRINT "TIME OF COLLECTION";T;"HOURS AFTER APPLICATION"
1970 PRINT N1,"CHARTS USED"
1980 FOR I = 1 TO N1
1990 PRINT "CHART",I,S$(I)
2000 NEXT I
2010 PRINT
2020 FOR I=1 TO M
2030 PRINT
2040 PRINT "PREPARATION",I,O$(I)
2050 LET A0=0
2060 LET A1 = 0
2070 FOR J=1 TO 5
2080 LET K=J-1
2090 PRINT "NUMBER OF";K;"'S:";TAB(22);T1(I,J)
2100 REM FORM SUMS OF 1'S, 2'S, 3'S, 4'S
2110 LET A=K*T1(I,J)
2120 LET A1=A1+4*T1(I,J)
2130 PRINT "TOTAL OF ALL";K;"'S:",A
2140 LET A0=A0+A
2150 NEXT J
2160 PRINT "SUM TOTAL OVER 1'S, 2'S, 3'S, 4'S:",A0
2170 REM CALCULATE PERCENTAGE OF MAXIMUM POSSIBLE SCORE
2180 REM (4 FOR EVERY SITE)
2190 LET A2 = (A0/A1) * 100
2200 PRINT "PERCENTAGE OF TOTAL POSSIBLE SCORE";A2
2210 REM
2220 NEXT I
2230 REM
2240 PRINT
2250 PRINT "RESULTS OF COMPARISONS"
2260 FOR J=1 TO N2
2270 LET A=E(J)
2280 LET A0=INT(A/10)
2290 LET A1=A-(A0*10)
2300 PRINT "COMPARING PREPARATION PAIR:";A
2310 PRINT "I.E.",O$(A0),"WITH",O$(A1)
2320 LET I=1
```

```
2330 PRINT "NUMBER OF 0",T2(J,I)
2340 LET I=2
2350 PRINT "NUMBER OF =",T2(J,I)
2360 LET I=3
2370 PRINT "NUMBER OF ]",T2(J,I)
2380 LET I=4
2390 PRINT "NUMBER OF [",T2(J,I)
2400 NEXT J
2410 SELECT PRINT 005
2420 PRINT "IS THERE ANOTHER TIME INTERVAL"
2430 INPUT "USING THE SAME CHARTS?",B$
2440 IF B$="YES" THEN 2470
2450 IF B$="NO" THEN 2480
2460 REM
2470 PRINT "PRESS RESET RUN 1110"
2480 STOP "END"
2490 REM ERROR MESSAGES
2500 REM --------------
2510 REM
2520 PRINT "ERROR. NUMBER OF CHARTS EXCEEDS 4"
2530 GOTO 90
2540 PRINT "ERROR. MAXIMUM 14 SITES ALLOWED"
2550 GOTO 110
2560 PRINT "ERROR. MAXIMUM 8 PREPARATIONS ALLOWED"
2570 GOTO 190
2580 PRINT "ERROR. MAXIMUM 12 PAIRS OF SITE NUMBERS"
2590 PRINT "CAN BE COMPARED"
2600 GOTO 520
2610 PRINT "ERROR. INVALID CHART NAME FOR CARD NUMBER",CO
2620 GOTO 1270
2630 PRINT "ERROR. NUMBER OF PREPARATION PAIRS MAY NOT"
2640 PRINT "EXCEED 12"
2650 GOTO 1630
2660 STOP "PROGRAM TERMINATING"
2670 END
```

APPENDIX 4

The tables in this appendix contain all the raw data collected at each reading time, the %TPS values and the AUC values for the various preparations, as well as the chi-squared values for the comparisons discussed in section 2.2.3. The digit before the point in the table numbers indicates the experiment number for which the table was compiled. This corresponds to the digit after the point in the subsection headings in chapter 3.

The following should be noted in the tables containing comparisons of adjacent sites : the values corresponding to ">" indicate the number of site pairs at which the first preparation in the table heading was recorded as eliciting a superior blanching response to the second preparation in the table heading, and "<" indicates the number of site pairs at which the first preparation was recorded as eliciting an inferior response to the second preparation.

EXPERIMENT 1

TABLE 1.1

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	39	28	22	19	9	2	5	5	96	105
1	51	43	32	30	36	44	49	53	47	39
2	35	39	31	20	23	31	27	41	1	0
3	14	26	40	43	28	37	40	36	0	0
4	5	8	19	32	48	30	23	9	0	0
%TPS	31,77	40,10	50,35	56,77	62,15	58,51	54,69	48,44	8,51	6,77

TABLE 1.2

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR PERSIVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	34	28	20	14	8	2	3	6	78	113
1	51	34	30	31	30	35	34	43	62	29
2	32	40	31	24	23	32	34	32	4	2
3	22	28	38	33	32	31	31	44	0	0
4	5	14	25	42	51	44	42	19	0	0
%TPS	34,90	44,10	53,13	60,07	65,28	63,89	63,02	54,69	12,15	5,73

TABLE 1.3

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	32	19	12	8	7	0	0	0	68	97
1	40	35	31	30	20	19	21	24	70	42
2	33	34	23	21	24	38	35	45	6	5
3	28	37	46	29	30	33	44	48	0	0
4	11	19	32	56	63	54	44	27	0	0
%TPS	40,63	50,35	59,55	66,49	71,18	71,18	69,27	63,54	14,24	9,03

TABLE 1.4

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	75	56	44	42	37	38	32	41	89	115
1	53	65	61	53	53	56	63	57	51	27
2	12	16	23	25	29	25	21	24	4	2
3	4	7	15	18	19	18	20	19	0	0
4	0	0	1	6	6	7	8	3	0	0
%TPS	15,45	20,49	27,08	31,42	33,33	32,64	34,21	30,21	10,24	5,38

TABLE 1.5

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR PERSIVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	46	34	23	16	16	14	10	10	62	91
1	49	42	34	37	31	27	27	31	76	40
2	32	41	34	21	24	30	32	37	6	13
3	15	21	35	43	41	36	43	37	0	0
4	2	6	18	27	32	37	32	29	0	0
%TPS	28,82	36,63	48,44	54,86	57,29	59,55	60,42	57,64	15,28	11,46

TABLE 1.6

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	64	51	42	39	35	31	28	36	95	105
1	63	67	65	57	58	59	67	68	49	37
2	15	18	25	23	24	26	22	16	0	2
3	1	6	8	19	21	18	17	17	0	0
4	1	2	4	6	6	10	10	7	0	0
%TPS	17,36	22,40	26,91	31,94	33,51	35,59	35,07	31,08	8,51	7,12

TABLE 1.7

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Betnovate cream	1017,14	620,56
Persivate cream	1135,97	1112,88
Celestoderm-V cream	1294,40	635,29

TABLE 1.8

CHI-SQUARED VALUES FOR CELESTODERM-V CREAM vs PERSIVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	4,50	14,35	21,01	21,83	25,36	25,36	25,54	23,51	2,72	0,83
Graded Response	4,38	4,23	4,82	4,11	3,42	8,34	8,39	15,15	1,57	4,89
Yes/No	0,02	1,63	1,72	1,23	0,00	0,50	1,35	4,26	1,13	3,96

TABLE 1.9

CHI-SQUARED VALUES FOR CELESTODERM-V CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	42,24	50,27	64,73	60,75	60,58	75,88	66,78	58,27	15,57	3,89
Graded Response	9,00	9,29	7,88	13,77	6,94	19,72	24,01	26,82	12,87	5,43
Yes/No	0,67	1,63	2,70	4,09	0,66	0,50	3,26	3,26	10,32	0,81

TABLE 1.10

CHI-SQUARED VALUES FOR PERSIVATE CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	17,51	7,44	11,92	4,90	4,16	19,78	12,25	16,16	11,03	0,30
Graded Response	2,26	2,78	1,03	3,81	0,96	4,22	10,71	6,61	5,73	3,76
Yes/No	0,29	0,02	0,03	0,55	0,00	0,25	0,13	0,00	4,20	0,93

TABLE 1.11

CHI-SQUARED VALUES FOR CELESTODERM-V CREAM vs PERSIVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	66,86	66,40	66,94	82,27	79,74	88,91	98,37	100,35	18,95	5,36
Graded Response	23,43	28,43	42,50	36,62	39,51	40,13	50,19	57,70	18,77	9,18
Yes/No	4,25	4,27	6,44	10,88	7,72	6,74	8,76	16,17	14,34	2,70

TABLE 1.12

CHI-SQUARED VALUES FOR CELESTODERM-V CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	1,27	1,87	0,42	0,86	0,00	0,00	0,00	1,44	0,10	3,70
Graded Response	4,87	2,46	4,19	0,37	0,85	1,34	0,88	4,60	4,24	2,02
Yes/No	1,39	0,24	0,02	0,07	0,02	0,69	0,19	0,28	0,38	1,56

TABLE 1.13

CHI-SQUARED VALUES FOR PERSIVATE CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	68,34	65,68	65,94	72,64	72,64	102,98	87,94	117,20	12,44	15,63
Graded Response	4,87	34,29	39,59	38,46	40,41	48,12	51,00	56,21	10,15	13,39
Yes/No	11,17	7,13	7,78	13,49	9,25	12,42	12,29	21,45	9,41	9,02

TABLE 1.14

COMPARISON OF ADJACENT SITES : CELESTODERM-V vs PERSIVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	25	18	8	5	1	1	0	0	62	101
=	21	20	17	29	28	28	34	29	29	13
>	60	73	85	80	85	85	82	84	33	18
<	38	33	34	30	30	30	28	31	20	12

TABLE 1.15

COMPARISON OF ADJACENT SITES : CELESTODERM-V CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	34	21	14	7	1	2	0	0	73	100
=	19	17	21	29	32	18	20	20	17	7
>	77	90	97	95	97	111	108	105	42	25
<	14	16	12	13	14	13	16	19	12	12

TABLE 1.16

COMPARISON OF ADJACENT SITES : PERSIVATE CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	27	24	16	12	5	4	1	2	86	105
=	21	22	31	24	33	33	43	38	18	9
>	69	63	66	66	64	77	68	73	31	17
<	27	35	31	42	42	30	32	31	9	13

TABLE 1.17

COMPARISON OF ADJACENT SITES : CELESTODERM-V CREAM vs PERSIVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	37	22	17	15	17	11	10	10	71	90
=	16	8	6	5	9	9	11	9	12	12
>	6	13	15	11	10	9	6	6	13	13
<	85	101	106	113	108	115	117	119	48	29

TABLE 1.18

COMPARISON OF ADJACENT SITES : CELESTODERM-V CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	60	46	33	32	33	30	20	29	89	110
=	20	21	26	18	26	25	27	31	16	7
>	37	45	46	52	42	45	48	48	21	19
<	27	32	39	42	43	44	49	36	18	8

TABLE 1.19

COMPARISON OF ADJACENT SITES : PERSIVATE CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	40	27	21	17	16	14	10	7	70	99
=	8	7	16	13	14	6	11	10	11	5
>	89	98	96	103	103	119	114	125	46	33
<	7	12	11	11	11	5	9	2	17	7

TABLE 1.20

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	74	65	58	39	25	27	29	28	77	84	102
1	53	53	40	51	39	33	28	29	54	53	42
2	12	17	26	23	30	34	35	48	12	7	0
3	2	8	13	19	31	27	35	31	1	0	0
4	3	1	7	12	19	23	17	8	0	0	0
%TPS	16,49	19,97	27,60	35,07	46,53	47,57	47,05	43,40	14,06	11,63	7,29

TABLE 1.21

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR PERSIVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	49	40	25	18	13	13	15	8	47	59	88
1	63	46	47	38	27	20	16	24	74	66	49
2	24	41	35	38	27	23	20	32	20	18	7
3	7	14	26	35	40	34	46	49	3	1	0
4	1	3	11	15	37	54	47	31	0	0	0
%TPS	23,61	31,60	41,49	48,44	60,59	66,67	66,32	62,33	21,35	18,23	10,94

TABLE 1.22

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	88	67	49	43	27	28	31	27	70	77	104
1	40	54	50	45	37	28	24	28	51	59	40
2	11	15	26	24	28	29	32	47	11	8	0
3	3	8	13	22	28	39	41	31	3	0	0
4	2	0	6	10	24	20	16	11	0	0	0
%TPS	13,72	18,75	28,65	34,55	47,40	49,13	47,74	44,97	14,24	13,02	6,94

TABLE 1.23

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	58	52	49	45	31	37	37	33	74	84	107
1	62	61	49	48	34	27	24	45	55	51	36
2	16	19	25	23	41	35	43	35	11	9	1
3	3	8	17	16	21	29	27	24	3	0	0
4	5	4	4	12	17	16	13	7	1	0	0
%TPS	21,35	24,13	28,82	32,99	42,88	43,06	42,18	37,33	15,63	11,98	6,60

TABLE 1.24

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR PERSIVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	45	41	30	26	20	17	15	9	48	61	94
1	50	44	39	31	23	20	16	28	69	67	48
2	34	33	35	38	34	27	39	44	21	15	2
3	8	21	27	33	34	39	32	35	5	1	0
4	7	5	13	16	33	41	42	28	1	0	0
%TPS	29,51	33,51	42,01	46,88	56,42	61,63	62,15	57,81	22,57	17,36	9,03

TABLE 1.25

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Response											
0	78	77	54	53	35	39	39	38	92	96	113
1	42	37	46	40	43	38	35	38	42	42	31
2	18	16	27	29	28	25	32	41	6	5	0
3	3	11	14	14	22	29	26	19	2	1	0
4	3	3	3	8	16	13	12	8	2	0	0
%TPS	17,19	19,79	26,74	29,86	39,76	39,41	39,06	36,28	11,81	9,55	5,38

TABLE 1.26

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Betnovate ointment	1019,72	987,23
Persivate ointment	1478,94	1427,75
Celestoderm-V ointment	1037,79	865,29

TABLE 1.27

CHI-SQUARED VALUES FOR CELESTODERM-V OINTMENT vs PERSIVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	38,76	38,28	35,27	68,81	46,02	66,78	64,93	70,35	21,83	7,79	7,48
Graded Response	23,00	24,16	15,01	17,96	11,37	23,48	25,48	27,04	14,92	7,62	9,24
Yes/No	20,10	10,05	9,62	11,98	4,91	5,57	5,82	10,54	13,56	4,03	3,52

TABLE 1.28

CHI-SQUARED VALUES FOR CELESTODERM-V OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	3,82	0,81	0,11	2,34	0,30	0,95	2,35	0,01	1,38	2,28	0,02
Graded Response	3,47	1,17	1,95	0,99	0,93	3,22	1,01	0,52	1,16	0,69	0,07
Yes/No	2,38	0,04	0,95	0,15	0,02	0,00	0,02	0,00	0,01	0,51	0,02

TABLE 1.29

CHI-SQUARED VALUES FOR PERSIVATE OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	43,19	64,04	61,36	52,89	51,20	62,79	86,13	72,38	30,01	15,21	11,37
Graded Response	13,72	19,02	20,23	18,40	13,06	23,50	27,38	32,40	13,38	11,63	8,57
Yes/No	8,17	8,63	17,33	8,75	3,67	4,91	4,53	11,46	11,91	8,00	2,61

TABLE 1.30

CHI-SQUARED VALUES FOR CELESTODERM-V OINTMENT vs PERSIVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	72,78	70,83	52,89	53,78	47,62	55,57	67,57	80,37	42,22	30,96	9,14
Graded Response	18,35	21,11	18,84	21,93	19,20	30,30	35,72	35,37	30,35	18,54	7,40
Yes/No	14,53	17,59	8,89	11,79	4,41	9,78	12,06	19,93	25,70	16,19	5,57

TABLE 1.31

CHI-SQUARED VALUES FOR CELESTODERM-V OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	13,30	14,24	3,56	7,15	2,31	4,16	1,77	2,77	9,45	1,64	0,02
Graded Response	7,41	11,60	0,85	3,01	3,80	3,89	3,78	2,06	5,70	3,81	1,54
Yes/No	5,03	8,09	0,24	0,76	0,18	0,02	0,02	0,30	4,11	1,79	0,48

TABLE 1.32

CHI-SQUARED VALUES FOR PERSIVATE OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
Comparison of adjacent sites	33,83	39,86	46,02	35,21	46,21	47,16	36,99	43,43	15,16	7,33	1,04
Graded Response	12,01	13,76	14,41	18,90	13,34	21,92	26,82	33,35	11,13	8,32	2,89
Yes/No	2,18	1,59	5,65	6,06	2,38	8,23	10,35	14,75	8,89	6,72	2,37

TABLE 1.33

COMPARISON OF ADJACENT SITES : CELESTODERM-V OINTMENT vs PERSIVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	42	28	19	17	9	10	11	7	29	31	62
=	6	9	16	4	16	10	11	6	5	5	5
>	17	21	23	15	22	16	16	17	30	39	26
<	79	86	86	108	97	108	106	114	80	69	51

TABLE 1.34

COMPARISON OF ADJACENT SITES : CELESTODERM-V OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	63	52	45	36	11	23	23	21	50	66	91
=	14	13	19	12	14	16	12	9	6	4	5
>	25	35	38	40	63	47	46	57	38	44	24
<	42	44	42	56	56	58	63	57	50	30	24

TABLE 1.35

COMPARISON OF ADJACENT SITES : PERSIVATE OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	43	33	20	14	9	9	11	7	26	37	66
=	12	6	9	9	10	6	5	7	2	7	4
>	76	94	100	101	103	110	117	114	88	70	52
<	13	11	15	20	22	19	11	16	28	30	22

TABLE 1.36

COMPARISON OF ADJACENT SITES : CELESTODERM-V OINTMENT vs PERSIVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	35	38	19	21	20	14	12	8	47	56	78
=	5	4	7	4	9	9	4	4	3	4	3
>	8	8	19	19	20	19	17	14	15	16	19
<	96	94	99	100	95	102	111	118	79	68	44

TABLE 1.37

COMPARISON OF ADJACENT SITES : CELESTODERM-V OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	50	40	34	35	22	26	23	23	58	76	99
=	17	13	19	7	11	12	10	4	3	7	3
>	22	27	36	37	47	42	48	49	27	25	22
<	55	64	55	65	64	64	63	68	56	36	20

TABLE 1.38

COMPARISON OF ADJACENT SITES : PERSIVATE OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52
0	24	30	14	16	14	8	11	7	21	32	63
=	10	8	11	8	5	7	8	4	1	5	3
>	86	86	97	93	101	104	97	105	83	68	44
<	24	20	22	27	24	25	28	28	39	39	34

EXPERIMENT 2

TABLE 2.1

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	17	5	3	1	0	1	6	10	85	87
1	76	64	55	36	24	24	34	51	57	54
2	38	64	73	70	66	74	66	63	2	3
3	13	11	13	34	40	41	35	17	0	0
4	0	0	0	3	14	4	3	3	0	0
%TPS	33,16	39,06	41,67	50,35	57,64	53,99	49,13	41,67	10,59	10,42

TABLE 2.2

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	13	4	0	2	0	1	3	7	76	90
1	77	61	54	30	18	23	44	49	61	48
2	41	62	67	65	59	67	46	62	7	6
3	12	16	22	42	52	47	43	24	0	0
4	1	1	1	5	15	6	8	2	0	0
%TPS	34,55	41,15	44,79	53,13	61,11	55,91	51,56	43,92	13,02	10,42

TABLE 2.3

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DIPROSONE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	16	9	4	4	1	0	0	2	58	75
1	62	42	32	14	5	7	12	15	75	52
2	43	59	62	39	35	38	30	40	10	15
3	23	31	34	60	48	39	48	60	1	2
4	0	3	12	27	55	60	54	27	0	0
%TPS	37,67	46,01	53,13	65,97	76,22	76,39	75,00	66,49	17,01	15,28

TABLE 2.4

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	67	49	50	43	49	54	66	72	83	64
1	68	84	84	83	85	76	68	64	60	78
2	9	11	9	14	10	14	8	7	1	2
3	0	0	1	3	0	0	2	1	0	0
4	0	0	0	1	0	0	0	0	0	0
%TPS	14,93	18,40	18,23	21,53	18,23	18,06	15,63	14,06	10,76	14,24

TABLE 2.5

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	73	51	47	42	43	66	62	74	87	79
1	61	80	89	84	91	59	70	61	56	57
2	10	13	7	14	10	19	10	8	1	8
3	0	0	1	4	0	0	2	1	0	0
4	0	0	0	0	0	0	0	0	0	0
%TPS	14,06	18,40	18,40	21,53	19,27	15,84	16,67	13,89	10,07	12,67

TABLE 2.6

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DIPROSONE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Response										
0	77	50	47	37	31	25	19	19	42	43
1	58	69	63	61	67	61	58	77	92	87
2	9	24	30	39	38	50	49	33	10	14
3	0	1	4	7	8	8	18	15	0	0
4	0	0	0	0	0	0	0	0	0	0
%TPS	13,19	20,83	23,44	27,78	28,99	32,12	36,46	32,64	19,44	19,97

TABLE 2.7

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Betnovate lotion	955,41	420,65
Celestoderm-V lotion	1016,50	410,07
Diprosone lotion	1352,57	705,70

TABLE 2.8

CHI-SQUARED VALUES FOR DIPROSONE LOTION vs CELESTODERM-V LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	0,15	0,54	9,80	18,78	31,58	55,71	62,02	63,13	24,75	24,74
Graded Response	6,43	11,29	21,70	31,29	37,49	62,47	59,06	62,57	5,39	7,38
Yes/No	0,15	1,29	2,28	0,17	0,00	0,00	1,35	1,85	4,03	2,78

TABLE 2.9

CHI-SQUARED VALUES FOR DIPROSONE LOTION vs BETNOVATE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	4,98	17,25	37,31	35,21	33,47	57,69	53,73	64,13	24,45	12,60
Graded Response	4,54	18,44	28,50	46,69	48,05	70,94	77,69	73,32	13,89	10,93
Yes/No	0,00	0,68	0,00	0,81	0,00	0,00	4,26	4,26	9,39	1,71

TABLE 2.10

CHI-SQUARED VALUES FOR CELESTODERM-V LOTION vs BETNOVATE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	0,15	0,06	0,51	0,55	0,02	0,02	1,75	0,57	1,56	0,00
Graded Response	1,69	2,14	6,58	2,40	2,85	1,18	8,95	1,97	3,42	1,40
Yes/No	0,33	0,00	1,35	0,00	0,00	0,50	0,46	0,25	0,90	0,06

TABLE 2.11

CHI-SQUARED VALUES FOR DIPROSONE LOTION vs CELESTODERM-V LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	1,17	0,68	0,88	5,68	8,20	44,84	46,29	50,42	29,47	24,50
Graded Response	0,23	5,09	20,54	16,58	29,92	40,42	62,53	61,88	31,82	18,51
Yes/No	0,13	0,00	0,02	0,28	2,20	25,70	30,30	49,31	27,18	17,42

TABLE 2.12

CHI-SQUARED VALUES FOR DIPROSONE LOTION vs BETNOVATE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	0,39	0,00	4,00	8,17	16,82	27,12	49,78	40,02	24,50	14,20
Graded Response	1,49	7,31	16,20	18,20	30,51	40,54	69,07	61,22	27,55	13,61
Yes/No	1,13	0,00	0,06	0,43	5,00	13,68	35,32	43,44	22,62	5,95

TABLE 2.13

CHI-SQUARED VALUES FOR CELESTODERM-V LOTION vs BETNOVATE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Comparison of adjacent sites	0,88	0,77	0,00	0,34	0,17	3,67	0,10	0,00	1,09	0,66
Graded Response	0,69	0,30	0,49	1,16	0,60	4,10	0,38	0,17	0,23	8,44
Yes/No	0,35	0,02	0,06	0,00	0,40	1,73	0,13	0,01	0,13	2,72

TABLE 2.14

COMPARISON OF ADJACENT SITES : DIPROSONE LOTION vs CELESTODERM-V LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	1	0	0	0	0	0	0	1	24	35
=	11	5	3	3	5	3	8	2	4	3
>	32	37	48	53	57	66	64	68	39	32
<	28	30	21	16	10	3	0	1	5	2

TABLE 2.15

COMPARISON OF ADJACENT SITES : DIPROSONE LOTION vs BETNOVATE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	4	0	0	0	0	0	0	1	17	27
=	3	5	5	1	6	1	5	1	2	3
>	42	51	59	61	57	68	64	69	45	33
<	23	16	8	10	9	3	3	1	8	9

TABLE 2.16

COMPARISON OF ADJACENT SITES : CELESTODERM-V LOTION vs BETNOVATE LOTION (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	1	0	0	1	0	0	0	1	24	32
=	9	3	1	6	10	12	15	8	7	5
>	33	33	39	36	31	29	34	35	25	18
<	29	36	32	29	31	31	23	28	16	17

TABLE 2.17

COMPARISON OF ADJACENT SITES : DIPROSONE LOTION vs CELESTODERM-V LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	27	15	11	10	10	9	7	7	20	17
=	3	4	5	5	3	5	2	5	3	5
>	17	30	32	38	41	55	59	58	44	43
<	25	23	24	19	18	3	4	2	5	7

TABLE 2.18

COMPARISON OF ADJACENT SITES : DIPROSONE LOTION vs BETNOVATE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	27	16	17	14	14	8	7	11	18	20
=	4	7	6	4	8	5	2	1	4	8
>	18	24	32	38	40	50	60	55	43	35
<	23	25	17	16	10	9	3	5	7	9

TABLE 2.19

COMPARISON OF ADJACENT SITES : CELESTODERM-V LOTION vs BETNOVATE LOTION (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
0	26	21	17	18	14	23	25	31	34	28
=	5	4	8	7	6	3	8	4	5	6
>	17	20	24	21	24	16	18	19	13	16
<	24	27	23	26	28	30	21	18	20	22

EXPERIMENT 3

TABLE 3.1

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	7	4	1	0	0	0	0	1	41	56	95	105
1	40	24	18	9	0	0	0	8	70	81	49	39
2	60	57	47	41	30	21	35	44	30	6	0	0
3	35	54	61	67	71	66	75	74	3	1	0	0
4	2	5	17	27	43	57	34	17	0	0	0	0
%TPS	47,40	55,56	63,02	69,44	77,26	81,25	74,83	67,01	24,13	16,67	8,51	6,77

TABLE 3.2

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	21	14	10	5	1	2	7	9	67	97	118	120
1	55	48	43	30	23	24	34	63	73	46	26	24
2	54	55	44	55	54	55	57	44	4	1	0	0
3	13	25	42	45	52	49	44	27	0	0	0	0
4	1	2	5	9	14	14	2	1	0	0	0	0
%TPS	35,76	41,84	48,09	53,99	59,55	58,51	50,00	40,97	14,06	8,33	4,51	4,17

TABLE 3.3

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	21	14	7	8	5	11	26	35	99	115	123	130
1	67	57	50	47	58	59	66	72	43	29	21	14
2	48	60	61	64	52	53	47	36	2	0	0	0
3	8	12	25	24	27	20	5	1	0	0	0	0
4	0	1	1	1	2	1	0	0	0	0	0	0
%TPS	32,47	37,67	43,58	43,58	43,58	39,76	30,38	25,52	8,16	5,03	3,65	2,43

TABLE 3.4

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	24	16	7	6	3	3	0	2	23	40	79	97
1	45	32	26	13	3	4	12	9	50	64	51	40
2	66	78	70	56	30	24	28	28	49	31	14	7
3	8	12	40	63	81	86	75	85	21	9	0	0
4	1	6	1	6	27	27	29	20	1	0	0	0
%TPS	35,59	43,06	50,35	58,68	71,88	72,57	71,01	69,44	37,33	26,56	13,72	9,38

TABLE 3.5

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	66	47	39	27	20	22	32	43	70	91	102	128
1	67	83	75	82	70	80	73	68	69	50	41	16
2	10	13	26	30	43	33	32	31	5	3	1	0
3	1	0	4	5	10	9	7	2	0	0	0	0
4	0	1	0	0	1	0	0	0	0	0	0	0
%TPS	15,63	19,62	24,13	27,26	32,99	30,03	27,43	23,61	13,72	9,72	7,47	2,78

TABLE 3.6

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	97	84	81	68	71	71	83	88	102	117	133	139
1	45	58	60	72	68	67	51	50	41	26	11	5
2	2	2	3	3	3	4	6	6	1	1	0	0
3	0	0	0	1	2	2	4	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
%TPS	8,51	10,76	11,45	14,06	13,89	14,06	13,02	10,76	7,47	4,86	1,91	0,87

TABLE 3.7

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Dermovate cream	1765,69	1934,78
Betnovate cream	1156,74	777,60
Eumovate cream	823,20	359,12

TABLE 3.8

CHI-SQUARED VALUES FOR DERMOVATE CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	60,90	75,78	75,78	96,81	93,89	110,62	132,18	120,59	59,65	52,20	20,08	7,85
Graded Response	20,10	25,52	27,76	31,67	48,55	69,77	82,78	85,10	29,20	25,20	9,54	4,57
Yes/No	6,69	4,80	6,05	3,25	0,00	0,50	5,27	5,08	9,26	22,31	8,73	3,98

TABLE 3.9

CHI-SQUARED VALUES FOR BETNOVATE CREAM vs EUMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	5,68	9,57	15,67	44,41	93,13	102,13	102,13	85,17	21,01	20,28	0,83	12,00
Graded Response	3,72	5,89	10,79	17,92	34,74	44,48	55,18	41,91	14,59	6,38	0,64	3,03
Yes/No	0,03	0,04	0,25	0,32	1,53	5,16	11,09	16,77	13,67	5,17	0,41	2,46

TABLE 3.10

CHI-SQUARED VALUES FOR DERMOVATE CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	90,37	102,33	105,11	106,09	123,57	126,35	133,17	127,35	84,20	72,37	27,36	30,42
Graded Response	71,63	99,87	96,65	126,81	155,91	174,03	161,43	181,90	84,64	53,63	15,28	21,56
Yes/No	27,17	18,29	24,86	13,69	12,10	14,19	33,79	42,14	33,60	35,01	7,20	18,29

TABLE 3.11

CHI-SQUARED VALUES FOR BETNOVATE CREAM vs EUMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	31,21	39,86	51,14	84,20	72,08	80,14	65,45	65,94	39,19	24,47	26,88	9,39
Graded Response	16,55	23,95	38,61	43,10	69,73	54,15	45,13	37,10	15,75	11,83	22,40	6,22
Yes/No	12,72	18,15	24,01	25,13	40,16	36,59	36,19	27,11	13,87	10,82	20,81	5,14

TABLE 3.12

COMPARISON OF ADJACENT SITES : DERMOVATE CREAM vs BETNOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	5	1	0	0	0	0	0	0	27	51	89	96
=	6	3	4	5	8	5	2	6	7	7	4	2
>	112	122	122	128	125	132	140	134	96	77	42	33
<	21	18	18	11	11	7	2	4	14	9	9	13

TABLE 3.13

COMPARISON OF ADJACENT SITES : BETNOVATE CREAM vs EUMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	13	10	2	0	1	0	2	5	49	80	110	116
=	12	6	7	7	1	3	2	12	15	7	4	1
>	73	82	91	108	129	131	130	116	61	46	18	23
<	46	46	44	29	13	10	10	11	19	11	12	4

TABLE 3.14

COMPARISON OF ADJACENT SITES : DERMOVATE CREAM vs BETNOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	19	14	7	2	2	3	0	1	13	32	69	94
=	3	3	0	4	1	1	1	2	5	5	1	0
>	114	121	129	130	137	137	141	138	115	98	60	45
<	8	6	8	8	4	3	2	3	11	9	14	5

TABLE 3.15

COMPARISON OF ADJACENT SITES : BETNOVATE CREAM vs EUMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	49	35	30	16	18	19	25	29	51	74	100	126
=	5	3	4	2	6	10	6	8	24	11	1	0
>	72	86	93	115	107	106	100	96	61	49	39	16
<	18	20	17	11	13	9	13	11	8	10	4	2

TABLE 3.16

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	5	1	1	1	0	0	0	0	20	27	77	75
1	33	21	11	5	5	0	2	5	69	79	57	61
2	51	46	45	28	12	14	8	22	39	33	10	8
3	52	61	61	71	55	59	85	72	16	5	0	0
4	3	15	26	39	72	71	49	45	0	0	0	0
%TPS	52,60	61,81	67,36	74,65	83,68	84,90	81,42	77,26	33,85	27,78	13,37	13,37

TABLE 3.17

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	36	25	11	7	8	8	13	14	56	62	113	127
1	77	63	53	43	13	15	23	37	65	72	31	17
2	21	36	50	49	61	58	65	50	19	10	0	0
3	10	15	23	32	53	51	37	42	4	0	0	0
4	0	5	7	13	9	12	6	1	0	0	0	0
%TPS	25,87	34,72	43,40	50,17	57,29	57,64	50,00	46,35	19,97	15,97	5,38	2,95

TABLE 3.18

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	24	16	12	6	9	18	48	71	100	111	127	137
1	74	69	58	52	53	78	65	53	40	32	17	7
2	35	35	52	53	61	33	24	17	4	1	0	0
3	11	20	17	25	18	15	7	3	0	0	0	0
4	0	4	5	8	3	0	0	0	0	0	0	0
%TPS	30,73	37,33	40,45	46,01	41,84	32,81	23,26	16,67	8,33	5,90	2,95	1,22

TABLE 3.19

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	6	2	1	1	0	0	0	0	17	31	67	78
1	31	16	11	4	4	1	3	3	57	60	71	60
2	57	64	51	43	24	19	29	52	43	43	6	6
3	44	46	65	68	63	65	82	65	26	10	0	0
4	6	16	16	28	53	59	30	24	1	0	0	0
%TPS	52,26	60,07	64,58	70,49	78,65	81,60	74,13	69,10	39,06	30,56	14,41	12,50

TABLE 3.20

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	61	46	29	22	14	16	35	30	74	82	116	125
1	60	69	60	55	47	52	51	61	54	50	28	19
2	17	13	36	31	48	45	34	32	14	12	0	0
3	4	11	14	24	32	24	19	18	2	0	0	0
4	2	5	5	12	3	7	5	3	0	0	0	0
%TPS	19,79	25,69	33,68	41,15	43,58	42,01	34,03	33,16	15,28	12,85	4,86	3,30

TABLE 3.21

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	18	6	4	1	2	10	25	22	74	91	125	134
1	60	52	35	23	26	45	65	77	62	50	19	10
2	52	63	69	69	72	62	34	37	8	3	0	0
3	10	17	30	40	39	23	19	7	0	0	0	0
4	4	6	6	11	5	4	1	1	0	0	0	0
%TPS	36,46	43,92	49,83	56,42	53,30	44,10	33,68	30,56	13,54	9,72	3,30	1,74

TABLE 3.22

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Dermovate ointment	2172,60	2160,85
Betnovate ointment	1266,71	964,54
Eumovate ointment	723,51	1024,24

TABLE 3.23

CHI-SQUARED VALUES FOR DERMOVATE OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	90,98	88,96	82,72	85,82	79,69	103,60	113,22	107,08	84,03	66,89	35,21	50,28
Graded Response	84,99	77,22	64,29	68,08	93,48	92,41	127,65	99,25	31,27	31,39	24,50	46,21
Yes/No	25,59	22,37	7,04	3,21	6,30	6,30	11,60	12,69	21,90	18,80	18,95	43,12

TABLE 3.24

CHI-SQUARED VALUES FOR BETNOVATE OINTMENT vs EUMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	3,41	0,03	7,39	20,03	65,52	95,83	99,67	117,37	40,46	38,88	7,61	4,05
Graded Response	6,01	3,09	1,54	3,14	44,55	85,03	85,47	92,12	32,15	36,63	4,90	4,55
Yes/No	2,55	1,82	0,00	0,00	0,00	3,42	24,04	52,34	25,86	33,35	4,22	3,68

TABLE 3.25

CHI-SQUARED VALUES FOR DERMOVATE OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	117,37	126,35	115,88	128,18	133,17	131,07	132,18	137,06	106,95	79,60	53,44	42,75
Graded Response	111,35	134,41	101,22	92,65	113,01	135,50	135,22	130,27	72,11	51,40	37,80	38,16
Yes/No	57,72	46,23	27,13	18,90	12,69	14,89	37,60	31,29	50,38	36,41	34,53	35,32

TABLE 3.26

CHI-SQUARED VALUES FOR BETNOVATE OINTMENT vs EUMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	61,95	56,46	48,24	43,43	26,67	5,21	0,99	3,61	3,36	2,84	1,29	1,75
Graded Response	44,40	67,43	41,80	50,79	21,03	5,43	6,02	9,29	4,19	5,87	2,06	3,11
Yes/No	30,77	35,69	19,71	18,90	8,01	1,06	1,71	1,15	0,01	0,93	1,63	2,45

TABLE 3.27

COMPARISON OF ADJACENT SITES : DERMOVATE OINTMENT vs BETNOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	5	0	0	0	0	0	0	0	16	21	70	69
=	6	3	3	3	3	5	6	5	9	15	3	1
>	122	127	125	126	124	130	132	131	110	97	61	68
<	11	14	16	15	17	9	6	8	9	11	10	6

TABLE 3.28

COMPARISON OF ADJACENT SITES : BETNOVATE OINTMENT vs EUMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	14	6	3	1	5	3	7	10	41	57	106	123
=	24	13	11	8	7	3	2	3	8	12	0	1
>	43	64	81	94	113	127	126	128	79	65	28	15
<	63	61	49	41	19	11	9	3	16	10	10	5

TABLE 3.29

COMPARISON OF ADJACENT SITES : DERMOVATE OINTMENT vs BETNOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	5	1	1	0	0	0	0	0	14	27	60	68
=	8	3	6	6	1	7	2	1	2	6	0	0
>	128	137	132	136	141	136	140	142	123	103	76	67
<	3	3	5	2	2	1	2	1	5	8	8	9

TABLE 3.30

COMPARISON OF ADJACENT SITES : BETNOVATE OINTMENT vs EUMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	12	3	2	1	1	3	5	3	48	61	106	116
=	7	10	6	10	8	21	17	19	10	14	0	0
>	18	22	27	28	37	47	67	72	52	42	23	18
<	107	109	109	105	98	73	55	50	34	27	15	10

EXPERIMENT 4

TABLE 4.1

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	19	11	6	5	9	13	26	107	119
1	81	55	40	51	56	82	96	37	25
2	39	60	66	65	61	44	22	0	0
3	5	18	31	23	17	5	0	0	0
4	0	0	1	0	1	0	0	0	0
%TPS	30,21	39,76	46,71	43,40	40,46	32,12	24,31	6,42	4,34

TABLE 4.2

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRALANUM CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	9	5	3	8	18	34	51	109	109
1	78	59	44	99	114	107	91	35	35
2	52	62	70	35	10	3	2	0	0
3	5	17	26	2	2	0	0	0	0
4	0	1	1	0	0	0	0	0	0
%TPS	34,20	41,32	46,18	30,38	24,31	19,62	16,50	6,08	6,08

TABLE 4.3

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	18	11	3	13	33	41	68	112	115
1	106	89	76	111	106	102	75	32	29
2	19	41	62	19	4	1	1	0	0
3	1	3	3	1	1	0	0	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	25,58	31,25	36,29	26,39	20,32	18,06	13,37	5,56	5,03

TABLE 4.4

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	84	55	34	36	48	48	67	99	121
1	53	82	98	98	85	91	74	44	23
2	7	7	12	10	11	5	3	1	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	11,63	16,67	21,18	20,49	18,58	17,54	13,89	7,99	3,99

TABLE 4.5

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRALANUM CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	78	65	49	69	78	89	99	115	124
1	59	74	89	74	65	55	44	28	20
2	7	5	6	1	0	0	1	1	0
3	0	0	0	0	1	0	0	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	12,68	14,59	17,54	13,20	11,81	9,55	7,99	5,71	3,48

TABLE 4.6

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Response									
0	80	60	37	57	81	84	101	116	128
1	63	82	105	85	60	59	41	28	16
2	1	2	2	2	1	1	2	0	0
3	0	0	0	0	2	0	0	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	11,29	14,93	18,93	15,45	11,81	10,59	7,81	4,86	2,78

TABLE 4.7

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Eumovate cream	707,16	376,27
Ultralanum cream	588,01	270,98
Ultradil cream	478,09	267,82

TABLE 4.8

CHI-SQUARED VALUES FOR EUMOVATE CREAM vs ULTRALANUM CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	7,77	4,63	0,11	48,19	54,74	49,61	39,01	0,24	0,94
Graded Response	5,49	3,45	1,75	42,69	72,26	53,46	24,92	0,07	2,11
Yes/No	3,20	1,65	0,46	0,32	2,62	10,17	10,21	0,02	1,71

TABLE 4.9

CHI-SQUARED VALUES FOR EUMOVATE CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	12,32	16,70	28,74	49,13	58,62	36,89	28,13	11,13	0,08
Graded Response	12,93	22,32	36,36	71,13	94,35	62,78	40,52	0,48	0,36
Yes/No	0,00	0,05	0,46	2,90	14,75	16,62	26,55	0,30	0,21

TABLE 4.10

CHI-SQUARED VALUES FOR ULTRALANUM CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	39,62	18,75	9,35	18,78	2,56	1,02	0,07	0,00	0,00
Graded Response	25,27	23,41	28,26	6,95	7,61	1,77	4,30	0,18	0,72
Yes/No	2,62	1,65	0,17	0,82	4,67	0,65	3,67	0,08	0,50

TABLE 4.11

CHI-SQUARED VALUES FOR EUMOVATE CREAM vs ULTRALANUM CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	0,30	2,22	0,77	4,56	10,09	31,74	21,84	5,04	0,36
Graded Response	0,54	1,58	5,14	21,08	21,81	26,15	14,80	4,75	0,25
Yes/No	0,35	1,16	3,32	15,35	11,87	22,27	13,67	4,09	0,11

TABLE 4.12

CHI-SQUARED VALUES FOR EUMOVATE CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	0,07	0,01	0,05	4,98	9,77	18,62	12,02	2,45	0,08
Graded Response	5,46	3,00	7,51	11,00	23,09	19,31	16,55	5,90	1,45
Yes/No	0,13	0,23	0,07	6,35	14,38	17,13	15,56	4,70	1,07

TABLE 4.13

CHI-SQUARED VALUES FOR ULTRALANUM CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
Comparison of adjacent sites	3,63	3,02	0,14	0,02	1,07	0,00	0,97	0,75	0,44
Graded Response	4,66	1,90	4,99	2,24	1,59	1,28	0,46	1,00	0,51
Yes/No	0,01	0,23	2,01	1,71	0,06	0,23	0,02	0,00	0,29

TABLE 4.14

COMPARISON OF ADJACENT SITES : EUMOVATE CREAM vs ULTRALANUM CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	0	0	0	2	2	2	10	68	68
=	9	18	14	9	12	14	14	11	11
>	30	29	43	75	75	72	63	10	6
<	57	49	39	10	7	8	9	7	11

TABLE 4.15

COMPARISON OF ADJACENT SITES : EUMOVATE CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	6	1	2	0	1	2	11	62	73
=	12	13	7	10	9	12	13	11	11
>	55	60	69	76	79	69	59	20	6
<	23	22	18	10	7	13	13	3	6

TABLE 4.16

COMPARISON OF ADJACENT SITES : ULTRALANUM CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	0	0	1	0	5	5	19	67	68
=	14	19	17	27	25	28	20	12	11
>	70	58	53	53	40	36	30	9	9
<	12	19	25	16	26	27	27	8	8

TABLE 4.17

COMPARISON OF ADJACENT SITES : EUMOVATE CREAM vs ULTRALANUM CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	35	18	13	10	16	23	40	60	73
=	8	13	19	15	13	12	12	12	12
>	24	26	28	45	47	53	38	18	4
<	29	39	36	26	20	8	6	6	7

TABLE 4.18

COMPARISON OF ADJACENT SITES : EUMOVATE CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	32	19	11	12	15	18	29	65	74
=	3	7	10	19	17	23	15	11	10
>	29	34	36	42	45	44	39	14	7
<	32	36	39	23	19	11	13	6	5

TABLE 4.19

COMPARISON OF ADJACENT SITES : ULTRALANUM CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	12	14	16	18	28	32
0	34	18	16	17	33	35	43	73	82
=	0	13	14	17	17	22	16	11	5
>	39	40	31	30	27	19	15	4	3
<	23	25	35	32	19	20	22	8	6

TABLE 4.20

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	9	5	9	5	7	23	29	84	86
1	46	34	36	33	37	53	68	24	22
2	50	53	39	45	36	26	10	0	0
3	3	15	23	23	26	6	1	0	0
4	0	1	1	2	2	0	0	0	0
%TPS	35,88	43,75	43,29	46,30	45,14	28,47	21,06	5,56	5,09

TABLE 4.21

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRALANUM OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	6	2	7	2	2	5	8	52	62
1	46	38	26	26	30	41	64	54	45
2	50	49	46	44	30	49	33	2	1
3	6	18	29	35	43	13	3	0	0
4	0	1	1	1	3	0	0	0	0
%TPS	37,96	44,91	47,69	51,62	53,47	41,20	32,18	13,43	10,88

TABLE 4.22

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	7	3	5	8	19	51	58	90	89
1	74	65	67	76	80	56	49	18	19
2	27	37	34	20	9	1	0	0	0
3	0	3	2	4	0	0	1	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	29,63	34,26	32,64	29,63	22,69	13,43	12,04	4,17	4,40

TABLE 4.23

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR EUMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	6	3	3	1	0	1	6	45	58
1	45	38	33	24	26	44	67	61	48
2	50	54	57	57	49	49	32	2	2
3	7	13	13	22	29	13	3	0	0
4	0	0	2	4	4	1	0	0	0
%TPS	38,43	42,82	44,91	50,93	52,55	42,82	32,41	15,05	12,04

TABLE 4.24

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRALANUM OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	12	8	6	4	2	5	7	34	46
1	58	45	38	25	33	43	70	58	57
2	36	49	48	57	44	42	25	16	5
3	2	6	15	22	25	18	6	0	0
4	0	0	1	0	4	0	0	0	0
%TPS	31,48	37,27	42,36	47,45	49,07	41,90	31,94	20,83	15,51

TABLE 4.25

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Response									
0	26	18	19	13	31	59	61	86	90
1	71	66	68	81	67	43	41	22	18
2	10	23	21	14	10	6	6	0	0
3	1	1	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
%TPS	21,76	26,62	25,46	25,23	20,14	12,73	12,27	5,09	4,17

TABLE 4.26

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Eumovate ointment	711,01	924,26
Ultralanum ointment	916,02	911,86
Ultradil ointment	476,92	411,68

TABLE 4.27

CHI-SQUARED VALUES FOR EUMOVATE OINTMENT vs ULTRALANUM OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	0,65	4,20	0,43	9,80	18,78	36,38	38,73	16,69	17,36
Graded Response	1,60	1,94	2,93	4,94	8,44	22,74	25,34	21,07	12,79
Yes/No	0,29	0,59	0,07	0,59	1,86	11,86	13,05	19,08	11,35

TABLE 4.28

CHI-SQUARED VALUES FOR EUMOVATE OINTMENT vs ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	17,56	27,11	32,55	34,30	36,38	33,59	27,16	0,94	0,41
Graded Response	16,65	22,05	29,46	42,64	65,54	39,83	22,75	1,06	0,27
Yes/No	0,07	0,13	0,69	0,33	5,29	14,99	15,09	0,74	0,12

TABLE 4.29

CHI-SQUARED VALUES FOR ULTRADIL OINTMENT vs ULTRALANUM OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	10,11	21,39	29,78	54,14	61,35	56,70	51,19	22,40	20,83
Graded Response	19,48	20,67	43,74	62,75	93,80	99,19	73,87	30,17	16,39
Yes/No	0,00	0,00	0,09	2,62	13,50	48,82	52,39	28,14	14,88

TABLE 4.30

CHI-SQUARED VALUES FOR EUMOVATE OINTMENT vs ULTRALANUM OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	2,73	2,09	0,25	0,06	1,27	0,02	1,64	10,02	14,38
Graded Response	8,70	5,68	2,60	5,82	3,40	5,02	2,00	12,50	3,44
Yes/No	1,52	1,53	0,46	0,82	0,50	1,54	0,00	2,00	2,24

TABLE 4.31

CHI-SQUARED VALUES FOR EUMOVATE OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	67,01	54,72	56,70	61,35	60,36	56,70	58,37	30,25	25,29
Graded Response	49,49	41,02	55,38	93,27	107,85	103,70	72,20	33,16	22,56
Yes/No	13,24	10,34	11,39	9,24	33,90	74,98	63,09	31,04	20,63

TABLE 4.32

CHI-SQUARED VALUES FOR ULTRALANUM OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
Comparison of adjacent sites	16,50	37,52	44,49	49,73	59,36	52,17	49,23	35,56	34,57
Graded Response	21,50	20,78	41,82	82,39	87,45	90,56	68,10	54,73	39,52
Yes/No	5,40	3,54	6,51	4,09	28,04	62,37	60,29	48,77	36,71

TABLE 4.33

COMPARISON OF ADJACENT SITES : EUMOVATE OINTMENT vs ULTRALANUM OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	1	0	1	0	0	2	3	34	33
=	16	11	13	3	3	4	7	9	3
>	24	22	26	21	16	8	6	3	5
<	31	39	32	48	53	58	56	26	31

TABLE 4.34

COMPARISON OF ADJACENT SITES : EUMOVATE OINTMENT vs ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	3	1	2	1	3	7	12	44	47
=	7	9	5	1	3	2	4	11	3
>	48	52	56	60	58	55	48	11	13
<	14	10	9	10	8	8	8	6	9

TABLE 4.35

COMPARISON OF ADJACENT SITES : ULTRALANUM OINTMENT vs ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	1	1	1	0	1	2	4	30	40
=	14	7	3	1	0	0	0	7	2
>	41	51	57	67	69	67	64	32	28
<	16	13	11	4	2	3	4	3	2

TABLE 4.36

COMPARISON OF ADJACENT SITES : EUMOVATE OINTMENT vs ULTRALANUM OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	2	1	0	0	0	0	0	19	21
=	8	13	7	3	8	8	11	9	4
>	38	35	35	33	27	31	25	11	10
<	24	23	30	36	37	33	36	33	37

TABLE 4.37

COMPARISON OF ADJACENT SITES : EUMOVATE OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	1	1	0	0	0	1	1	28	30
=	2	3	2	1	2	1	3	8	4
>	69	65	67	69	68	67	66	35	35
<	0	3	3	2	2	3	2	1	3

TABLE 4.38

COMPARISON OF ADJACENT SITES : ULTRALANUM OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	10	12	14	16	18	28	32
0	4	4	2	0	1	1	2	17	27
=	2	4	2	2	2	2	4	10	1
>	50	57	62	65	67	65	62	43	42
<	16	7	6	5	2	4	4	2	2

EXPERIMENT 5

TABLE 5.1

FREQUENCIES OF BLANCHING SCORES AND % TPS VALUES FOR DERMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	7	2	1	0	0	0	0	0	20	23	71	87
1	44	21	15	10	4	5	8	10	68	72	36	19
2	41	44	41	40	27	22	32	50	20	13	1	2
3	15	36	44	38	47	51	43	42	0	0	0	0
4	1	5	7	20	30	30	25	6	0	0	0	0
%TPS	40,51	54,86	59,49	65,74	73,84	74,54	69,68	60,19	25,00	22,69	8,80	5,32

TABLE 5.2

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR NERISONE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	4	0	0	0	0	1	3	8	51	40	76	92
1	32	11	13	7	8	12	29	54	55	66	32	16
2	45	49	38	36	31	41	43	40	2	2	0	0
3	27	45	48	46	50	47	30	6	0	0	0	0
4	0	3	9	19	19	7	3	0	0	0	0	0
%TPS	46,99	59,26	62,27	67,82	68,52	60,88	50,23	35,19	13,66	16,20	7,41	3,70

TABLE 5.3

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR FLORONE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	22	10	3	2	2	3	1	5	44	46	92	92
1	68	51	50	41	26	17	28	44	60	59	16	16
2	18	37	35	40	46	56	56	49	4	3	0	0
3	0	10	19	23	33	27	20	9	0	0	0	0
4	0	0	1	2	1	5	3	1	0	0	0	0
%TPS	24,07	35,88	41,90	45,83	51,16	53,24	49,07	40,05	15,74	15,05	3,70	3,70

TABLE 5.4

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	10	2	3	1	12	19	29	39	61	52	95	99
1	71	50	43	44	64	77	72	62	45	56	13	9
2	25	49	42	50	30	10	5	6	2	0	0	0
3	2	7	19	12	2	2	1	1	0	0	0	0
4	0	0	1	1	0	0	1	0	0	0	0	0
%TPS	29,40	39,12	43,52	42,59	30,09	23,84	20,60	17,82	11,34	12,96	3,01	2,08

TABLE 5.5

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	22	8	3	2	1	1	3	0	17	16	46	60
1	55	41	31	21	13	4	5	7	54	47	51	39
2	27	46	51	48	27	22	31	61	34	44	10	8
3	4	12	22	33	59	61	57	35	3	1	1	1
4	0	1	1	4	8	20	12	5	0	0	0	0
%TPS	28,01	40,05	46,99	53,70	63,89	71,99	66,20	58,80	30,32	31,94	17,13	13,43

TABLE 5.6

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR NERISONE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	37	12	4	6	7	10	19	30	61	56	89	96
1	55	68	64	49	50	50	64	66	45	50	19	12
2	14	25	33	39	45	39	19	11	2	2	0	0
3	1	2	6	13	5	8	6	0	0	0	0	0
4	1	1	1	1	1	1	0	1	0	0	0	0
%TPS	20,83	29,63	35,19	39,35	36,81	36,11	27,78	21,30	11,34	12,50	4,40	2,78

TABLE 5.7

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR FLORONE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	63	39	27	15	12	5	4	4	26	30	80	90
1	43	53	52	49	31	25	34	43	74	65	24	18
2	2	15	27	35	50	47	43	48	7	13	4	0
3	0	1	2	9	15	29	26	12	1	0	0	0
4	0	0	0	0	0	2	1	1	0	0	0	0
%TPS	10,88	19,91	25,93	33,80	40,74	49,54	46,76	41,44	21,06	21,06	7,41	4,17

TABLE 5.8

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	57	33	34	27	43	39	47	53	71	59	101	102
1	50	74	70	78	65	69	60	55	37	48	6	6
2	1	1	4	3	0	0	1	0	0	1	1	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
%TPS	12,04	17,59	18,06	19,44	15,05	15,97	14,35	12,73	8,56	11,57	1,85	1,39

TABLE 5.9

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Betnovate cream	1735,78	1864,53
Nerisone cream	1367,96	824,11
Florone cream	1132,63	1160,18
Ultradil cream	795,29	503,83

TABLE 5.10

CHI-SQUARED VALUES FOR FLORONE CREAM vs DERMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	57,64	58,13	61,76	66,95	65,61	70,83	71,27	56,08	45,01	39,62	12,60	2,89
Graded Response	37,87	38,13	34,74	39,26	52,66	49,61	44,34	51,34	20,17	15,21	11,40	2,40
Yes/No	7,81	4,32	0,25	0,50	0,50	1,35	0,00	3,28	11,75	10,31	10,00	0,52

TABLE 5.11

CHI-SQUARED VALUES FOR FLORONE CREAM vs NERISONE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	71,27	65,28	65,61	54,22	57,40	22,54	0,18	0,92	0,33	1,64	7,23	1,14
Graded Response	63,99	62,75	43,81	47,72	34,13	9,92	4,72	4,22	1,40	1,02	6,86	0,00
Yes/No	12,64	8,49	1,35	0,50	0,50	0,25	0,25	0,33	0,68	0,48	6,03	0,04

TABLE 5.12

CHI-SQUARED VALUES FOR FLORONE CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	7,10	4,50	0,01	0,04	60,95	73,75	78,12	78,64	8,11	6,15	0,00	0,06
Graded Response	7,70	7,55	1,16	5,34	55,01	108,55	106,32	70,35	5,56	3,45	0,36	2,22
Yes/No	4,44	4,32	0,17	0,00	6,19	11,39	28,22	31,08	4,74	0,47	0,16	1,63

TABLE 5.13

CHI-SQUARED VALUES FOR FLORONE CREAM vs DERMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	56,11	74,71	78,22	62,72	71,41	80,61	79,21	61,45	33,01	51,01	27,19	30,19
Graded Response	46,80	48,04	49,56	40,89	57,70	53,04	44,54	45,39	23,79	25,01	22,47	22,74
Yes/No	31,04	24,47	20,48	9,19	8,18	1,54	0,00	2,29	1,86	4,67	20,74	18,35

TABLE 5.14

CHI-SQUARED VALUES FOR FLORONE CREAM vs NERISONE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	17,75	31,51	28,87	6,83	0,04	30,45	67,37	86,49	48,19	36,01	4,97	2,04
Graded Response	19,23	19,99	21,91	5,80	12,04	23,00	41,76	59,94	24,93	17,88	5,06	1,39
Yes/No	11,64	17,35	18,23	3,38	0,92	1,15	9,54	21,82	22,25	12,08	1,74	0,97

TABLE 5.15

CHI-SQUARED VALUES FOR FLORONE CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	0,93	1,23	4,71	7,92	73,72	88,76	102,01	102,01	60,49	52,22	16,46	6,05
Graded Response	1,16	17,22	22,52	46,00	94,51	124,87	110,54	104,59	41,21	22,29	15,04	6,75
Yes/No	0,47	0,52	0,82	3,58	21,95	31,08	45,28	54,91	36,23	14,98	13,64	5,67

TABLE 5.16

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs DERMOVATE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	5	0		0	0	0	0	0	13	18	66	79
=	8	6	12	10	8	6	9	5	4	8	0	1
>	10	12	9	8	9	8	7	13	13	12	9	9
<	85	90	87	90	91	94	92	90	78	70	33	19

TABLE 5.17

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs NERISONE CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	3	0	0	0	0	0	0	0	23	27	68	85
=	6	5	8	7	10	10	21	20	9	7	0	1
>	7	10	9	13	11	25	41	49	41	31	11	8
<	92	93	91	88	87	73	46	39	35	43	29	14

TABLE 5.18

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs ULTRADIL CREAM (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	6	1	0	0	0	1	1	1	28	35	85	88
=	14	9	4	7	3	2	1	4	9	8	0	2
>	31	38	53	52	93	97	99	97	48	43	11	10
<	57	60	51	49	12	8	7	6	23	22	12	8

TABLE 5.19

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs DERMOVATE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	20	2	3	1	0	0	2	0	10	10	38	55
=	8	7	6	10	2	3	6	9	3	10	2	0
>	6	6	5	9	9	6	5	10	19	10	12	6
<	74	93	94	88	97	99	95	89	76	78	56	47

TABLE 5.20

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs NERISONE CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	27	5	2	1	2	0	4	3	18	21	72	84
=	8	7	5	8	13	5	9	5	5	3	2	0
>	18	20	23	36	45	80	88	97	75	70	24	16
<	55	76	78	63	48	23	7	3	10	14	10	8

TABLE 5.21

COMPARISON OF ADJACENT SITES : FLORONE CREAM vs ULTRADIL CREAM (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	33	13	12	6	5	2	3	4	23	27	73	88
=	6	14	11	3	5	0	1	0	4	5	0	0
>	39	46	53	64	92	102	104	104	76	70	30	16
<	30	35	32	35	6	4	0	0	5	6	5	4

TABLE 5.22

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	0	0	0	0	0	0	1	0	19	18	67	72
1	11	5	2	3	1	0	6	1	56	67	41	36
2	66	52	27	25	16	13	22	31	30	22	0	0
3	31	48	71	44	50	50	48	64	3	1	0	0
4	0	3	8	36	41	45	31	12	0	0	0	0
%TPS	54,63	61,34	69,68	76,16	80,32	82,41	73,61	70,14	28,94	26,39	9,49	8,33

TABLE 5.23

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR NERISONE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	3	0	0	0	0	0	4	6	66	62	95	93
1	47	29	12	15	10	12	53	51	42	46	13	15
2	52	57	49	44	44	59	39	48	0	0	0	0
3	6	22	44	42	45	35	11	3	0	0	0	0
4	0	0	3	7	9	2	1	0	0	0	0	0
%TPS	39,12	48,38	58,80	59,49	62,27	56,25	38,89	36,11	9,72	10,65	3,01	3,47

TABLE 5.24

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR FLORONE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	17	5	0	1	0	0	3	0	57	58	99	103
1	67	51	36	31	19	15	20	25	47	47	9	5
2	23	40	44	41	46	39	48	65	4	3	0	0
3	1	12	28	33	37	49	34	18	0	0	0	0
4	0	0	0	2	6	5	3	0	0	0	0	0
%TPS	26,85	38,66	48,15	50,93	56,94	60,19	53,24	48,38	12,73	12,27	2,08	1,16

TABLE 5.25

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	30	10	6	9	31	44	59	56	83	85	102	102
1	64	80	71	80	67	61	45	49	25	23	6	6
2	13	15	28	17	9	3	3	3	0	0	0	0
3	1	3	3	2	1	0	0	0	0	0	0	0
4	0	0	0	0	0	0	1	0	0	0	0	0
%TPS	21,53	27,55	31,48	27,78	20,37	15,51	12,73	12,73	5,79	5,32	1,39	1,39

TABLE 5.26

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR DERMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	0	0	0	0	0	0	0	0	16	19	73	74
1	11	5	3	3	0	2	9	2	61	66	35	34
2	76	55	30	23	21	19	23	53	31	22	0	0
3	20	46	71	67	61	60	52	47	0	1	0	0
4	1	2	4	15	26	27	24	6	0	0	0	0
%TPS	52,55	60,42	67,59	71,75	76,16	75,93	71,06	63,19	28,47	26,16	8,10	7,87

TABLE 5.27

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR NERISONE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	23	8	3	6	3	5	24	24	71	72	94	99
1	52	53	38	38	40	57	47	66	36	36	14	9
2	27	43	52	46	43	37	28	17	1	0	0	0
3	6	4	15	16	21	9	9	1	0	0	0	0
4	0	0	0	2	1	0	0	0	0	0	0	0
%TPS	28,70	34,95	43,29	43,06	44,68	36,57	30,09	23,84	8,80	8,33	3,24	2,08

TABLE 5.28

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR FLORONE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	37	17	5	5	3	3	10	7	65	67	87	97
1	59	70	64	54	45	45	44	63	43	40	21	11
2	12	20	32	37	41	48	42	30	0	1	0	0
3	0	1	7	12	18	12	12	8	0	0	0	0
4	0	0	0	0	1	0	0	0	0	0	0	0
%TPS	19,21	26,16	34,49	37,96	42,82	40,97	37,96	34,03	9,95	9,72	4,86	2,55

TABLE 5.29

FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Response												
0	47	28	20	21	36	55	57	61	88	82	103	106
1	57	74	80	83	71	51	51	45	19	26	5	2
2	4	6	8	4	1	2	0	2	1	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
%TPS	15,05	19,91	22,22	21,06	16,90	12,73	11,81	11,34	4,86	6,02	1,16	0,46

TABLE 5.30

AREA UNDER THE CURVE VALUES

PREPARATION	OCCLUDED	UNOCCLUDED
Dermovate ointment	2007,10	1897,44
Nerisone ointment	1123,28	827,95
Florone ointment	1165,26	891,82
Ultradil ointment	484,25	405,94

TABLE 5.31

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs DERMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	103,01	94,45	72,37	80,61	56,86	98,23	75,70	96,24	73,29	55,74	21,79	23,08
Graded Response	106,11	68,95	61,17	59,93	58,72	60,01	43,64	72,00	42,67	40,00	26,65	28,93
Yes/No	16,35	3,28	0,00	0,00	0,00	0,00	0,25	0,00	27,79	30,88	25,01	27,09

TABLE 5.32

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs NERISONE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	50,65	38,16	29,45	18,13	4,32	3,47	43,13	42,29	4,34	4,34	2,45	4,76
Graded Response	28,09	16,97	18,82	10,53	4,22	8,03	28,75	28,17	4,94	3,14	0,81	5,51
Yes/No	9,31	3,28	0,00	0,00	0,00	0,00	0,00	4,29	1,21	0,17	0,46	4,46

TABLE 5.33

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	10,45	27,25	56,08	60,01	89,76	102,08	101,08	102,08	15,25	21,12	0,31	0,00
Graded Response	6,44	24,85	41,17	67,42	122,79	156,70	134,90	138,31	15,55	16,33	0,64	0,10
Yes/No	3,92	1,15	4,29	5,14	33,90	52,77	68,43	72,92	12,69	13,99	0,29	0,00

TABLE 5.34

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs DERMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	100,08	98,23	106,01	88,76	94,45	98,23	88,76	89,76	61,45	64,45	7,22	12,60
Graded Response	137,46	134,75	117,12	107,19	101,00	113,89	87,67	104,27	63,76	53,34	4,73	14,85
Yes/No	42,27	16,35	3,28	3,28	1,35	1,35	8,49	5,32	45,51	42,68	4,07	13,59

TABLE 5.35

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs NERISONE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	36,10	24,27	33,98	18,13	0,49	9,24	27,88	35,64	5,68	7,85	0,35	0,06
Graded Response	15,48	15,79	14,80	6,42	0,57	3,76	9,09	18,43	1,89	1,39	1,67	0,22
Yes/No	3,90	2,90	0,13	0,00	0,17	0,13	5,90	9,64	0,50	0,32	1,23	0,06

TABLE 5.36

CHI-SQUARED VALUES FOR FLORONE OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
Comparison of adjacent sites	1,78	6,97	23,54	44,89	73,75	92,46	85,49	83,80	18,15	14,38	7,68	5,82
Graded Response	5,23	11,34	32,18	54,55	90,85	101,32	87,49	78,38	13,75	5,48	11,19	6,63
Yes/No	1,58	2,81	8,87	9,84	32,04	61,31	45,78	60,29	10,85	4,24	9,84	5,24

TABLE 5.37

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs DERMOVATE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	0	0	0	0	0	0	0	0	12	13	61	69
=	3	2	1	3	1	0	1	2	2	2	0	0
>	0	3	9	6	14	2	8	2	5	10	7	4
<	105	103	98	99	93	106	99	104	89	83	40	35

TABLE 5.38

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs NERISONE OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	3	0	0	0	0	0	1	0	45	43	88	87
=	11	4	9	6	6	4	6	5	4	6	0	0
>	12	20	22	29	40	62	84	85	38	38	6	5
<	82	84	77	73	62	42	17	18	21	21	14	16

TABLE 5.39

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs ULTRADIL OINTMENT (OCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	10	0	0	0	0	0	1	0	45	47	95	98
=	6	1	5	4	1	0	0	0	4	3	0	1
>	62	81	90	92	103	107	106	107	45	47	8	4
<	30	26	13	12	4	1	1	1	14	11	5	5

TABLE 5.40

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs DERMOVATE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	0	0	0	0	0	0	1	0	9	15	58	66
=	2	0	0	2	0	0	1	1	0	1	0	0
>	1	2	0	4	3	2	4	4	10	7	15	9
<	105	106	108	102	105	106	102	103	89	85	35	33

TABLE 5.41

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs NERISONE OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	14	1	0	0	0	0	4	3	47	59	79	91
=	4	4	9	6	8	4	7	4	4	3	3	1
>	16	26	20	29	46	68	75	81	38	33	15	8
<	74	77	79	73	54	36	22	20	19	13	11	8

TABLE 5.42

COMPARISON OF ADJACENT SITES : FLORONE OINTMENT vs ULTRADIL OINTMENT (UNOCCLUDED)

Time (hrs)	7	8	9	10	12	14	16	18	28	32	52	56
0	23	5	2	1	0	2	7	5	48	58	85	97
=	4	6	4	4	3	0	2	2	0	3	1	0
>	47	62	76	86	97	103	96	97	47	37	18	10
<	34	35	26	17	8	3	3	4	13	10	4	1

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