A CRITICAL EVALUATION OF THE HUMAN SKIN BLANCHING ASSAY AND COMPARATIVE BIOAVAILABILITY STUDIES ON TOPICAL CORTICOSTEROID PREPARATIONS

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This thesis is dedicated to those Soviet Jews
who through no fault of their own
have been frustrated in their pursuit of religion and education

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ABSTRACT

Several aspects of the human skin blanching assay were evaluated in an attempt to suggest improvements in the methodology of this assay. Three trials were performed in the unoccluded application mode, using two proprietary creams containing 0,1% betamethasone (as the 17-valerate). Preliminary observations of the influence of ambient temperature and relative humidity on the blanching response did not allow definite conclusions to be drawn. Studies on the number of observers required for reliable results of comparative blanching indicated that at least two trained observers should be employed. Analyses of the results of individual volunteers demonstrated the expected biological variability, and suggest that subjects selected for trials should represent a range of blanching responses. No sex-related differences in blanching responses were found, and both arms exhibited similar sensitivity to corticosteroids. Retrospective analysis of 95 040 observations of blanching responses showed that in the unoccluded application mode blanching is lowest close to the wrist, and in the occluded mode blanching is lowest close to the elbow.

Studies on the method of transportation of Betnovate preparations suggest that topical formulations should not be exposed to temperature extremes during transportation. It is proposed that patients should not transport topical formulations in the holds of ships or aircraft, and that exporters and manufacturers should make use of special transportation and storage conditions.

In a study of ten topical formulations from three countries it was found that there was no trend of products from one country consistently

exhibiting superior blanching to products from the other two countries, or products from one country consistently exhibiting the lowest degree of blanching, although considerable differences in blanching responses were found in some cases. Interpretation of the results of these studies demonstrated the importance of employing a combination of statistical analyses, blanching profiles and AUC values when drawing conclusions regarding comparative bioavailability.

A study of the blanching profiles of Betnovate cream included in all 16 trials performed during this work indicated that this preparation behaved in a similar fashion during all trials, thereby giving credence to the results of the trials.

CONTENTS

CHAPTER 1

INTRODUCTION

The usefulness of hydrocortisone acetate (compound F) in the treatment of dermatoses was first reported by Sulzberger and co-workers in 1952 (1) and 1953 (2). The use of topically applied corticosteroids has, since then, had a substantial influence on the approach to the treatment of various skin disorders (3-12), with the discovery of the efficacy of hydrocortisone acetate having been described as one of the most important contributions to medical therapeutics this century (13). Corticosteroids are amongst the most frequently prescribed and efficacious compounds in modern dermatological therapy (14-17), and several reports have discussed the extensive use of topical corticosteroid-containing preparations in the United States of America and the United Kingdom (3, 4,18,19). The medical practitioner in South Africa can choose from 90 available preparations (20) and in the United Kingdom from 125 preparations (21), depending on the desired vehicle, concentration of the corticosteroid and whether or not additional pharmacologically active agents are indicated.

The extensive employment of topical corticosteroid therapy has stimulated researchers to investigate the pharmacology of these drugs, as well as various chemical, physical and pharmaceutical properties.

1.1 PHARMACOLOGY

Topical corticosteroids are used extensively in medicine 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 (22,23).

Skin disorders have been reported to be the second most common minor illness (24) and it is well known that many of these are effectively controlled by topical corticosteroid therapy. 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 erythematosus, 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 (5,8,18,23,25,26).

1.1.1 Mode of Action

The physiological actions of corticosteroids allow them to be divided into two major groups, namely mineralocorticoids (those influencing fluid and electrolyte balance) and glucocorticoids which influence metabolism of carbohydrates, fats and proteins (26,27). It has thus far been impossible to dissociate the glucocorticoid and anti-inflammatory properties of synthetic corticosteroids for topical application, although the unwanted activity of the mineralocorticoids is no longer a problem during therapy (26,27,section 1.2) now that fludrocortisone acetate (28) is not used topically.

Although the mechanism of action of topical corticosteroids remains unresolved and has been the subject of much speculation, it is thought that the efficacy of these agents is associated with their anti-inflammatory, antipruritic (with questionable efficacy (21,29,30)) and vasoconstrictive actions (31). The complexity of the inflammatory response is recognized and it is generally accepted that corticosteroids influence many aspects of this response (5,32,33). The degree of anti-inflammatory activity of corticosteroids appears to be quantitatively related to the concentration of the drug present at the site of inflammation (34). It has been shown that the concentration of intravenously injected corticosteroid is higher in inflamed skin than in normal skin and this has led to the suggestion that these agents act directly on the cells of the lesions of dermatoses, not indirectly through their action on blood vessels (35). The suppressive action of corticosteroids on blood vessels therefore does not seem to be the main mechanism of action in the treatment of dermatoses (35) but may act in parallel to other anti-inflammatory effects by blocking circulating mediators of inflammation from the local site (26). The vasoconstrictor activity also decreases swelling,

discomfort and serum extravasation (7). Some factors that are involved in the mechanism of vasoconstriction are discussed in section 1.7.

Some of the suggested mechanisms of anti-inflammatory action include the decrease in membrane permeability by a lysosomal membrane stabilizing effect (13,33,36,37) and inhibition of the formation, release and action of endogenous mediators of inflammation (for example kinins, histamine and prostaglandins) by the corticosteroids attaching themselves to tissue receptors (22,33,34,38-47).

Corticosteroids have been found to inhibit endogenous phospholipase activity (44,47), which in turn leads to suppression of the production of arachidonic acid, a substance required for the synthesis of prostaglandins and other cutaneous mediators of inflammation (44). Corticosteroids are also immunosuppressive and may inhibit allergic reactions by preventing the production of antigen/antibody complexes (7,33), although the production of antibodies *per se* does not appear to be significantly inhibited (32).

It is well established that corticosteroids exhibit an antimitotic effect on various cells in the body (7,22,48-51) and it appears as though this may be caused by enzyme inhibition (52). Marks *et al.* (53) demonstrated a decreased uptake of thymidine in corticosteroid-pretreated skin, probably reflecting decreased epidermal DNA synthesis due to steroid treatment. This is possibly an important factor in the antimitotic effect of topical corticosteroids and may explain their well documented effectiveness in the treatment of psoriasis (26,51,54-60).

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 the use of occlusion) or by increasing the inherent potency of the drug, has led to the incidence and severity of side effects becoming increasingly more significant (25,26). The side effects of topical corticosteroids can, in general, be divided into two categories, namely local effects and systemic effects (7,22).

The most common side effect of topical corticosteroids (other than burning, itching, irritation and dryness) appears to be skin atrophy which occurs with the application of corticosteroids over a prolonged period. The mechanism of atrophy seems to be multifactorial and the condition manifests as thinning of the epidermis (including the stratum corneum) and dermal tissue (7,9,25,26,61-64). 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 (65). An accompanying decrease is found in the diameter of collagen fibrils and in the number of fibroblasts (65). *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 (66). 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 (65,67). Two of the abovementioned studies have further shown a positive dose-response effect with the corticosteroids used (66,67).

Telangiectasia, which is often found within the area of atrophy (25), is another well documented side effect of topical corticosteroids (22,25, 30,64). A correlation has been observed between the severity of telangiectasia and atrophy (64). Data obtained from two independent studies on corticosteroid-containing creams further indicate that the rank order of blanching correlates with the severity of atrophy and telangiectasia caused by these preparations (64,68). It should be noted that the experiments were performed about seven years apart in different laboratories with different proprietary preparations. Differences in the extent of percutaneous absorption at various sites and due to different methods of application also have an influence on the side effects, which increase with an increase in percutaneous absorption (7,25,26).

Other local side effects include striae (which are irreversible), allergic reactions (usually 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), delayed wound healing and ophthalmic effects such as

glaucoma (7,9,22,25,30,33,69-74).

Systemic side effects due to the absorption of topically applied corticosteroids include reduction in endogenous cortisol levels either by direct action on adrenocortical function or by inhibition of ACTH production in the anterior pituitary lobe. Clinical manifestations have not been reported in a very large number of cases, although there are reports of iatrogenic disease (7,28,75-77). The occurrence of Cushing's syndrome and a depression of cortisol levels have been reported in patients and healthy experimental volunteers after the use of topical corticosteroids (22,25,26,75-84). In some experiments cortisol suppression was not seen in normal subjects, but occurred in psoriatic patients, suggesting enhanced percutaneous penetration through psoriatic skin (82,85). It has further been noted in the clinical situation (28, 76,83) that the penetration of corticosteroids appears to decrease as the dermatitis clears, thus suggesting that the problems associated with percutaneous absorption are temporary and occur mainly in the initial stages of treatment. The possibility of iatrogenic suppression of plasma cortisol levels should therefore be considered during treatment with topical corticosteroids. This is especially important when occlusion is required and percutaneous absorption occurs more readily (22,25,26,82, 83), as well as in the case of children (19,25,26,33,75,83,86-88) where the surface to weight ratio is greater than that for adults. Hepatic dysfunction in patients has been found to increase the possibility of systemic effects of topical corticosteroids and should therefore be considered before prescribing potent corticosteroids for long periods (89,90).

It should be noted, however, that although the morbidity of systemic side 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 seldom used over large body areas for prolonged periods (5,8,26,30,76,80,82-84,91).

As mentioned previously, an increase in the incidence and severity of side effects will more than likely follow an increase in the therapeutic efficacy of a drug. The inclusion of a halogen atom is the most common

and generally most effective means of increasing the efficacy of topical corticosteroids (see section 1.2). This has led to the terms "fluorinated" and "halogenated" being used to describe the superior therapeutic efficacy of the newer corticosteroid molecules over hydrocortisone, and "fluorinated steroids" being most often implicated in the production of various side effects (9,25,92). The terms "fluorinated", "strong" and "halogenated" should, however, not be used synonymously (83,93). Hydrocortisone 17-butyrate (92), desonide (93-95), budesonide (96) and domoprednate (97-99) do not contain halogen atoms but have been shown to elicit similar or superior responses to halogenated corticosteroids in blanching assays (68,93,95,96,100,101) and clinical trials (92-95,97,98, 102). Their local and systemic side effects have also been found to be similar to or more severe than those of the halogenated analogues (61,64,67,72,82,83,92).

1.2 STRUCTURE ACTIVITY RELATIONSHIPS

The basic structure to which all corticosteroids are related is that of the fully reduced phenanthrene to which is fused a 5-membered ring structure, giving rise to a cyclopentanoperhydrophenanthrene nucleus consisting of three 6-membered rings and a 5-membered ring, fused to form the basic nucleus depicted in figure 1.1 (5,33,103-105). The D-homocorticosteroids, which are also effective anti-inflammatory agents, have a 6-membered ring instead of the 5-membered ring (97-99). A further common feature of steroids is the presence of methyl groups attached at positions 10 and 13 and designated 19 and 18, respectively (22,103). The four rings of the corticosteroid skeleton do not exist in a flat

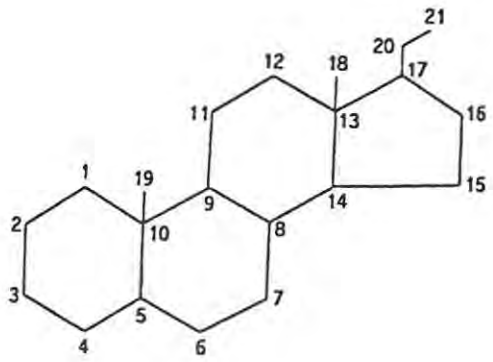

Figure 1.1 The basic corticosteroid nucleus

plane (106) and the structure has no elements of symmetry, with each of the 19 positions being chemically distinct from each of the others (103). The corticosteroid skeleton is a rigid structure, and small changes in the position of a substituent will often result in a large change in biological activity (22,103).

Some of the essential structural features required for glucocorticoid and anti-inflammatory action and binding to receptors include (3,33,34, 104-108):

a) a double bond between carbon atoms 4 and 5,
b) a $-CO.CH_2OH$ moiety at carbon 17 (producing a C21 steroid),
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.

This gives rise to corticosterone and the simplest modification to this is hydrocortisone which has, in addition to the above, an hydroxyl group at carbon 17 (5,109). Hydrocortisone retains little of the mineralocorticoid activity of corticosterone, but has the glucocorticoid and anti-inflammatory activity. It is interesting to note that cortisone, the 11-keto analogue of hydrocortisone is ineffective topically (109), although it is absorbed through the skin to the same extent (110).

The constant drive to find molecules with increased anti-inflammatory activity and reduced unwanted effects has given rise to several alterations in the synthetic analogues of cortisone. The potential number of changes that can be made to the basic structure is unlimited and the literature abounds with such reports. As a complete review of these reports does not lie within the scope of this thesis, only some of those changes that have had a significant effect on topical corticosteroid therapy and research will be discussed.

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 (22,33,34,107,109,111). The synthesis of the 6α- and 9α-halogenated derivatives (22,33,34,103,109,111,112) had a major influence in enhancing both glucocorticoid and mineralocorticoid effects, the latter being overcome by the introduction of a substituent

at the 16 position, for example the β-methyl group in betamethasone (22,103,107,111), the α-hydroxyl group in triamcinolone (22,103,111) and the 16,17-acetonide group in fluocinolone acetonide acetate (fluocinonide) (109). In addition to decreasing the unwanted mineralocorticoid effects, the 16,17-acetonide group has been found to increase the corticosteroid-induced blanching (112). Substitution of the 21-hydroxyl group with a chlorine atom to produce clobetasol 17-propionate resulted in an increase in lipophilicity and has given rise to an extremely powerful topical corticosteroid (103). Combination of the 21-fluoro substituent with the 16,17-acetonide moiety of fluocinolone acetonide has resulted in halcinonide (107), a topical corticosteroid that exerts a very potent blanching response (101,113). Alclometasone contains a 7α-chlorine atom and has been found to be effective in clinical trials (102,114-116). Halometasone is a trihalogenated molecule which is clinically efficacious (117-121) and elicits a blanching response similar to that of clobetasone 17-butyrate (122).

Halogenation (especially fluorination) has therefore given rise to some very potent topically active corticosteroids, but the terms "fluorinated steroid" and "potent steroid" should not be used synonymously, since some of the non-fluorinated compounds undoubtedly fall into the potent group (5,22,section 1.1.2). An interesting exception to the effect of fluorination is seen in the comparison of triamcinolone acetonide and desonide (93,95). The only difference in the structure of these compounds is the absence of the 9α-fluorine atom in desonide. Desonide, however, has been found to penetrate the skin to a greater extent than triamcinolone acetonide in *in vitro* human (93) and *in vivo* animal studies (123), to elicit a similar response to triamcinolone acetonide in blanching studies (93,95) and not to be statistically different in a clinical trial using a preparation containing a 50% lower concentration of desonide than triamcinolone acetonide (95).

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 (105), although these two effects may be divorced from each other (124). The stratum corneum is known to be an effective barrier to most compounds (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. An increase in lipophilicity, generally by masking one or more of the hydroxyl groups on the side chains of the corticosteroid molecule, is regarded as an important and useful means of improving topical activity (3,22,106,107).

The structure of the drug will therefore influence its oil/water solubility which will in turn influence 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 (125-128). 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.* (125) 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. The authors 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. The solubility of corticosteroids is increased appreciably when the solvent possesses both polar and non-polar character, and the lipid and relatively polar protein components of the stratum corneum seem to aid in solvating these molecules (125). The water within the stratum corneum greatly impedes migrating molecules, particularly those which possess hydroxyl groups available for hydrogen bonding (129).

Generally drugs possessing dual solubility in both oil and water require the least hydration of the stratum corneum for percutaneous penetration, followed by drugs soluble only in water. Those soluble only in oil appear to require the greatest hydration of the stratum corneum for penetration to occur (130). It is known that the hydrated stratum corneum has an affinity for both water-soluble and lipid-soluble compounds (127,131). It would seem reasonable to extrapolate from this that the hydrated stratum corneum has affinity for compounds with dual solubility.

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. *In vitro* studies performed by Ponec and Polano (132) have however shown that the amount of corticosteroid that penetrated the epidermis after application in an ethanolic solution decreased with decreasing polarity of the corticosteroid. Osamura (128) points out that polarity and lipid solubility of corticosteroids are not the only factors that influence 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 a 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 (133) showed that the absorption of corticosteroid salts was different to that of the parent alcohols when compared by using the blanching assay. Intradermal injections of dexamethasone and its phosphate derivative elicited similar blanching responses, whereas after topical application the relative activity of the alcohol was far superior to that of the salt, a phenomenon suspected to be due to the difference in penetrability of the two compounds. After topical administration, the phosphate salt of prednisolone elicited less blanching than the acetate and similar blanching to the parent alcohol. The general conclusion was that the acetates are better absorbed than the parent alcohols, whilst the phosphate salts are poorly absorbed.

The removal or masking of the hydroxyl groups to increase lipophilicity often occurs at the 17 and/or 21 positions (111). The length and position of the substituent side chains are important (134) as can be seen in the comparisons of betamethasone 17-valerate and 21-valerate (22,135) and by comparing different corticosteroid esters in the 17 and 21 positions using the blanching assay (135,136). It has further been found that the introduction of a sulphur atom into the 17 side chain (137) or 21 side chain (138) could be effective in enhancing anti-inflammatory activity and decreasing side effects. The marked variance in effect of

derivatization at positions 17 and 21 has been attributed to the difference between a tertiary alcohol enveloped by the steroid nucleus and the relatively unhindered, mobile primary alcohol (134).

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) (124), but betamethasone 17-valerate elicits a substantially greater blanching response than that of the parent alcohol (112,124,135). Conversely, fluocinolone acetonide and fluocinolone acetonide acetate have similar penetration abilities and both penetrate better than fluocinolone alcohol, which also has a much lower blanching activity than these esters (124). Although the fluocinolone esters have similar penetration abilities, fluocinonide was found to be five times more potent than fluocinolone acetonide in the blanching assay (112, 124). 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 (6,112). Similarly, fluocinolone acetonide was absorbed to a very much greater degree than betamethasone 17-valerate, but their blanching responses were similar (124).

Although penetration of the corticosteroid molecule is essential for it to exert its physiological and pharmacological action (section 1.3) it is, to a large extent, the inherent pharmacological activity of the molecule that allows one corticosteroid to be classed as superior to another (5,6,128). The binding affinity of corticosteroids to receptors in the skin has generally been found to correlate well with the degree of blanching elicited by the corticosteroid as well as with clinical efficacy (108).

1.3 PERCUTANEOUS ABSORPTION

It is generally accepted that topical corticosteroids and other drugs that 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 (5,22,

126,130,139-141). Ostrenga (142) described a striking similarity between blanching profiles and *in vitro* penetration. It is suggested from this and other experiments that the rate-controlling step lies 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 (22,105,106,129,142-144). If, however, the skin is damaged and the barrier is thus absent, the skin may be functioning as a perfect sink (106,144) and the release of the drug from the vehicle becomes the rate-limiting step (106,109,145,146). It is therefore reasonable to assume that in the clinical situation, as the healing process proceeds, the rate-controlling step will shift from release of the drug from the vehicle to penetration of the drug through the skin (147). This assumption is strengthened by the observation that systemic side effects become less significant a short while after therapy with topical corticosteroids has commenced (28,76,83,84,86).

The stratum corneum is thus the first and often principal barrier encountered by a corticosteroid after topical application (22,26,125, 127,131,148-150). It has been found that the isolated stratum corneum is almost as impermeable as the entire skin (150,151). There are several factors which influence the percutaneous absorption and consequently the efficacy and blanching ability of topically applied corticosteroids. There are numerous reports in the literature dealing with the absorption of various substances through human and animal skin *in vivo* and *in vitro*. These have been reviewed elsewhere (129,131,143,147-149,152-156) and this review deals only with the percutaneous penetration of corticosteroids through human skin.

1.3.1 Anatomy of Normal Skin

The structure of the skin is depicted diagrammatically in figure 1.2. Although the skin may be discussed as a singular entity, it is in fact a highly heterogeneous organ consisting of several functionally discrete sub-organs and varying greatly in different body areas (157), exhibiting characteristic thickness, pliability, surface markings, pigmentation, glandular activity and hair growth (157,158). The role of regional variation in percutaneous absorption is discussed in section 1.3.2.3. It can be seen from figure 1.2 that the skin consists of several layers which

include the epidermis at the surface, with the dermis (corium), which supports the epidermis, below this (157,158). The dermis has a considerable supply of blood vessels (146,156,158), and because these approach the dermal/epidermal interface, the dermis cannot be regarded as a significant permeation barrier (146,156,159). Therefore once a topically applied substance traverses the stratum corneum its absorption into the blood stream is assured (160).

The epidermis consists of four layers, namely the deep-lying germinative layer or stratum malpighii, the stratum granulosum, the stratum lucidum and the superficial stratum corneum. The stratum corneum can be subdivided into two layers, namely the exterior horny layer of loosely packed cells (stratum corneum dysjunctum) and the tightly packed stratum corneum conjunctum (compactum) below this (146). The stratum malpighii consists of the basal layer (stratum germinativum) and the stratum spinosum, and it is the cells of the basal layer that multiply rapidly and progressively rise to the top (156-158).

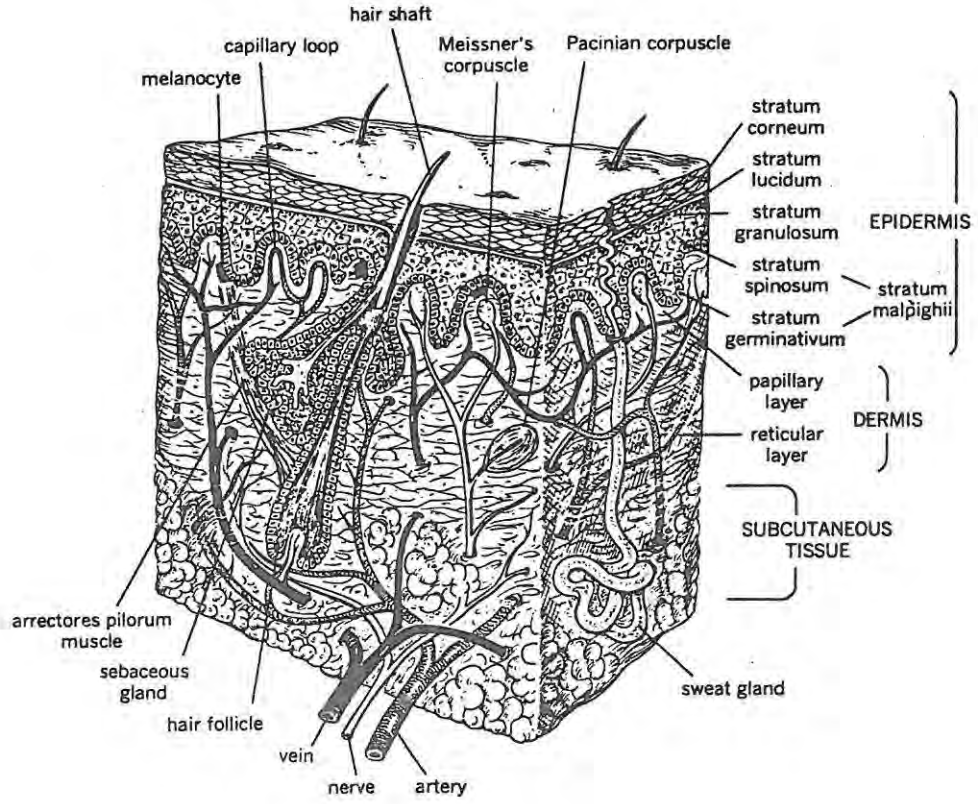

Figure 1.2 Anatomy of normal skin

As the cells of the basal layer ascend to the surface, they enlarge and accumulate basophilic cytoplasmic granules, thereby forming the granular layer (157). These cells finally become the essentially uniformly impermeable stratum corneum (127,131,157) and it is these densely packed, keratin filled, biologically inactive shrunken cells (corneocytes) that are of primary concern in the barrier to percutaneous absorption (129, 131,150,153,156). The mainly hexagonal (161) cells of the stratum corneum are arranged in an interlocking structure somewhat akin to bricks and mortar (156). The hexagonal arrangement is efficient for covering large surface areas of the body with the least mass (161).

The barrier function of the skin depends to a considerable extent on the dead horny layer of keratin in the stratum corneum (157,158,160,161). The cells of the stratum corneum conjunctum are rich in protein-bound phospholipids (162). It has been said that the loosely arranged upper two or three cell layers (stratum dysjunctum) are readily permeated and cannot be regarded as a barrier to absorption, while the tightly packed stratum compactum below this is the major barrier (161). Although the cornified cells in the upper region of the horny layer have no appreciable bound phospholipid derived from the epidermal cells, they do contain absorbed sebum, a fatty material formed by the sebaceous glands (157,162). The normal horny layer of the arm is about 0,01 mm in thickness (162) and remains relatively constant irrespective of age (160).

Keratin is a fibrous protein of high molecular weight which is synthesized by epidermal cells (162). It differs chemically from both collagen and elastin which are secreted by specialized cells of the dermis (162). Keratin is a strong material and is extremely resistant to changes in pH, temperature and enzymatic digestion (158). It is found in closely packed filaments suspended in an osmophilic matrix, and is the main component of the corneocytes (161).

Experiments performed to ascertain the location and distribution of corticosteroid receptors in the skin have shown that the distribution of receptors in the epidermis and papillary (upper) dermis is similar, and is higher than in the reticular (lower) dermis (163). These workers also found a variation in the number of receptors from different anatomical sites and in different age groups. These findings indicate probable

sites of action for topical corticosteroids (163) and may elucidate one of the mechanisms for the influence of age and anatomical regions in the severity of local side effects. It has been shown that cutaneous effects of corticosteroids are mediated by occupancy of glucocorticoid receptors (164), and as the thickness of the stratum corneum is the same in children and in adults the role of the corticosteroid receptors may be worth considering in the study of local side effects.

Certain other aspects of the stratum corneum are pertinent to section 1.3.2 and are discussed in that section.

1.3.2 State of the Stratum Corneum

1.3.2.1 Intact or Damaged

Whether the stratum corneum is intact or not is a major factor in dermatological therapy and investigations (130). Penetration is greatly enhanced when the skin is abraded, broken or inflamed (154) as is the case in numerous dermatoses (107,129,148,165). Percutaneous absorption of radioactive hydrocortisone has been found to be approximately three times more rapid in the initial stages of absorption through irradiated skin compared to normal skin (166). Enhanced absorption of certain topical corticosteroids has also been noted after removal of the stratum corneum by stripping with cellophane tape (6,129,167). Regeneration of the barrier has been found to be just beginning 25 hours after stripping and was complete 72 hours after stripping in 3 of the 4 subjects used, although it was almost complete after 57 hours (167). Feldmann and Maibach (168) 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 may well be significant in the case of damaged skin but will be of less importance in intact skin due to the barrier efficiency of the stratum corneum. It is the diffusion of corticosteroids through this second barrier that gives rise to systemic side effects, and the ideal situation would thus be the retention of the drug in the epidermis with only slow migration to the dermis (107,155). Improved formulation

has indeed allowed more penetration into the dermis with a reduction in systemic absorption (section 1.6.1.3).

1.3.2.2 Hydration and Temperature

Hydration of the stratum corneum *in situ* is achieved by structural lipids and water soluble substances contained within the cells of this tissue (150). The major constituents and their approximate percentages in hydrated stratum corneum are water 75%, protein 20% and lipid 5% (129,148) combined in an ordered structure (129). The amphoteric protein of the stratum corneum is hygroscopic and softens when it contains sufficient water (130); the stratum corneum is therefore always partially hydrated *in situ* (131). It has been suggested that hydration may increase the size of the pores in the membrane (129,130,147-149,153). Additional hydration appears to increase the rate of absorption of all substances which penetrate the skin (22,109,129,130,149,155). The mechanism of percutaneous penetration may therefore be different in hydrated as opposed to normal skin (155). Hydration of the skin can be artificially induced by means of the vehicle (130) or by the application of an occlusive dressing (169,170). Occlusion can increase the water content of the stratum corneum from the normal 5 - 15% to as much as 50% (155) and the thickness from about 15 microns when dry to about 48 microns when fully hydrated (129,159). The water content of the stratum corneum, as expected, decreases after removal of the occlusive dressing. The situation for the first hour after removal of the occlusive dressing seems unclear, where infrared spectroscopy has shown a further increase in water content, while direct current resistance measurements indicated a decrease (170).

McKenzie and Stoughton (169) and McKenzie (133) were the first to attempt to quantitate the effect of occlusion by using the blanching assay, although plastic dressings had been successfully employed in topical corticosteroid therapy prior to these studies. 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 without occlusion, displayed vasoconstrictive properties when applied under occlusion (133). Conversely it has been found that hypohidrosis or anhi-

drosis, whether due to a disease state or induced artificially, caused a decrease in blanching after the application of topical corticosteroids under occlusion (171). When the skin in these subjects was artificially hydrated (with physiological saline) blanching was noted in 13 of the 17 sites. The other four sites remained resistant to the corticosteroid-induced blanching, even in the presence of water and occlusion.

Occlusion with plastic film has thus far been found to be the single most effective mechanism for increasing penetration (6,22), a fact that has been demonstrated in numerous experiments since the original observations of McKenzie and Stoughton (6,169,172). 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 (173). The authors offer three possible explanations for this. Firstly the 10 hour occlusion period may have elicited the maximum blanching attainable by betamethasone 17-valerate in the particular formulation tested, implying that no more corticosteroid could be released from that formulation and/or that even if more corticosteroid was released the maximum blanching response attainable by betamethasone 17-valerate had been reached. The second possibility was that occlusion for 10 hours causes maximum hydration and permeability of the stratum corneum (presumably specific to the corticosteroid tested in the particular formulation used) and that further occlusion could therefore not lead to an increased blanching response. The third possibility involves the subjective nature of the assay in that the ability to observe blanching may have been a limiting factor. The authors, however, go on to point out that the maximum %TPS observed was substantially below 100, thereby strengthening the first two possible explanations. It has further been shown by Szadurski *et al.* (22) that little blanching could be seen after 1- and 2-hour occlusion periods, with blanching becoming evident after occlusion for more than 3 hours.

In an experiment to compare the urinary excretion of radiolabelled 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 (168).

Since many corticoid-unresponsive dermatoses become responsive when occlusion is used in combination with the corticosteroid therapy, it is reasonable to assume that this increase in penetration is clinically significant (6,78). Occlusion has been found to facilitate penetration of the corticosteroid into the deeper layers of the stratum corneum, thereby increasing the amount closer to the living epidermis and consequently closer to the site of anti-inflammatory action (174). It should however be remembered that in the clinical situation occlusion can lead to miliaria and increased microbial (bacterial and candidal) infections, and the occlusion time should be kept to a minimum (5,22,26). Where possible the hydration should rather be attained by using occlusive vehicles such as ointments (130,section 1.6.1.1).

Besides hydration, occlusion also has the effect of increasing the skin temperature (155,169) to core temperature (161). It is known that the penetration rates of some substances are altered by a change in temperature (154,175) 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 (109), as *in vitro* experiments have shown that the permeability of the stratum corneum is only slightly affected by temperatures below 60°C (129,153), while irreversible changes have been noted above 65°C (176). It is further worth noting that experiments on polar non-electrolytes (not corticosteroids) have indicated that percutaneous absorption could decrease significantly in cold weather (131). Some authors have pointed out that under normal *in vivo* conditions, substances penetrate the skin only "within a very narrow temperature range" (155), whilst it has also been said that the skin temperature "fluctuates considerably" (177) and "rapidly" (154). Unfortunately these rather vague terms were not defined.

1.3.2.3 Thickness and Regional Variation

It has been recognized since 1956 that the thickness of the skin has an effect on the rate of absorption of topically applied corticosteroids. An experiment using hydrocortisone showed that it took 6 hours for the corticosteroid to penetrate lichenified skin to the same extent that it had penetrated normal, thinner skin after 2 hours (166). In a comprehen-

sive study of the regional variation in percutaneous penetration it was found that hydrocortisone was absorbed at different rates and to different extents from various anatomical sites (178). Absorption varied from a trace through the heel to a 42-fold increase for the scrotum when expressed relative to absorption through the skin of the ventral aspect of the forearm. Various regions of the head showed greater penetration, whereas the foot showed lower 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 (129,158,179) and may be as thick as 600 microns (158). The structural differences of the stratum corneum at different body areas may also be a factor in the varying degrees of percutaneous absorption (131,178). The variation in absorption at different anatomical sites may explain the difference in response of different areas to the same corticosteroid formulations in clinical practice (5,22).

Foetal and infant skin have been reported to be more permeable than adult skin (129). This is difficult to explain as it has been established that the thickness of the stratum corneum is the same in children and in adults (160), although it is possible that the cells of the stratum corneum are less densely packed in infant skin. Percutaneous absorption of topical corticosteroids therefore occurs more readily in children than in adults (19,86). These factors are also noted in a review by Keipert (86) which 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.

1.3.3 Concentration of Steroid

The concentration of the corticosteroid applied to the skin has been found to influence 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 (section 1.3.5).

It has been demonstrated in blanching assays that increasing the concentration of the corticosteroid leads to an increased degree of blanching, up to a certain point at which a plateau is reached (165,180,181). A plateau in the degree of blanching has also been found after applying different amounts of a cream containing the same concentration of beta-

methasone 17-valerate (173). It should be noted, however, that in this experiment the thickness of the cream applied possibly produced a physical barrier between the corticosteroid in the outer layers of the base and at the skin surface. Continued percutaneous absorption requires diffusion of the drug from the outer layer of the vehicle to the vehicle/skin interface (106,143,182). Barry and Woodford (68) have, on the other hand, found no significant differences in the blanching response when between 3 mg and 8 mg of the preparation was applied, while the plateau effect described in the abovementioned experiment (173) was observed with the application of 4,8 mg of cream. The size of the application sites was equal in both sets of experiments. It has further been found that there is a decrease in the percentage of applied material that will penetrate the skin as the concentration or volume of an agent is increased on the surface area (165,183). Clinical evidence does, however, suggest that increasing the concentration of the corticosteroid increases the therapeutic response (5), which suggests that an increase in the concentration or amount applied may be beneficial in certain cases.

There are a number of possible explanations for the abovementioned plateau. The duration of application is closely related to the concentration of the drug in the vehicle. The concentration of the drug in the vehicle may increase to some extent as the aqueous phase of the vehicle evaporates and will decrease as the drug passes through the stratum corneum (130,184). It has also been suggested that there may be a decrease in the rate of percutaneous absorption as the tissue becomes saturated with the drug (167). 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 (173). The amount of undissolved corticosteroid in the base has also been implicated in the relationship between concentration and vasoconstriction, undissolved drugs not being able to diffuse out of the vehicle (109,144,185). 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 (185). This possibly indicates that the plateau is of biological origin. In the measurement of urinary excretion of radiolabelled corticosteroid after topical application, Maibach and Feldmann (186) however reported

an increase in absorption following an increase in the amount of corticosteroid applied. It is noteworthy that a 500-fold increase in the amount applied (from 4 µg to 2 mg) led to a 100-fold increase in absorption. Another possible explanation is that the relevant receptors in the skin become saturated at a certain point, an increase in the number of corticosteroid molecules thus having no further effect (181). The application of high concentrations of the corticosteroid may also cause deviations from Fick's Law which may be due to membrane changes induced by the high concentrations or due to the partition coefficient between the donor phase and the skin barrier not being constant over the entire concentration range (147).

It is, however, probable that a combination of the above effects contributes to the maximum observed blanching, with the predominant effect depending on the specific experimental variables, such as the inherent blanching ability of the steroid (Altmeyer and Zaun (181) found no concentration dependency in the case of hydrocortisone) or the vehicle (alcoholic or semi-solid) into which the corticosteroid is incorporated.

1.3.4 Routes 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 of corticosteroids, and various conclusions have been drawn over the last 30 years (160,179). When a corticosteroid comes into contact with the skin it has a number of potential routes of entry into the subepidermal tissue, namely *via* the hair follicles and sweat ducts (intra-appendageal), across the continuous stratum corneum between these appendages (intracellular or transcellular) or between the cells of the stratum corneum (intercellular) (127,129,131,149,150,154,159,179,480). The total volume of the intercellular space has been estimated to be 0,01 - 0,1% of the volume of the stratum corneum (149), although this volume has been said to be larger (480). Researchers have attempted to ascertain which of the abovementioned routes are predominant in the percutaneous penetration of topical corticosteroids; some reports have been complementary whilst others have been contradictory. Some of the complexities related to the study of percutaneous absorption are

summarized in a review by Marzulli and Maibach (160). However, an inviolate rule of penetration is that molecules follow the path of least diffusional resistance (481).

Penetration of most substances almost always includes some penetration *via* the skin appendages (129). A study of the percutaneous absorption of radiolabelled hydrocortisone showed an accumulation of the corticosteroid in the orifices of glands and in hair follicles (166). 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. In his review of 1960, Shelmire (130) draws the conclusions that there can be little doubt that drugs which penetrate the stratum corneum do so most easily at the follicular ostia and that all drugs, regardless of solubility, penetrate the skin in exactly the same manner and by the same route, views which could certainly not be agreed with today. Several workers (107,125,127,129,178) have, however, concluded that the follicular component of absorption would be less prominent in substances which are rapidly absorbed (for example ^{32}P tributyl phosphate) when compared with a poorly absorbed substance like hydrocortisone, where all penetration routes are significant. The very small proportion of hair follicles and sweat glands should also be borne in mind (127,129,131,148,149,161) leading to the possible assumption that the transepidermal route is the principal mode of entry (107,129). Barry (129) does however 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 shared by other workers (110, 125,127,148,187). Scheuplein *et al.* (125) have further stated that the large molecular volume and polyfunctional character of corticosteroids have a profound influence on skin permeability, and Barry (129) concludes that the polarity of a molecule could influence the mechanism of penetration.

The role of lipids in percutaneous absorption and the barrier properties of the stratum corneum have also received attention in the literature, and it has been suggested that percutaneous absorption of lipid soluble substances occurs primarily *via* the lipid-rich intercellular spaces (482,483). Whilst intercellular skin lipids are considered to be a major

constituent of the epidermal barrier to some substances (150,484), there is evidence that lipid soluble molecules penetrate the stratum corneum *via* this intercellular route (127,485). The selective penetration of most nonpolar materials across the stratum corneum (131,149) suggests that lipids are important determinants of skin penetration (480). It is also known that facial skin, which has higher lipid content than other skin sites, provides ready access to topical corticosteroids (485). The intercellular spaces of the stratum corneum are rich in lipids and have a greater lipid content than the cells themselves, with lipid composition shifting from polar to nonpolar during keratinization (483). About 75% of the lipids in the stratum corneum are nonpolar with a carbon chain length of lower than 20 (486-488). In a comprehensive review of the mechanism of percutaneous absorption, Flynn (481) concludes that the general concept of at least two pathways, "one lipoidal and the other watery" seems feasible, whilst Cooper (489) has commented on a polar pathway associated with the protein component of the stratum corneum and a nonpolar pathway associated with the lipid component of the stratum corneum. The precise nature of these pathways and their location within the stratum corneum however require further elucidation. It has been suggested that the intercellular route, whilst being physically longer than the transcellular route, may be preferred by some substances because of the presence of continuous intercellular lipids (156). Permeation studies of hydrocortisone esters through mouse skin have indicated that the more nonpolar molecules permeate the skin as if significantly controlled by an aqueous diffusion barrier, whilst the more polar analogues exhibit a penetration behaviour associated with passage across a lipid medium within the membrane (481). In a study of the permeation of several steroids through excised human skin, it was concluded that both the lipid and relatively polar protein components of the stratum corneum are involved in the mechanism of penetration (125). It was found that diffusion constants for steroid molecules generally decreased with the introduction of polar groups onto the molecule (125). It was postulated that this may either have been due to decreased solubility of the steroid in the lipid component of the barrier or increased binding of the steroid to the intracellular protein. It has further been found that there is a positive correlation between the flux of lipophilic substances and increased fluidity of the lipids in the stratum corneum (490). An increase in fluidity is postulated as the mechanism of action

of fatty acids as penetration enhancers (491) and of the enhanced percutaneous penetration due to hydration of the stratum corneum (section 1.3.2.2,490).

It was mentioned previously (section 1.3.2.2) that the mechanism of percutaneous penetration may be different in hydrated as opposed to normal skin. However, 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 mechanism of penetration (168), although it is thought to affect the structure of the stratum corneum (130,153).

The regional variation in percutaneous penetration has provided some interesting data (178). 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 3 times as many sweat glands than other skin sites, but being less permeable than other skin sites (154,178). The possibility of structural specialization of the stratum corneum in these regions should be considered (178). It has, however, been suggested that neither the thickness of, nor the number of cell layers in the stratum corneum are determinants of percutaneous transport, and that the total lipid concentration may be the critical factor governing skin permeability (492).

It is generally accepted that percutaneous penetration of topical corticosteroids is by passive diffusion rather than by a specialized active transport system (22,126,129,142,147-149,154-156). It is well known that the stratum corneum consists of dead keratinized cells (section 1.3.1) and there is no reason to suspect an active transport system (131). Corticosteroids are thought to maintain steady-state penetration through the skin following a lag period (125,149), which may last from several hours up to a few days in the case of the more polar corticosteroids

(150). The steady-state penetration through the stratum corneum is considered by some workers (109,125,149) not to be primarily intercellular or intra-appendageal, but a combination of the two. Others workers (159), however, 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 and that steady-state transport occurs directly through the cells (149,159).

Cognizance should be taken of the fact that most of these experiments were conducted on healthy human skin. This is acceptable in the case of normal comparative 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 (107), although it seems highly probable that the basic principles will be similar (148). Scott and Kalz (166) found differences in the penetration rate of radiolabelled hydrocortisone through normal, lichenified and irradiated skin, but concluded that the mechanism of percutaneous penetration did not appear to be dependent on the condition of the skin.

It should also be borne in mind that several of the experiments cited in this section on percutaneous absorption were performed by measuring urinary excretion of the corticosteroids after topical administration (126,168,178,186), or were performed *in vitro* (125,139). Measuring the urinary excretion of radiolabelled corticosteroids does not take into account metabolism of the corticosteroid in the skin, and *in vitro* techniques, in addition to this, do not take into account the removal of corticosteroid from the skin by the dermal blood supply (129,149). These two factors will probably influence the concentration gradient across the barrier and could conceivably be a source of error in the interpretation of results. In *in vivo* studies the vasoconstriction induced by corticosteroids could also alter the rate of removal of the molecules from the skin (109,126,129,145,149,154,188), this being dependent on the inherent vasoconstrictive ability of the corticosteroid. Feldmann and Maibach (126) tested this by applying hydrocortisone to the skin prior to the application of radiolabelled testosterone and the subsequent measurement of urinary excretion of the testosterone. They concluded that hydrocortisone did not hamper the absorption of testosterone. This

experiment would probably have been more valuable had the pretreatment been with a corticosteroid that is known to induce more vasoconstriction than hydrocortisone.

1.3.5 Biophysics of Skin Transport

The biophysics of transport across the skin is important for the understanding of percutaneous penetration. The subject has been extensively reviewed by Flynn *et al.* (189) but a short description is warranted here.

Several mathematical formulae have been derived to explain the relationships between permeability, diffusion and concentration, and the first to be considered is the simplified version of Fick's Law in equation 1:

$$Js = Kp.\Delta Cs \qquad (1)$$

where Js = steady state flux of the drug (quantity of drug absorbed per unit area and unit of time)
Kp = permeability coefficient
ΔCs = concentration difference of solute across the membrane.

The permeability coefficient in equation 1 can be expressed as:

$$Kp = (D.k_1)/d \qquad (2)$$

where D = diffusion constant of the drug in the stratum corneum
k_1 = stratum corneum/vehicle partition coefficient of drug
d = thickness of the stratum corneum.

It can be seen from equations 1 and 3 (below) that the absorption of the drug is proportional to the concentration of the drug in the vehicle (109,131,149,150,155,190), to the contact time (150,190), the application area (155) and the thickness of the barrier (149). This can be expressed as

$$Q = Js.A.t \qquad (3)$$

where Q = quantity of solute which penetrates the skin
A = area of the membrane covered
t = time of contact.

The diffusion constant (D in equation 2) represents the rate of migration of the drug through the stratum corneum or within the vehicle (109, 150,191,192) and is a measure of the mobility of the solute in the membrane or the vehicle (148). It is inversely proportional to both the size of the molecule and the viscosity of the medium. The stratum corneum exhibits a very high apparent viscosity so the diffusion constant of the molecules is very low, namely between 10^{-12} and 10^{-13} cm^2s^{-1} for corticosteroids (131,150). These values are much higher in the skin below the stratum corneum (10^{-6} cm^2s^{-1}) which is why the stratum corneum is the rate-limiting step in percutaneous absorption. It has been shown that for corticosteroids the diffusion constant decreases with an increase in polar groups (section 1.2). Corticosteroids possess a lower diffusion constant than smaller molecules which penetrate the skin more readily (125), although D varies approximately only as the cube root of molecular weight (152,192). This arises from the increased degree of chemical interaction between the larger corticosteroid molecule and the lipid/protein/water matrix within the stratum corneum (125). It has been suggested that the size and polyfunctional nature of the corticosteroid molecule may cause a weakening in the relationship between Kp and k_1 (125).

The stratum corneum/vehicle partition coefficient (k_1 in equation 2) shows the importance of the solubility characteristics for a substance to penetrate the skin. When k_1 is high, the drug favours the stratum corneum above the vehicle and penetration is enhanced (109,140,150,190, 192). Therefore as the solubility of the drug in the vehicle is decreased, k_1 and Js increase, provided however that the drug is solubilised for adequate penetration. The effects of this are seen in that greatest penetration is attained when the corticosteroid is just dissolved in the vehicle (see below). It can further be seen that the permeability coefficient (Kp) is proportional to the partition coefficient which directly influences permeability (153) and the rate of transport across the skin (155). The partition coefficient plays a role because the skin normally does not provide sink conditions for release (144,149), but where sink conditions do exist, the rate-limiting step is the diffusion of the drug within the vehicle (144).

Equation 2 also takes the thickness of the stratum corneum (d) into consideration. Where Kp and k_1 have been determined experimentally, and d is known, D can be calculated (148,149,155). The effects of different thicknesses of stratum corneum are discussed in sections 1.3.2.2 and 1.3.2.3.

It should be pointed out that equations 1 and 2 were derived from studies of small non-electrolytes penetrating the skin at steady state flux; the lag time for corticosteroids is quite substantial (125). These equations are nevertheless still valid in predicting the behaviour of large corticosteroid molecules (125,131,152).

Another consideration in percutaneous absorption is that of thermodynamic activity. In theory, under strictly defined ideal conditions, the bioavailability of a drug from a topical formulation depends only on the thermodynamic activity of the medicament in the base (193,194). Thermodynamically, the activity of the drug in the vehicle is the product of the concentration and the activity coefficient of the drug in the vehicle (149,155,188). Because the rate of penetration is limited by the impermeability of the skin, the highest thermodynamic potential in the applied phase is necessary to obtain the maximum rate of penetration (149,155,188). Higuchi (152) has pointed out that the driving force behind the movement of the drug from the vehicle to the skin is the thermodynamic potential between the vehicle and the skin, and the direction of flow is from high thermodynamic potential to lower thermodynamic potential.

The thermodynamic activity of the penetrating agent in its vehicle can be expressed in terms of equation 4 (152):

$$\frac{dq}{dt} = (a.D.A)/(\gamma.d) \qquad (4)$$

where a = thermodynamic activity of the drug in its vehicle

γ = effective activity coefficient of the drug in the skin barrier phase

$\frac{dq}{dt} = J_s$ (see equations 1 and 3).

In order to obtain the maximum rate of penetration it is evident that the highest thermodynamic potential possible for the penetrating substance must be used (152,195). The thermodynamic activity is therefore

closely related to the relative solubilities of the drug in the vehicle and the stratum corneum (152,155), and is highest when the drug concentration equals the saturation solubility in the vehicle (192). Drugs with greater affinity for the vehicle than the skin will have lower activity coefficients and consequently lower thermodynamic activity, with decreased release of the drug from the vehicle (195). 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 (14,140,144,191,192,196, 197,section 1.6.3.5). It can be seen from figure 1.3 that it is the soluble fraction of the drug that crosses the barrier. 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 (109,144,198).

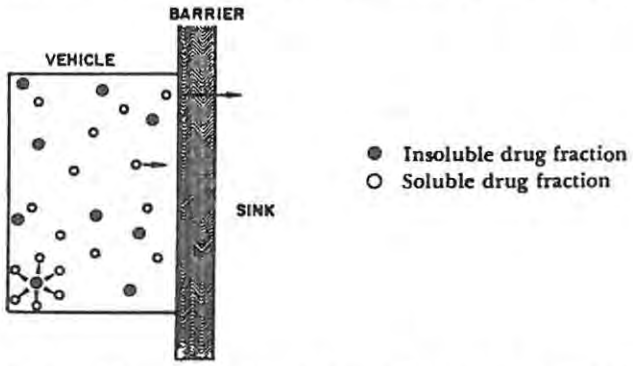

Figure 1.3 Diagrammatic sketch of drug environment

A further aspect of percutaneous penetration that has been investigated is the particle size of the corticosteroid in the vehicle. The blanching responses (180,199,200) and clinical efficacies (199,201) of dispersions of micronized corticosteroids have been found to be superior to a dispersion of the coarse particle, but inferior to the response of dissolved drug. *In vitro* release of micronized halometasone has also been found to be better when the drug is dissolved as opposed to a micronized suspension (146).

It should also be borne in mind that the possible increase in temperature caused by occlusion will increase the thermodynamic activity of the corticosteroid in the vehicle (161). This, however, does not appear to have been reported in detail as yet.

It is important to note that the above discussion assumes that the character of the vehicle remains the same after application to the membrane and that the membrane acts as a perfect sink. The stratum corneum however does not exhibit perfect sink conditions and the character of the vehicle may well change after application to the membrane (193). The equations discussed are nonetheless considered to be good guidelines to the biophysics of skin transport.

1.4 RESERVOIR

The existence of a reservoir was first suggested in 1955 by Malkinson and Ferguson (202) 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. It should be noted that in this experiment the corticosteroid was allowed to remain on the skin for the duration of the experiment and it is possible that the continued excretion may have been due to continued absorption from the surface depot (203). Feldmann and Maibach (168) also noted a levelling off of the excretion of hydrocortisone after topical administration, and in their experiment occlusion was left on for 4 days and urine was collected for 10 days, whilst Asche *et al.* (146) noted a continued increase of radiolabelled halometasone in guinea-pig urine for 120 hours after a 6-hour occluded application period.

An extensive study by Vickers (203) led to the conclusion that some corticosteroid is retained in the stratum corneum after topical application. The blanching noted after the topical application of corticosteroids was observable for up to 15 days after repeated occlusion. Similar initial blanching was noted after intradermal injection, but occlusion after 2 days did not induce a blanching response. It is noteworthy that the quantity of corticosteroid injected was the same as had previously been found to produce the reservoir effect when applied topically under occlusion. This implies that although more corticosteroid reached the site of action because of the absence of the stratum corneum barrier, the reservoir effect was not seen. Corticosteroids applied to the skin from which the stratum corneum had been stripped produced the expected blanching response after 1 hour, but not after 16 hours, and re-occlusion of the sites had no effect. This indicates that the cortico-

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 (82). In a further experiment by Vickers (203), the stratum corneum over one half of some of the blanching sites was removed by stripping, whilst the other sites were either completely stripped or left unstripped. Re-occlusion 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, however, observed at the completely stripped sites. In the third experiment of this series, sites were partially stripped, completely stripped or left intact after the establishment of a reservoir. Blanching was elicited after re-occlusion in the unstripped and partially stripped sites and thereby excluded the possibility that the blanching after re-occlusion 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.

It has been established that the amount of corticosteroid stored in the reservoir depends on the amount absorbed through the skin. In a series of *in vitro* experiments, between 82% and 99,9% of the corticosteroid applied was recovered from the horny layer after 5 hours (139). The blanching intensity in the initial stages of the trial has, in most cases, been found to correlate with the intensity of blanching observed after re-occlusion at a later stage. This has been found in the cases of increased corticosteroid-induced blanching due to occlusion (68,174,203), due to the vehicle (14,204-206) and due to the inherent potency of the corticosteroid or the ability of the preparation to elicit blanching (14,68,113,204,205,207,208). Similar results have been found in the case of studies utilizing radiolabelled corticosteroids in *in vitro* (125,209, 210) and *in vivo* (174,211) experiments in human and animal (212) studies. Stoughton (213) has also reported that hydration of the stratum corneum prior to application of corticosteroid enhanced the reservoir capacity of the skin when compared to normal intact skin.

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 (107,139). Another suggestion is that one of the main causes of the maintenance of the reservoir is the slow removal of the drug by capillaries, which is further affected by the vasoconstrictive properties of topical corticosteroids (214). The author interestingly goes on to say that drugs which cause vasodilation would exhibit no storage characteristics. Considering, however that *in vitro* studies have shown a reservoir effect (125, 209,210), it would seem reasonable to speculate that vasodilators may reduce the reservoir *in vivo*, but not that the reservoir may necessarily be absent.

The question of the clinical significance of the reservoir remains unanswered (174,210), but sufficient quantity of corticosteroid may be retained to influence epidermal cell division (210).

The time up to which blanching can be induced after re-occlusion of the sites of initial application of corticosteroid does not appear to have been reviewed in the literature. In the original experiment by Vickers (203) the duration of the reservoir effect varied from 3 to 15 days after re-occlusion for 16 hours "every second or third day".

Barry and Woodford (68,204) re-occluded the sites for twelve hours 8, 12 and 14 days after the commencement of their experiments. In general, re-occlusion elicited blanching for up to 12 days after the initial application, with the exception of Synalar ointment and Dermovate cream and ointment at which sites the reservoir effect disappeared after 14 days. (A list of active ingredients found in proprietary products discussed in this thesis is presented, with their tradenames, in Appendix A). In an experiment on 30 creams and gels the reservoir could be demonstrated by occlusion of originally unoccluded sites on the eighth but not the twelfth day after the commencement of the experiment (68), whereas in an experiment on 31 ointments occlusion of the originally unoccluded sites produced very similar results to those of the occluded sites, indicating that the capacity of the ointment to establish a reservoir was apparently not enhanced by the occlusive dressing (204). In an experiment in which betamethasone benzoate was used, blanching re-appeared for up to 8 days, but could not be demonstrated after a further 4 days even in cases where the sites were originally occluded (14).

Barry and Woodford (68,204) have reported that a 12 hour re-occlusion period facilitated estimation of pallor and that blanching was maximal 2 hours after removal of the occlusive dressing and usually disappeared after a further 10 hours. They have however reported several cases in which blanching was maximal 5 hours after removal of the occlusive dressing used for re-occlusion (101,113,194,205,207,208) and 3 hours after removal of the dressing in the assessment of the reservoir after multiple application of the corticosteroids (215).

In experiments performed by Woodford and Barry (14,101,113,205,208), they routinely re-occlude sites in selected volunteers for twelve hours 8 days and sometimes again 12 days after the commencement of the experiment, although they have varied this regimen (138,195,206).

Carr and Wieland (174), using radiolabelled triamcinolone acetonide in ethanol, occluded the application sites on one arm of their volunteers for 24 hours, after which the sites were alternatively re-occluded and left open for 12 hour cycles for a total of 7 days after the commencement of the experiment. Blanching was induced after each re-occlusion.

Preparations containing corticosteroids that elicit a weak blanching response (for example hydrocortisone) have been found not to demonstrate a reservoir effect on re-occlusion 8 days after the initial application (208,216), although radiolabelled hydrocortisone studies have indicated the presence of a reservoir (125,168,202,209-211).

One aspect of the reservoir that does not appear to have been extensively studied is the ability of the corticosteroid to induce blanching after frequent re-occlusion or re-occlusion of various durations. The fact that blanching is observed after re-occlusion implies that the corticosteroid is released from the reservoir in the stratum corneum to the site of action below the stratum corneum. The blanching of different corticosteroids after repeated re-occlusion of various durations may give an idea of the mechanism of binding within the reservoir and the dynamics of the depletion of the reservoir. From the clinical viewpoint, the hydration of the stratum corneum due to bathing may affect the release to the site of action for some time after the application of the corticosteroid-containing preparation.

Movement of the corticosteroid into newly formed stratum corneum also does not appear to have been studied. Considering that the stratum corneum is replenished about every 2 weeks (131), some corticosteroid may be continuously migrating into the newly formed stratum corneum or moving into the stratum corneum whilst bound to cells of the epidermis below the stratum corneum.

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 different regimens (217,218). The 1 to 4 times a day application has been accepted as the norm (5,26,30,219-224) with this also generally being the recommendation of the manufacturers (20,21).

Du Vivier and Stoughton (218) and du Vivier (217) 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.* (222) and Clement *et al.* (225) further found that neither a twice daily nor an alternate day regimen was able to maintain a continuous suppression of DNA synthesis, but the systemic effect was less marked with the alternate day regimen. Application after a resting period was found to again inhibit DNA synthesis. Clement *et al.* (225) have investigated a 72-hourly application for 9 days and whilst tachyphylaxis was found with Metosyn ointment and fluocinonide solution, none was demonstrated with Dermovate or Betnovate ointments. These workers have also reported that the sustained inhibition of DNA synthesis produced by Dermovate was lost if the corticosteroid was changed at the second or third application.

In studies of urinary excretion rates of hydrocortisone in monkeys, Wester *et al.* (219,226) found greater absorption after a single application of corticosteroid when compared to the same amount of corticosteroid applied in divided doses over a period of 12 hours (219). Enhanced penetration of hydrocortisone was also found after one week of daily applications of corticosteroid (226). It therefore appears as though frequent application leads to an increase in absorption but a partial decrease in physiological effects, although comparisons of this kind

should be made with caution.

Numerous workers have also demonstrated the development of tolerance to the blanching effect of topical corticosteroids. Several workers have reported a decreased blanching response after the multiple application of corticosteroid, followed by recovery after a rest period (215,218, 227,228) with subsequent tachyphylaxis after a further multiple application (215,227,228), although this has not been found in all studies (223). The blanching response during the period after the resting period has been found to be smaller than the original response in some cases (215,218,227), similar in some cases (218,227,228) and larger in some cases (223). It has further been found that tachyphylaxis was induced more rapidly by corticosteroid preparations that elicited a more intense initial blanching response (218,227), although this was not found to be true in all cases (215). 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 (68,215). Control experiments using the vehicle alone prior to application of the corticosteroids have shown that the vehicles did not cause blanching, nor were they responsible for the tachyphylaxis (218,223,227). 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 (228), while the reverse of this was noted in other cases (223,228). Jacobs *et al.* (229) have found that tachyphylaxis can be reduced by treating the skin with phosphatidylcholine prior to the application of the corticosteroid. They proposed that pretreatment with phosphatidylcholine increased the total lipid content of the stratum corneum, leading to a more controlled delivery to the lower skin layers and a reduced tachyphylactic response.

A clinical study (221) has shown that although once daily and 3 times daily regimens are both effective, the 3 times daily regimen is superior overall, whereas other studies have shown that once daily treatment appeared to be as effective as twice daily applications (230) and 3 times daily applications (220). The onset of action was however more rapid with the 3 times daily application. A study using the *Rhus* dermatitis assay (equivalent to intense vesicular dermatitis) has indicated that a 3 times daily application was superior to the once daily application (231).

Tachyphylaxis may also have occurred in a study of the depigmentation of guinea-pig skin. After initial depigmentation, melanogenesis was found to resume after 45 days of treatment of the skin with corticosteroid (232). The authors do however point out that the corticosteroids were applied in combination with DMSO, which is known to stimulate melanogenesis in human skin. They further state that this may be due to the concentration of the corticosteroid arriving at the basal layer becoming constant at that time. It would, however, be reasonable to expect the concentration to be constant before 45 days.

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. The fact that patients observe that they become resistant to a topically applied corticosteroid after constant use may imply the occurrence of tachyphylaxis in the clinical situation (22,25,227). The above experiments have made it possible to suggest improved dosage regimens for topical corticosteroid therapy, with obvious clinical advantages and the reduction of side effects which can arise with continuous therapy (215,221,228), in addition to reducing the cost of therapy and improving patient compliance (220,228). Suggestions have included daily applications for 5 day courses separated by 2 day resting periods as opposed to continuous therapy (215), and once daily applications as opposed to applications twice daily (228) or 3 times daily (220). The abovementioned clinical studies have indicated the need for individualised therapy. Once daily treatment should probably be the regimen of choice, at least initially, for young children, pregnant women and in cases where long-term therapy is likely (221). The frequency of application can then be increased if this regimen is unsatisfactory.

1.6 FORMULATION ASPECTS

The percutaneous absorption of a drug is frequently an extremely inefficient process (109) and the importance of the vehicle into which a topical corticosteroid is incorporated is becoming increasingly recognized. In a recent study of generic and trade name topical corticosteroid preparations, Stoughton (233) found some striking differences in the blanching activities of the preparations tested. The vehicle should, to the largest possible extent, facilitate the migration of the drug to the

target site (109,139,149) so as to allow the drug to exert its pharmacological effect (190). 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 in low concentrations is readily released (109,144,190,192,234). It is however possible that release of the drug from the vehicle and stability of the drug in the vehicle may be in conflict with each other. A case has been reported where the stability of hydrocortisone is unsatisfactory in the vehicle which allows the most efficient release of the drug (235). Maximization of the release and penetrability of the drug, without unfavourably altering other relevant vehicle properties, should therefore be a general goal in topical vehicle design (109,144,149). The role of the vehicle in the therapeutic efficacy of a topical corticosteroid is illustrated in the diagram by Katz and Poulsen (109) reproduced in figure 1.4.

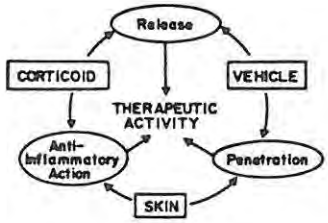

Figure 1.4 Interactions of release, penetration and anti-inflammatory activity

It can be seen from the diagram that therapeutic activity is dependent on release, penetration and anti-inflammatory action which in turn are dependent on the corticosteroid, the vehicle and the skin. The arrival of the drug at the site of action requires the release of the drug from the vehicle and the subsequent penetration through the skin barrier. In the event of this barrier being damaged, which is often the case in diseased skin, the rate-limiting step is the release of the drug from the vehicle (191).

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 penetra-

tion enhancers. The barrier properties of the stratum corneum have been discussed in section 1.3, and it should be remembered when considering formulation aspects that whilst the vehicle can materially affect the pharmacokinetic behaviour of a drug with some potential for skin penetration, no vehicle can force a drug, which for molecular reasons cannot penetrate the membrane, through the skin (190). Movement of the drug from the dosage form to the site of action is therefore dependent upon the physical properties of the drug, the vehicle and the barrier (142).

1.6.1 Vehicles

Reports on the effect of the base on percutaneous absorption and therapeutic activity are plentiful, but often contradictory (129,149). 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 elucidated. The design of a vehicle for topically administered drugs should take into consideration the formulation aspects discussed below, the physical characteristics of the drug (section 1.2) and the skin barrier (section 1.3.2), as well as the biophysics of skin transport (section 1.3.5). It is also important to bear in mind that a vehicle that provides good release of one drug may not be suitable for another drug (109).

1.6.1.1 Ointments

The effect of hydration of the stratum corneum on the percutaneous absorption of corticosteroids after topical application is described in section 1.3.2.2. It has been found that the vehicle has a substantial influence on the hydration of the skin barrier, with greases and oils being the most occlusive types of vehicle and consequently inducing the greatest degree of hydration (130). The inclusion of a surfactant into these vehicles results in water-in-oil emulsions which induce less hydration than the greases and oils. Oil-in-water emulsions, formed by an increase in the water/oil ratio, induce the lowest degree of 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 (130). 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 experiment in which hydrocortisone was incorporated into four different ointment bases indicated that the different therapeutic effects found with the different bases 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 vehicle (166). In an experiment using fluclorolone acetonide in petrolatum ointment, hydrophilic petrolatum and propylene glycol ointment, no difference was found in the blanching activity of the corticosteroid in the first two vehicles, whilst a significantly superior response was obtained from the propylene glycol ointment (180). A blanching trial on two fatty ointments and two ointments containing the same concentration of diflucortolone valerate (Nerisone and Temetex) indicated equivalent release of the corticosteroid from all four preparations (236).

A series of experiments by Ostrenga *et al.* (144) illustrates the importance of not drawing hard and fast conclusions regarding the release of a corticosteroid from ointment bases after an *in vitro* assay. Release of fluocinonide into isopropyl myristate from five different ointment bases 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 was associated with the small diffusion coefficients of the corticosteroid in the external and internal phases of the emulsions, whereas in the latter the vehicle itself may have been absorbed, thereby carrying the corticosteroid into the skin, or that the phases separated after application to the skin. Similar results were found in an *in vivo* blanching trial using betamethasone benzoate in seven different ointment bases (237). The inclusion of propylene glycol or isopropyl myristate into the formulations was found to increase the blanching response elicited by 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 same corticosteroid in Macrogol ointment B.P.C. base containing 5% propylene glycol 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 statistically significantly inferior response to the white soft paraffin/propylene glycol base. This further illustrates 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.

1.6.1.2 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 1.3.5 and 1.6.3.5).

Creams hydrate the stratum corneum to a lesser degree than ointments (130). They wet the skin initially, but the degree of hydration decreases as the aqueous phase evaporates. 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, provided relief significantly sooner than the other (238). This difference in onset of action is presumed to be due to enhanced penetration from one of the bases.

A factor that needs to be considered in emulsions is that of phase inversion, which could occur as the aqueous phase of the emulsion evaporates. The penetration of the drug into the stratum corneum occurs mainly from the continuous phase (177). Although this will generally not influence the absorption of drugs with dual solubility, the preferential solubility of the molecule should be considered in cases of poorly soluble substances like corticosteroids (130).

1.6.1.3 Gels

Gels are aqueous vehicles that contain only a small proportion of material not soluble in water, and often show physical characteristics similar to the oil-in-water emulsions (130). These vehicles maintain the

lowest level of hydration of the stratum corneum after equilibrium has been reached. Gels are single phase systems and therefore do not give rise to the problem of the corticosteroid partitioning between the phases of the two phase emulsion systems (14). The reservoir effect has also been found to be greater with gels than with creams (14). As is the case with ointment and cream formulations, the proportion of propylene glycol in a gel has an effect on the release of the corticosteroid from the vehicle (239).

Higher corticosteroid concentrations have been found in the dermis and epidermis from a gel containing liposomes than from the control vehicle, but the corticosteroid concentration in the blood was lower from the former (240,241), the implication of this being a greater reservoir effect from the liposomal preparation, lower systemic absorption and consequently a smaller chance of systemic side effects (241). Conversely, in a blanching study in which the responses of three different hydrocortisone esters were compared in a liposome formulation and cetomacrogol cream, the release of the corticosteroids was better from the cream vehicle (242).

1.6.1.4 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. Two proprietary lotions containing equal concentrations of betamethasone 17-valerate have been found to elicit similar blanching responses in both modes of application (243,244). This similarity in bioavailability could be expected due to the good surface coverage, skin contact and spreadability of lotions. 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 (5,55,74,107).

1.6.1.5 Aerosol Quick-Break Foams

This form of vehicle has not been extensively reported, but it does appear to be promising. The quick-break aerosol foam has the advantages of

high activity (the corticosteroid is in solution), ease of application, controlled dosage from a metering valve, economy in use, suitability for smooth or hairy skin and reduced possibility of inhaling the corticosteroid compared with aerosol sprays (205). The blanching elicited by betamethasone benzoate was found to be better from this formulation than from the ointment, cream and gel formulations tested.

1.6.2 Comparison of Vehicles

Blanching assays using equal concentrations of the same corticosteroid have shown statistically significant differences in the corticosteroid-induced blanching response from different vehicles, although there are exceptions to this (245,246). 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 (14,205,236,247,248). The superior blanching obtained from ointment bases is almost certainly due to an increase in the hydration of the stratum corneum. White soft paraffin has been found to depress transepidermal water loss for up to 16 hours when left on the skin (249), the high occlusivity of white soft paraffin (249) being due to its inability to absorb water (188). The alteration of a component within a formulation has also been found to significantly alter the corticosteroid-induced blanching response. Fluocinolone acetonide in white soft paraffin was found to elicit an inferior response to the corticosteroid in aqueous cream (245), whereas the inclusion of 5% propylene glycol into the white soft paraffin led to a superior blanching response (199). The vehicle has in some cases been found to increase the area of pallor, even when the degree of blanching remained the same (245) or was less intense (250).

When assessing the bioavailability of the same corticosteroid from different types of vehicles, it should be borne in mind that the release of the drug may be influenced by a small change in the formulation. For example, a small change in a cream base may improve the release of the drug to an extent that blanching is equivalent to an ointment (possibly poorly formulated) containing the same concentration of the same corticosteroid. Current literature does, however, indicate that the ranking of formulation type presented above is an accurate guideline. A further

point that is worth noting, from the point of view of the blanching assay, is that occlusion has been found, in some cases, to mask the effect of the vehicle (section 2.2.2). The duration of occlusion has also been found to mask vehicle effects (248). This is important when using blanching assays to predict the clinical situation, where topical corticosteroids are normally applied without occlusion. From the clinical point of view, less cream, lotion or gel is required, because these less viscous preparations spread more easily than ointments (25).

1.6.3 Penetration Enhancers

The importance of the formulation of vehicles for the optimum delivery of topically applied corticosteroids has been described in the preceding sections. The well documented inefficiency of percutaneous penetration has led to the search for non-irritating substances of low toxicity to enhance penetration of corticosteroids and other drugs administered transdermally (109,251,252). Since the study of penetration enhancers appears to be receiving increasingly more attention in the scientific literature, and their mechanism of action allows some insight into the mechanism of percutaneous penetration, some of the more common and/or promising agents are discussed below.

1.6.3.1 Dimethylsulphoxide (DMSO)

DMSO has been known for some time to be a good solvent and to enhance the percutaneous absorption of corticosteroids in man (253).

A blanching study using various concentrations of both fluocinolone acetonide and DMSO showed that DMSO in concentrations of 10% and 25% decreased the minimum concentration of corticosteroid required to cause blanching in the unoccluded mode with no significant changes observed in the occluded mode (253). The effect of the concentration of DMSO was further elucidated in a study using varying concentrations of DMSO and triamcinolone acetonide in alcoholic solution (254). A greater concentration of corticosteroid was required to elicit similar blanching responses when less DMSO was incorporated into the vehicle. The amount of radiolabelled hydrocortisone measured in urine over a 5 day period was found to be greater when applied in a vehicle containing DMSO (255,256),

with the greatest increase having been observed in the first 2 days (256). *In vivo* and *in vitro* experiments using excised human skin also showed increased penetration of corticosteroids (183,209,253). It is interesting to note (253) that if the solvent and corticosteroid were left in contact with the skin for 24 hours, greater penetration was achieved with water than with DMSO as opposed to greater penetration from DMSO with transient exposure (25 minutes). The authors suggest that this may be due to the facilitation of rapid initial penetration of the corticosteroid from DMSO through the stratum corneum. The fact that skin from different parts of the body was used for the different studies may have had an effect on the results. The rapid penetration of corticosteroids from a solution containing DMSO was further shown by the blanching induced by the corticosteroid after having been left on the skin for only 1 to 5 minutes (211,254). The presence of radiolabelled 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 (211). No radiolabelled corticosteroid was however observed on day 2 in the case of hydrocortisone and day 4 in the case of fluocinolone acetonide when applied in 95% ethanol. 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 treatment of psoriasis, where triamcinolone acetonide in 90% DMSO was found to be superior to this corticosteroid applied in a cream base (256).

Problems with the use of DMSO as a penetration enhancer are its odour, possible toxicity, irritant effect on the skin and high concentrations required to enhance penetration (129,156,175,192,209,245,254,255,257), although some workers have reported no irritation (255) or toxicity (254).

A suggested mechanism for the increased percutaneous penetration of corticosteroids 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 (131,254). The hydrogen bonding between water and DMSO has been found to be greater than between water molecules (258). *In vitro* experiments using human abdominal skin specimens have further shown that DMSO itself penetrates

the skin (183). DMSO has been found not to destroy the dense intercellular cement substances which bind the horny cells and therefore not to influence the integrity of the membrane even after lengthy exposure (254), although this has been disputed (179). It is also worth noting that pretreatment of the skin with DMSO did not significantly enhance the penetration of testosterone (254). DMSO seems to exert its effect on skin permeability by producing structural changes, such as swelling of the stratum corneum (109,192) because of its hygroscopicity (149,254) and by possible replacement of water as the continuous membrane phase of the skin barrier (109), although another report claims that it does not alter the structure of the skin (149).

1.6.3.2 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. ointment and B.P.C. cream bases produced no visible blanching (245). The observation by Feldmann and Maibach (255) of urinary excretion of hydrocortisone over a 5 day period showed that the extent of percutaneous absorption of the corticosteroid from DMA was less than that from 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) (209). An increase in the blanching elicited by hydrocortisone, fluocinolone acetonide and triamcinolone acetonide was also found after examination of four semi-solid dosage forms (259). The absorption of all three corticosteroids was greatest from a base containing DMA. 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.

1.6.3.3 Urea (Carbamide)

In vitro studies of the absorption of radiolabelled hydrocortisone through 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 (260). Contrary to this, the addition of 10% urea has resulted in an increase in the amounts of hydrocortisone and triamcinolone acetonide in the epidermis and dermis of excised human skin (141). Urinary excretion of radiolabelled hydrocortisone acetate was found to be approximately double 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 (261). An urea-induced increase in corticosteroid penetration has further been reported in a study using excised hairless mouse skin (106).

Clinical trials comparing the topical application of corticosteroid preparations with and without urea have shown that better results are obtained when urea is included into the preparation (251,262). It has further been reported from clinical trials that a hydrocortisone/urea combination in powder-cream base (Alphaderm) has performed as well as or better than preparations containing more potent corticosteroids (266-268). 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 disorders (251,263-265).

Blanching assays using a triamcinolone acetonide cream containing urea indicated a significant increase in bioavailability of the corticosteroid, with the placebo base (containing urea but no corticosteroid) showing minimal blanching (269). However, a similar study using betamethasone 17-valerate in a cream containing urea showed a significant decrease in the blanching activity of the corticosteroid in the urea-containing creams when compared with the steroid-containing cream with no urea (270). In another study, lower intensity blanching was elicited by betamethasone 17-valerate in a buffered cream formulation containing urea when compared in the unoccluded mode with the same cream containing no urea, whilst comparative blanching between these preparations was similar in the occluded application mode (271). Barry and Woodford (272) found no increase in the blanching activity of hydrocortisone in an 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 (215). This apparent disparity in the above results is most likely related to the period of

skin/vehicle contact (179,215). The mechanism by which urea increases absorption of some corticosteroids is unknown, although it may be a combination of its keratolytic activity and hydration of the stratum corneum due to its ability to increase the water binding capacity or reduce the transepidermal water loss of the stratum corneum (179,251,264). The keratolytic and hydration effects of urea appear to require a contact time of more than 6 hours, which may explain the lack of increased blanching in two of these experiments (270,272) and the observed increase in the 16 hour (269) and 5 day (215) applications. This, however, does not explain the decrease in blanching in the case of betamethasone 17-valerate, which was postulated to be due to the urea either retarding the release of the corticosteroid from the base employed or enhancing the removal of the drug from the biophase (270).

The problem of stabilising an urea solution at a near-neutral pH, thus making it compatible with hydrocortisone stability, has been overcome by adsorbing the urea in high concentrations onto a polysaccharide powder matrix under controlled conditions (267,268). This system is used in Alphaderm which has been found to be clinically efficacious (266-268) and to elicit superior blanching to some other urea-containing corticosteroid preparations (215,272).

1.6.3.4 Salicylic Acid and Resorcinol

An increase in the penetration of triamcinolone acetonide through excised human skin has been noted after the addition of salicylic acid to an ethanolic solution of the corticosteroid (273), with peak penetration having been noted at the same time with and without salicylic acid. Salicylic acid is known to be a keratolytic agent (274-276), and reduces the intercellular cohesiveness of the horny cells (274). Blanching studies using triamcinolone acetonide and salicylic acid however showed a decrease in the blanching activity of the corticosteroid in conjunction with salicylic acid (269), whereas salicylic acid with flumethasone pivalate showed an increase in blanching (276).

Using skin thickness in guinea-pigs as an indicator of anti-inflammatory action, Sarkany and Gaylarde (175) found that the anti-inflammatory action of hydrocortisone alcohol was completely suppressed by the addition

of salicylic acid and acetylsalicylic acid, although in the same paper the authors note the topical anti-inflammatory action of both of these salicylates. Similar results have been observed in urinary excretion studies of radiolabelled hydrocortisone in monkeys where a decrease in percutaneous penetration was reported after the addition of salicylic acid (275).

Resorcinol is another substance that has keratolytic and exfoliative properties (265) and has been found to enhance percutaneous absorption of betamethasone 17-valerate. Studies in our laboratories have shown that the inclusion of resorcinol into an extemporaneous preparation led to an increased blanching response in the occluded and unoccluded application modes (277). It was further found that the addition of 0,5% and 2,5% resorcinol elicited a similar degree of penetration enhancement (278). Another study performed in our laboratories has indicated that the release of betamethasone 17-valerate from three buffered cream formulations (containing 0%, 2% or 5% resorcinol) was essentially the same, although a rank order difference suggested superiority in drug release from the 2% resorcinol formulation over the 0% or 5% resorcinol formulations (271).

1.6.3.5 Propylene Glycol

In a report by Baker (249), the addition of propylene glycol to soft white paraffin was found to decrease the occlusivity of the vehicle, and propylene glycol in water produced a minor but constant increase in water loss through the skin. Experiments on the combined effects of propylene glycol and relative humidity appear to be contradictory. *In vitro* penetration of hydrocortisone butyrate from a cream containing 40% propylene glycol was found to increase under dry atmospheric conditions when compared to moist conditions (273). 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 differing relative humidity (279). It is interesting to note that 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 (280). Considering the complexity of the interaction between the vehicle, skin and corticosteroid, the conclu-

sions that these differences were due to alteration in stratum corneum hydration should be drawn with caution.

Blanching assays using fluocinolone acetonide (199) and betamethasone benzoate (237) showed enhanced blanching with the addition of propylene glycol to white soft paraffin base, the corticosteroid being dissolved in the propylene glycol. Increased blanching has also been seen in the case of fluclorolone acetonide in FAPG (fatty alcohol/propylene glycol) base (234) and halometasone with the addition of propylene glycol to a base consisting mainly of white soft paraffin (146). In the latter experiment (146) it was found that increasing the propylene glycol content from 1% to 5% led to an increase in blanching with a 5 hour, but not a 16 hour contact time, apparently indicating more rapid penetration with higher solvent concentration. It appears as though it may be worthwhile in future to differentiate between accelerants and penetration enhancers (or sorption promotors), whilst bearing in mind that one of the desirable properties of these agents is immediate penetration enhancement (179). Experiments should possibly be designed to allow a distinction to be made between enhanced and more rapid penetration (accelerants), and enhanced penetration with little alteration in the penetration rate (penetration enhancers). The effect of contact time in the case of urea (section 1.6.3.3) would indicate that urea is a penetration enhancer, but not an accelerant.

Percutaneous penetration of radiolabelled hydrocortisone when measured by urinary excretion in man was found to decrease when applied in propylene glycol in acetone as compared to the control in pure acetone (255). Percutaneous penetration in guinea-pigs, however, remained the same when halometasone was applied under occlusion with or without propylene glycol (146). The release of fluocinolone acetonide, fluocinonide 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 (142,185,281,282), findings which correlate well with blanching studies (142,185,282). 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 cortico-

steroid between this mixture and isopropyl myristate (185,283).

An excess and an insufficiency of propylene glycol both lead to a decrease in the amount of corticosteroid released into the receptor phase, the excess causing increased affinity of the corticosteroid for the vehicle, whilst the insufficiency leads to dissolution of the corticosteroid becoming the rate-limiting step (185,281,283). 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 (142,281,282).

An *in vitro* study of hydrocortisone butyrate indicated an enhancement of percutaneous penetration of the corticosteroid from a formulated product containing propylene glycol (273). Further experiments (279) have shown that the best penetration of 0,1% hydrocortisone butyrate is obtained from a Plastibase/propylene glycol vehicle followed by an ethanolic solution and an ethanol/propylene glycol gel, whilst the least penetration was from an oil-in-water cream containing propylene glycol. In the case of 0,2% hydrocortisone butyrate, better penetration was obtained from the cream than from the ethanol-containing preparations. Increasing the concentration of the corticosteroid from 0,1% to 0,2% in a cream containing propylene glycol led to an increase in penetration which was not seen in the cream without propylene glycol. The aqueous phase of the cream was saturated at a concentration of 0,07% corticosteroid. A similar, but less pronounced increase was obtained in Plastibase containing propylene glycol, but not in a gel containing propylene glycol. Increasing the concentration of the propylene glycol in Plastibase from 6% to 18% did not diminish the penetration, 9% having been required to dissolve the corticosteroid (273). 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.* (239) where an *in vivo* and two *in vitro* methods were used in the assessment.

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 (284).

It should be noted that in addition to its solubilising properties, propylene glycol is a keratolytic agent (285). It softens keratin, loosens cornified epithelium and desquamates the epidermis.

1.6.3.6 Miscellaneous

Some of the other penetration enhancers that have been studied in corticosteroid-containing formulations are discussed below or presented in table 1.1.

Tetrahydrofurfuryl alcohol (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% corticosteroid, whereas in the DMA formulations used, 0,1% of hydrocortisone was required to produce blanching (245). Enhancement of blanching activity has also been observed with triamcinolone acetonide and fluocinolone acetonide in the presence of THFA (259), but in this case the corticosteroid induced more blanching in the vehicle containing DMA than the base containing THFA. It should again be noted that other constituents of the vehicles were different. THFA, however, has the disadvantage of causing skin irritation and is used as an irritant in bioassays to assess topical corticosteroid activity (286-288). A further consideration is that THFA and DMA increase water loss from the stratum corneum *in vivo* (249).

In a study using radiolabelled testosterone and hydrocortisone, the urinary excretion over a 5 day period was found to double when the steroid was applied in dimethylformamide (DMF) (255), while an *in vitro* study of radiolabelled hydrocortisone showed an approximately 6-fold increase in penetration from DMF compared to 95% ethanol (209). An assay comparing the blanching produced by betamethasone 17-benzoate when dissolved in DMF and dimethylisosorbide indicated no significant differences in the blanching response (206), while aqueous DMF without dimethylisosorbide was found to enhance blanching activity (195).

TABLE 1.1 SOME MISCELLANEOUS SUBSTANCES STUDIED IN RELATION TO ENHANCEMENT OF PERCUTANEOUS PENETRATION OF CORTICOSTEROIDS

Enhancer	Corticosteroid	Reference
diethylbenzamide	experimental	106
N,*N*-diethyl-*m*-toluamide	hydrocortisone	252
	hydrocortisone acetate	252
	hydrocortisone 17-butyrate	252
	hydrocortisone 17-valerate	252
	betamethasone 17-benzoate	206,494
	experimental	106
isopropyl myristate	betamethasone 17-benzoate	237
	experimental	106
lactic acid	hydrocortisone	260
liposomes	hydrocortisone	241,495
	hydrocortisone 21-acetate	242
	hydrocortisone 21-hexanoate	242
	hydrocortisone 17-butyrate	242
	triamcinolone 16,17-acetonide	240
oleic acid	betamethasone 17-benzoate	195,496
2-pyrrolidone	betamethasone 17-benzoate	195,206,494,496
	hydrocortisone 17-butyrate	405
1-ethyl-2-pyrrolidone	betamethasone 17-benzoate	206,494
N-methyl-2-pyrrolidone	betamethasone 17-benzoate	195,206,494,496
	hydrocortisone 17-butyrate	405
N-vinyl-2-pyrrolidone	hydrocortisone 17-butyrate	405
polysorbates	hydrocortisone	497
propyl myristate	hydrocortisone	498
propyl oleate	hydrocortisone	498

Azone (1-dodecylazacycloheptan-2-one) is a relatively new chemical agent that has been found to enhance the percutaneous absorption of topically applied corticosteroids (289). *In vitro* penetration of radiolabelled triamcinolone acetonide was enhanced by the addition of azone to the vehicle. Blanching elicited by triamcinolone acetonide, desonide and amcinonide was enhanced with the addition of azone, the increase in blanching activity apparently not having been influenced by the concentration of azone (2% - 100%). Azone functions as a penetration enhancer by partitioning into the lipid regions of the stratum corneum and thereby disrupting the structure (290).

α-, β- and γ-Cyclodextrins have received considerable attention in pharmaceutical research (291) as sorption promotors. It has been found that cyclodextrins increase the solubilities of steroids, and that this is dependent on cyclodextrin concentration and on the size of the cyclo-

dextrin cavity (291), where $\gamma > \beta > \alpha$ (291,292), suggesting that the larger the cavity, the more favourable is the fit of the steroid molecule (291). The *in vitro* release of betamethasone 17-valerate from gels and ointments was significantly improved by cyclodextrin (β and γ) complexation, due to increases in the apparent rates of dissolution and membrane permeation of the drug (293). Blanching studies have also shown enhanced percutaneous penetration of beclomethasone dipropionate in an ointment vehicle containing γ-cyclodextrin (493). Experiments performed in our laboratories have indicated that the inclusion of β-cyclodextrin in an extemporaneously prepared formulation has little effect on the blanching elicited by betamethasone 17-valerate (294). It is felt that optimal thermodynamic potential was obtained in the control vehicle and that the inclusion of a penetration enhancer had little effect.

1.6.4 Dilution

The importance of careful, scientific considerations during the design of vehicles for topical corticosteroids are illustrated in the preceding sections. The undesirable practice of dilution is, however, still requested by prescribers (12,295-299) even though the very large range of proprietary topical corticosteroid preparations available today (20,32, 299) makes dilution unnecessary (7,22,299,300). The dangers of dilution have been reviewed by Busse (301) under the headings of pharmaceutical considerations (such as the physical compatibility of the steroid and the vehicle with the diluent), bacteriological considerations and biopharmaceutical considerations. Dilution may also lure the prescriber and patient into a false sense of safety if they are under the impression that the potency of the diluted preparation is reduced by the same magnitude as the dilution factor (10,87,299,301), a fact that has been disproved by several workers (16,101,295-297,302-305). The 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 preparation (295,304). Similar findings have been reported for numerous corticosteroid preparations where dilutions ranging from 4-fold to 32-fold have shown similar blanching responses (16,101,303), as well as for marketed dilutions of Kenalog creams ranging from 0,025% to 0,5% (233).

Microbial contamination is another potential problem associated with extemporaneous dilution. The possibility of contamination, especially by *Pseudomonas aeruginosa* in hospital dispensaries, is real (306,307) and the efficacy of preservatives may be inhibited by incompatibilities with the diluent or by dilution to below the minimum inhibitory concentration (301). *Pseudomonas* has also been found to utilize glucocorticoid esters as a metabolic substrate (308). 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 (309). Other studies, in which Propaderm and Betnovate creams and ointments were diluted with cetomacrogol cream and white soft paraffin respectively, indicated that the viable microbial counts were well within pharmacopoeial limits for the 12 month duration of the experiment (310).

The blanching activities of corticosteroid-containing creams diluted with either Unguentum Merck or cetomacrogol cream have been found to be equipotent, thus suggesting that Unguentum Merck is a suitable alternative to cetomacrogol cream as a diluent for the tested corticosteroid creams (101,309). High performance liquid chromatographic investigations of several proprietary topical corticosteroid creams have further indicated that the corticosteroid is chemically stable for 32 weeks after dilution with Unguentum Merck (309). Unguentum Merck has also been found by Ryatt *et al.* (303) not to cause degradation of betamethasone 17-valerate for up to 5 months, and Cornarakis-Lentzos and Cowin (310) found that Propaderm and Betnovate creams and ointments were stable for 12 months when diluted with cetomacrogol cream and white soft paraffin, respectively.

Reports on the stability of corticosteroids after dilution of proprietary preparations are, however, contradictory. Magnus *et al.* (302) found that the blanching activity and concentration of betamethasone 17-valerate was constant for up to 14 months after dilution of Betnovate cream with various diluents. The only exception was with the use of E45 cream in which degradation was noted after 14 months. Ray-Johnson (10) found that the concentrations of various corticosteroids remained 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 con-

version to the 21 isomer) 2 weeks after dilution of Betnovate cream with emulsifying ointment. Several workers (16,311,312) have however reported remarkably rapid degradation of betamethasone 17-valerate after dilution of Betnovate ointment with emulsifying ointment and other diluents. When an ointment containing betamethasone 17-valerate 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 (311) and approximately 4 hours for a 25% dilution (16,312). It has further been found that the rate of corticosteroid decomposition increases with an increase in the dilution factor (305,311,312).

Decomposition of the corticosteroid in diluted preparations at different storage temperatures has also been studied. Decomposition of a variety of corticosteroids was not influenced by temperatures of up to 32°C when diluted with Unguentum Merck (10), but decomposition rates increased considerably between 20°C and 30°C when betamethasone 17-valerate was diluted with emulsifying ointment (311) and between room temperature and 40°C when diluted with other diluents (305). A storage temperature of 32°C has been described as "unrealistically high", and whilst this may be true in certain parts of the world it is by no means an accurate generalization.

A factor that should be considered in these experiments is the method of dilution which is usually cold mixing on a tile, thereby simulating the practice in hospital and community pharmacies (301). The possibility, however, does exist that the particular aliquot of the preparation assayed (*in vivo* or instrumentally) may not be representative of the entire batch. The patient may therefore also be administering different amounts of the corticosteroid during the treatment period.

The clinical significance of using products with smaller amounts of active ingredient has been debated in the literature. Ryatt *et al.* (16, 303) have said that diluted products could be used more liberally, with greater efficacy and with fewer side effects. Kirsch *et al.* (313) and Gibson *et al.* (304) have, however, countered this. While agreeing that this may be true for systemic side effects, they caution that this should not be assumed to be true for local side effects until this has been proven. Wohlrab (141) has further stated that a reduction in side

effects cannot be expected if the same therapeutic effect is achieved with a smaller amount of drug.

It would seem reasonable to assume that similar blanching responses induced by the same corticosteroid applied to the skin in different concentrations would imply that approximately the same number of molecules are reaching the relevant corticosteroid receptors. If it is assumed that clinical efficacy can be predicted from blanching data, and that the severity of side effects coincides with clinical efficacy, it would probably follow that similar blanching intensities induced by different quantities of the same corticosteroid could cause similar side effects.

This assumption is possibly only accurate if it is further assumed that, although the mechanisms of clinical activity, side effects and blanching may be different, they are interrelated and all depend on the effect of the corticosteroid at the receptor site.

1.7 MECHANISM OF BLANCHING

The mechanism of steroid-induced blanching has not yet been fully elucidated (47), although reports in the available literature seem to favour vasoconstrictor activity of the corticosteroid. This is also evident from the large number of workers in the field who refer to the assay as the vasoconstrictor assay, and from the speculation that vasoconstriction is partly responsible for the therapeutic effects of topical corticosteroids in the treatment of eczema and psoriasis vulgaris (314), although it has been argued that the vasoconstriction has little to do with the treatment of disease (172).

Several corticosteroids tested have been found to increase the sensitivity of ocular blood vessels to topical norepinephrine (315). In an experiment using fluocinolone acetonide, Juhlin (316) 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*. Other workers (38,317) have, on the other hand, found that certain corticosteroids significantly increased the vasoconstrictor response of epinephrine, norepinephrine and serotonin and have suggested that the measurement of clearance of radioactive $Na^{131}I$ could

be used as an objective assessment of topical corticosteroid activity (317). It should however be noted that not all the corticosteroids in the study of Mahajani *et al.* (317) 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 (318). It has been suggested that this may be due to an indirect action of corticosteroids on the sympathetic nerve supply by the potentiation of norepinephrine (314). Solomon *et al.* (318) did, however, point out that this is limited to normotensive patients with cutaneous disease. In view of the more recent work on tachyphylaxis (section 1.5) it should be borne in mind that the experiments by Solomon *et al.* (318) were performed at 2 and 3 week intervals. The demonstration of tachyphylaxis may however support the involvement of norepinephrine in the blanching response and tachyphylaxis may be a result of norepinephrine store depletion in the adrenergic nerve endings (218,227). A potential problem with the systemic administration of antagonists is that the control experiment can naturally not be performed simultaneously. This is, however, unlikely to be a problem if sufficient volunteers are used in the study and the study is carefully designed. Although corticosteroid-induced vasoconstriction has been observed in canine skin, it appeared as though this was not due to endogenous norepinephrine, but the authors did not offer an alternative explanation (319).

Cyclic adenosine monophosphate (cAMP) or guanosine monophosphate have also been implicated in the mechanism of blanching, with the involvement of the latter being more likely (227), as Halprin *et al.* (320) have found no corticosteroid-induced influence on cAMP. It has also been postulated that corticosteroids reduce endogenous phospholipase activity, thereby possibly sensitizing the blood vessels to norepinephrine (47).

Clearance of ^{133}Xe has indicated a decrease in peripheral blood flow after topical treatment with corticosteroids (321), and plethysmographic recordings of skin pulses have indicated that corticosteroid-induced blanching in normal skin is possibly due to decongestion of the capillaries (322-324), an idea previously suggested by Baker and Sattar (325).

In his studies on the topical application of corticosteroids to human lips, Stüttgen (326) observed narrowing and shortening of the columns of erythrocytes which represent the capillaries, and blanching of the underlying tissue with narrowing of the venules. The prior application of phentolamine (an α-receptor inhibitor) did not inhibit the constrictive effects of the corticosteroids. Stüttgen further observed that corticosteroids initially induce a narrowing of the capillaries which is then followed by constriction of the deeper venous vessels. In a discussion on this work, Holti suggested that blanching may be a vasocompressor phenomenon brought about by the swelling of collagen or effects upon mucopolysaccharides. Whilst Stüttgen agreed that vasocompression may be a component of blanching, he felt that this effect was not strong enough to cause blanching.

The influence of prostaglandins (which are implicated in the anti-inflammatory activity of topical corticosteroids (section 1.1.1)) on blanching has been suggested (42). It is possible that corticosteroids prevent the release of prostaglandins from fat cells thereby preventing their vasodilatory action on the blood vessels (42) and it is known that corticosteroids inhibit the biosynthesis of prostaglandins (section 1.1.1). While this may explain the corticosteroid-induced inhibition of vasodilation, it does not appear at this stage to elucidate the mechanism of blanching in normal skin.

Studies of the effects of various glucocorticoid antagonists on the corticosteroid-induced blanching response have shown that blanching was reduced by these agents (327,328), although not by steroids that are inactive in glucocorticoid systems (327). Epinephrine-induced vasoconstriction was further found not to be influenced by glucocorticoid antagonists (327). The results of these studies have led to the conclusion that the vasoconstrictor effects of topical corticosteroids are mediated by occupancy of classical glucocorticoid receptors, rather than by non-specific pharmacological mechanisms.

It has also been suggested that corticosteroids may exert a direct action on the smooth muscle of the wall of the blood vessel (314,315). It has further been suggested that this is a local phenomenon and not secondary to systemic changes, namely the carbohydrate regulation and salt

retention seen with adrenocorticoids (315,329). Altura (38) has, however, found that corticosteroids had no visible effect on the diameters of muscular microvessels.

Aiache *et al.* (330) have proposed that blanching is a function of corticosteroid-induced melanosome degradation. *In vitro* studies on skin with a high melanin content have demonstrated the disruption of the dense matrix of melanosomes after 48 hours of treatment with 1 mg/ml of hydrocortisone, and the absence of melanosomes after 24 hour treatment with 2 mg/ml. Whilst melanolysis may be in part responsible for the blanching phenomenon, more studies, possibly utilising more potent corticosteroids, are required to ascertain the extent to which melanolysis is involved in the blanching response.

1.8 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. Certain aspects of the blanching assay were investigated during this project and are reported in Chapter 3. For this reason the blanching assay and the correlation of results of the blanching assay and clinical trials are discussed fully in Chapter 2. The assays discussed in this section tend to be more tedious and more painful than the blanching assay, or require the sacrificing of animals, and although some of them are more closely related to the clinical uses of topical corticosteroids than the blanching assay they have, in the main, not gained popularity over the years.

1.8.1 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 (179).

1.8.1.1 Chemical Irritants

The anti-inflammatory activity of a corticosteroid can be assessed by

measuring the extent to which it inhibits inflammation elicited by a chemical irritant. Croton oil, for example, is applied to the skin and the corticosteroid is injected intradermally, administered orally or applied topically before, at the same time as, or after the application of the irritant (13,172,184,331-336). The experiment can be conducted on humans (13,172,184,331) and on animals (332-334,336). The method has been modified (335) by the application of a mixture of croton oil and sulphuric acid or benzenesulphonic acid, thereby causing more severe inflammation than croton oil alone and allowing tests to be performed on more intense inflammation.

Tetrahydrofurfuryl alcohol (THFA) acts both as the irritant and the solvent for the corticosteroid (286-288). The activity of the corticosteroid is indicated by its ability to inhibit the contact dermatitis induced by THFA. Since the irritant acts as the vehicle for the corticosteroid, this assay cannot be used for formulated products (287).

Other chemical irritants that have been used are kerosene (184,337), histamine (338), *Rhus* oleoresin (231), mustard oil (339), nitric acid (339), cantharidin (340) and picryl chloride (341).

Irritants are also used in the rat or mouse ear assay (332,334,336,340, 341). Use is made in this assay of the fact that rat ears increase in size after the application of an irritant. Corticosteroid activity is ascertained by noting the suppression of increase in the weight or size of the ears to which the corticosteroid and irritant were applied, compared to the ears onto which only the irritant was applied.

The systemic activity of corticosteroids can be ascertained by measuring the inhibition of thymus activity in young rats after application or oral administration of the corticosteroid and application of the irritant (332,334,336,340-342). It should be noted that the correlation of potencies of corticosteroids between rat and man (in induced inflammation) has been found to become less reliable with an increase in corticosteroid potency (333).

1.8.1.2 Ultraviolet Irradiation

The ability of the corticosteroid to suppress UV-induced inflammation is determined by exposing the test site to UV radiation before (172,343, 344) or after the application of the corticosteroid and measuring the degree of erythema inhibition. Kanof (345), who first reported this assay procedure in 1955 in the assessment of hydrocortisone, reported that erythema was diminished only if the corticosteroid was applied within 24 hours before exposure to UV rays. Scott and Kalz (339) 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 inhibition when the corticosteroid was applied more that 16 hours before UV exposure. Unfavourable responses were obtained if the interval between corticosteroid application and UV exposure was too short, as well as when the corticosteroid was applied after exposure to UV irradiation. Similar results with respect to the period between irradiation and corticosteroid application have been found by Altmeyer and Krumrey (344). Kalz and Scott (346) have described a similar assay utilizing Grenz rays instead of UV rays.

UV irradiation studies have also been performed in *in vitro* (36) and *in vivo* animal studies (347). In the *in vitro* study (36) hydrocortisone was found to reduce and retard the cellular breakdown of irradiated foetal rat skin. In the *in vivo* studies (347), which were performed on rats, the corticosteroid was either applied in ethanolic solution immediately after irradiation, or injected subcutaneously 1 hour prior to irradiation. Both the topically applied and subcutaneously administered corticosteroids demonstrated a dose-related response, although the erythema inhibition was found to be more effective after topical administration.

1.8.1.3 Stripping

Wells (348) described a method of assessing the activity of corticosteroids by applying hydrocortisone to areas of the skin that had been traumatized by stripping with cellophane tape. This method has since been employed to assess the effect of vehicles (349), to compare diffe-

rent concentrations of triamcinolone acetonide with hydrocortisone (350) and to screen new corticosteroid compounds (351).

1.8.1.4 Antigranuloma

In this assay the potency and activity of corticosteroids are measured by their ability to inhibit the formation of scar tissue. The assay involves the insertion of cotton pellets that have been impregnated with the test steroid into the subcutaneous tissue of experimental animals (333,352). Control pellets, which contain no steroid, are similarly inserted. It is important to note that this assay provides no information on the ability of the corticosteroid to penetrate the skin. This disadvantage also applies to the assay described by Lerner *et al.* (353) in which the corticosteroid was administered parenterally after insertion of the cotton pellets. DiPasquale *et al.* (342) have described a modified assay in which corticosteroids are applied topically, thereby taking into account the percutaneous absorption of the drug. The granuloma pouch technique, first described by Selye (354), requires the injection of croton oil into an air pouch and the corticosteroid into the wall of the pouch.

1.8.2 Cytological Studies

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

The fibroblast inhibition assay, which is performed *in vitro*, gives an indication of the anti-inflammatory activity of corticosteroids by observation of the corticosteroid-induced inhibition of fibroblasts (355-359), which are known to be involved in the inflammatory process.

The activity of corticosteroids may also be assessed by measuring the extent to which they produce thinning of the epidermis in rat tails (360). Barnes *et al.* (284) have described a modification of this assay in which the activity of the corticosteroid is measured by its ability to suppress the epidermal thickening caused by the vehicle.

The corticosteroid-induced inhibition of mitosis has been well established and well documented (48-51), and it has been suggested that this antimitotic effect be used as a bioassay for corticosteroids (49). Corticosteroid potency is ascertained by measuring the mitotic index of the epidermis after oral administration or topical application of the corticosteroid. Marks *et al.* (50) have described a similar assay using hairless mouse skin instead of human skin.

1.8.3 Correlation of these Bioassays with Blanching Assays and Clinical Trials

Few of the above studies allow direct comparisons of the results with the blanching assay or clinical trials. Where more than one type of experiment was reported in the same paper, it can normally be seen or assumed that the same batch of the corticosteroid preparation was utilized in the different studies and only these direct comparisons are discussed here.

Correlation has been found between the suppression of experimentally induced inflammation and blanching. The correlation was higher with kerosene than with croton oil (184), and the degree of correlation was not consistent for all the preparations tested. Zaynoun and Kurban (337) found good correlation with three of the four corticosteroid preparations used in their blanching and kerosene-induced inflammation assays, where fluocortolone showed marked blanching, but relatively poor anti-inflammatory activity.

In a comparison of four novel corticosteroid compounds with betamethasone 17-valerate, Bagatell and Augustine (351) found that the rank order of activity predicted by the blanching and stripped-skin assays was borne out in studies of clinical efficacy, with one exception.

Burdick (172) studied fluocinolone acetonide and fluocinonide in alcoholic solution and in a cream base. The vehicle was the commercial base for fluocinolone acetonide and theoretically ill-suited for efficient release of fluocinonide. The activity of fluocinonide was greater than that of fluocinolone acetonide in the alcoholic blanching assay, whereas the responses of the two corticosteroids in the cream

base were similar in the blanching, UV and croton oil assays, as well as in a clinical trial. Correlation was also found in the comparison of 3 proprietary preparations in the blanching assay and in UV erythema suppression (343).

CHAPTER 2

THE BLANCHING ASSAY:

METHODOLOGY AND EVALUATION OF RESULTS

The pallor induced by the topical application of certain corticosteroids to human skin has been the basis of a valuable bioassay since its publication by McKenzie and Stoughton (169) in 1962. Modified versions of the relatively crude assay described by these workers have been used extensively over the past 25 years to assess the blanching abilities, and by inference the clinical efficacies, of novel corticosteroid molecules in alcoholic solution, as well as the release of corticosteroid molecules from formulated topical preparations (179,361). It is interesting to note that several of the criteria proposed by Redman (362) for an effective screening method are met by the blanching assay. Some of these are that the method should be inexpensive, produce results in 10 days or less, require small amounts of sample, provide statistically reproducible results and have a sound biological basis.

The aim of the first part of this chapter is to review certain aspects of the methodology of the assay and cite evidence for the good correlation that has been found between results of the blanching assay and clinical trials. The second part of the chapter comprises a description of the methodology employed in this study.

2.1 BIOAVAILABILITY

The concept of bioavailability of topically applied drugs has been a topic of debate in the literature. As the blanching assay allows an assessment of the availability of the drug molecule to its site of action, it seems apt to clarify the use of the term bioavailability before discussing the assay.

Bioavailability includes a study of factors which influence and determine the amount of active drug that gets from the administered dose to the site of pharmacologic action, as well as the rate at which it gets there (363,364). The usual procedure for conducting a bioavailability

study is to collect plasma and/or urine samples from each of a number of subjects following administration of a dose of the drug (365). It is well known, and is evident from the review in Chapter 1, that penetration of corticosteroids into the skin depends on the release of the active substance from the vehicle, the diffusion of the substance into the horny layer and the transfer of the substance from the horny layer to the epidermis and dermis (17).

A major distinction between drugs administered topically for dermatological disorders and those administered for their systemic action is that in the former the general circulation does not distribute the drug to the target tissue, but removes it from the site of action (147,366). Measurement of the amount of corticosteroid in the blood has the additional complication that the state of the skin barrier depends on the intensity and type of disease and may influence bioavailability of the same drug from different vehicles when the stratum corneum is intact (147). In dermatological preparations the bioavailability could be referred to as the difference between the amount of a specific drug that is applied to the skin and the amount that reaches the site of action (366). It has been suggested (367) that comparative bioavailability, as assessed by the blanching assay, could be expressed as

$$\text{Bioavailability} = \frac{\text{score achieved by the (test) product}}{\text{score achieved by the most active formulation}}$$

which may be compared to the more usual relationship in pharmacokinetics where

$$\text{Bioavailability} = \frac{\text{amount systemically available from a dosage form}}{\text{amount systemically available from the optimum dosage form}}$$

It should be noted that slightly different values could be obtained depending on whether the scores referred to above are percentage of the total possible score values (see section 2.4.1) or area under the curve values (see section 2.4.2) (205,367).

As the traditional definition of bioavailability refers to the absolute concentration of the drug in flowing blood it would only refer to topical corticosteroids once they have been removed from the site of pharmacological action into the bloodstream, and it has therefore been sugges-

ted that another word be used for the comparative amounts of corticosteroid released from the vehicle to the skin (368). Rectifying this problem would involve either the introduction of a new term relating specifically to topical corticosteroids or the extension of the existing term to include the measured pharmacological response of the corticosteroid from the vehicle (367). The term bioavailability is a contraction of "biological availability" (364), and as the pharmacological response requires the corticosteroid to be available at the receptor site in the biophase (147,366), it would seem logical that the definition of bioavailability of topical corticosteroids given below would be acceptable.

In the blanching assay the term bioavailability can be used to refer to the relative absorption efficiency for a corticosteroid as determined by its release from the preparation, followed by its penetration through the epidermis into the dermis to produce the characteristic blanching effect (14,68,138,194,207,367).

It should be noted that this is different to the "activity" of a preparation. The activity of a preparation is its ability to produce skin pallor, while the bioavailability of a formulation refers to the activity of a given corticosteroid in a given base monitored as a function of time compared with this activity in different bases (205).

2.2 THE BLANCHING ASSAY

Considering the extensive use of the blanching assay, and the general acceptance of the correlation between this assay and clinical efficacy (section 2.2.8), it is surprising that there appears to be no consensus about how best to conduct the assay (15).

2.2.1 Selection of Volunteers

In general the volunteers employed in blanching trials have been healthy persons (101,135,138,173,243,250,302,361,369-371) of either sex (14,68, 101,113,135,140,205,207,208,215,243,302,305,309,361,369-372) who have previously shown a consistent response to a standard corticosteroid (14,68,101,113,138,207,215,309,370), but without reference being made to

sensitivity of the response (14,68,101,113,135,140,205,207,208,215,243, 305,309,370,371). Variations of this have included the use of males only (16,184,269,337,373,374), volunteers of "mixed sex and age range" (15), an equal number of males and females (295,296,375-377), random selection of subjects for the occluded assay but only subjects that had previously shown conspicuous blanching for the unoccluded assay (373) and the use of subjects that had not been exposed to topical corticosteroids at any time prior to the study (16). The stated age of the volunteers has varied from young adult volunteers (250,253,296,297,304,377,378) and adult volunteers (138,369) to various ages between 12 and 74 years (124, 140,184,206,250,269,314,373-376,378-380).

The recognition of tolerance to the blanching response (section 1.5) has necessitated that volunteers should be allowed a recovery period between trials (367,381). This has varied, with resting periods being at least 2 weeks (124,269), at least 2 weeks but usually 3 - 4 weeks (135), at least 4 weeks (93,96,144,237,296,302,377,382), at least 6 weeks (140, 173,243,361,371,383), at least 2 months (194,195,206,208,250,325,381, 384), at least 3 months with 1 month between successive trials (370), at least 3 months with 2 months between trials (68), at least 3 months (14,101,113,138,180,205,207,215,228,309,369,378,385,386) and at least 6 months (223,380).

The number of volunteers used in a trial should depend on the method of statistical analysis used to evaluate the responses and the number of preparations being tested. Most papers have reported the use of 10 volunteers (14,16,68,96,101,135-138,146,194,195,205,215,224,228,236,242, 247,295-297,302,304,314,367,370,372,377,383,384,387-390), but this too has varied from 4 (391), 6 (180), 7 (136,137), 8 (223,305,381,391,392), 9 (393), 11 (380), 12 (15,135,243,371,375,379,394,395), 16 (351,381, 396), 18 (140), 16 - 20 (343,397), 20 (96,128,314,351), 24 (369,373), 30 (398), 32 (382,385,386), 35 (325) to 50 (93).

Moore-Robinson and Christie (385,386) used 32 subjects so that no steroid was ever applied to the same area more than once and therefore variability between blanching at different sites was eradicated. Whilst this is an important consideration, a similar effect can be obtained with fewer subjects (see section 2.3).

2.2.2 Mode of Application

Several comments have been made in the literature about whether the blanching assay should be performed in the occluded or unoccluded mode of application. A few workers have reported the use of only the unoccluded mode (96,233,242,247,252,269,289,381,398-400), while the majority have used either the occluded mode (14-16,23,93,95,96,101,112,113,124, 128,135,140,142,146,171,184,194,196,199,203,205,207,208,215,224,237,245, 248,272,295-297,302,305,309,314,318,325,327,337,351,369,372,374,378,379, 385-388,392-394,396,401-404) or both the occluded and unoccluded modes (68,133,138,144,169,172,180,206,236,253,343,367,371,373,375,380,383,384, 391,397,405), and in one study polythene rings secured to the arms with adhesive tape were described as semi-occlusive (250).

The unoccluded mode has the advantage of not masking enhanced penetration due to skin hydration (195,247). Reports have appeared in which the difference between preparations is smaller in the occluded mode than in the unoccluded mode (206) and where differences were found in the unoccluded mode but not in the occluded mode (253,383,391,406). It has been reported that a 6 hour occluded application masked vehicle effects, whereas a 6 hour unoccluded and a 19 hour occluded did not (373). The hydration caused by the occlusion tends to favour poorly designed formulations and thereby masks more subtle effects of the base and corticosteroid interaction (397). Theoretically, in an optimum formulation the results from the unoccluded sites should be almost equal to those from the occluded sites (397,406), whilst in suboptimal formulations a truer picture of release should be obtained from unoccluded sites (172). It has been noted that because of the occlusive nature of ointment vehicles, the difference in blanching between the occluded and unoccluded ointments is generally smaller than between occluded and unoccluded creams (138,343). Differences in onset and duration of action from formulated products may also be masked by occlusion (68). A further consideration is that many of the potent topical corticosteroid preparations, when administered in therapeutic doses under occlusion, may provoke a maximal response, which makes assessment of differences in percutaneous penetration and blanching activity difficult, and for this reason it has been suggested that the assay be performed in both modes of application (391).

It has further been pointed out that occlusion promotes percutaneous absorption to a greater extent than achieved in normal clinical practice (382), where corticosteroids are usually not applied under occlusion (381,383).

The variability in sweating from one subject to another has led to substantial variations in responses when occlusion was used with ointments, creams and lotions (247). This may exacerbate the effects of occlusion masking the influence of the vehicle, in that pooled results which include the results of volunteers in whom more hydration of the stratum corneum is achieved by occlusion, may mask significant differences found in other volunteers. The author suggested that this may be avoided if the sites are left unoccluded and if the duration of application is less than 20 hours.

2.2.3 Site of Application

The site of application has generally varied little, with several workers having used the forearm (15,93,140,172,215,247,295,302,304,343, 373,378,381,382,391,394,402) and most having specified the flexor aspect of the forearm (14,16,23,68,95,96,101,113,124,133,135,138,169,173,180, 195,199,203,205-208,233,236,237,243,248,250,253,296,305,309,318,351,367, 369-372,377,379,380,383-387,392,397,398,400,401).

Other application sites have included the dorsal and flexor sides of the forearm (146), the back (128,375,399), the lower back (252), the upper back (344,374) and the back, 5 cm on each side of the spinal cord (269). The lower back has been found to be more sensitive and less variable to the blanching response than the upper back (184).

A precaution that has been mentioned with respect to the forearm is to avoid areas with large blood vessels or skin blemishes (384). Areas close to the elbows and wrists should also be avoided (296,305,377,384), as sites about 4 cm from the elbows and wrists have been reported to show poor responses (397). Sites located on the lateral and medial surfaces have been reported to be more difficult to read (397). It has therefore been suggested that volunteers with short and narrow forearms should, where possible, not be used (397). Interestingly, it has been

reported that responses seemed to be "most definite" near the wrist (407). Variable blanching responses on different sites of the forearm are discussed in more detail in Chapter 6.

2.2.4 Size of Application Area and Amount Applied

It can be seen from table 2.1 that these two aspects of the blanching trial have varied tremendously in reports on blanching assays.

Few comments have appeared in the literature about the size of the application area or the amount applied. Magnus *et al.* (173) found differences in the blanching response depending on the amount of preparation applied, whereas Barry and Woodford (68) did not (see section 1.3.3).

It has been suggested (15) that the amount applied should be sufficiently small to obtain a surface film thin enough to have a minimal effect on the penetration rate, but sufficiently large that the application period can be as short as possible (for example 3 hours) in order to obtain the fullest picture of the response-time profile. It would appear as though the amount of material applied and the size of the application area will not be critical as long as the above suggestion is adhered to. Whilst the influence of the application of different amounts of the same preparation has been studied, it would be worthwhile ascertaining experimentally whether results of comparative blanching responses between different preparations differ when, for example 5 mg or 10 mg of each preparation is applied to sites of the same size. Similarly, the effects of applying the same amount of different preparations to sites of varying sizes requires investigation. Until this aspect of the blanching assay has been thoroughly investigated, authors should clearly report the amount of material applied and the size of the sites to which it was applied, so as to allow comparisons of results from different trials or laboratories to be drawn more reliably.

With respect to the shape of the application area, it has been pointed out that a possible disadvantage of circular test sites is that circular corticosteroid-induced blanching areas may be confused with nonspecific discolorations of the skin (112,369,397).

Table 2.1 Sizes of Application Sites and Amounts Applied

Size	Amount	References
4 mm x 4 mm	4 mg	382
7 mm x 7 mm	1 mg	402
7 mm x 7 mm	3 mg	96,144,172,343,397,400
7 mm x 7 mm	3,2 mg	173,371
7 mm x 7 mm	4 mg	236,383
7 mm x 7 mm	5 mg	14,68,101,113,138,194,195,206-208, 215,223,228,242,309,370,384,387
7 mm x 7 mm	5,5 mg	140
7 mm x 7 mm	12,5 mg	296,377
7 mm x 7 mm	30 mg	305
7 mm x 7 mm	0,8 mm diameter strip	180
7 mm x 7 mm	5 µl	194,195,205,206,243
7 mm x 7 mm	10 µl	96,112,327,367,370,385,386
7 mm x 7 mm	-----	237,387
8 mm x 8 mm	10 µl	378
10 mm x 10 mm	20 µl	93
10 mm x 10 mm	0,5 ml on paper patch	369
10 mm x 10 mm	a filled plastic cup	93
13 mm x 13 mm	a liberal amount of cream	337
15 mm x 15 mm	75 mg	184
16 mm x 16 mm	-----	199
20 mm x 20 mm	-----	252
25 mm x 25 mm	0,25 ml on absorbent lint	407
35 mm x 15 mm	0,1 ml	16
6 mm diameter	25 µl on nylon sponge	396
7 mm diameter	2,7 mg	380
8 mm diameter	3 mg	96
8 mm diameter	15 µl	375
9,5 mm diameter	0,1 ml	95
10 mm diameter	10 µl	15
10 mm diameter	50 µl	23,250
12 mm diameter	40 mg	399
16 mm diameter	360 mg	408
19 mm diameter	10 µl	373
20 mm diameter	20 µl	135,325
20-25 mm diameter	10 mg	247
21 mm diameter	20 µl	392
25 mm diameter	20 µl	133,169
30 mm diameter	10 µl	289
40 mm diameter	0,1 ml	344
19 mm diameter x 0,365 mm deep	-----	373
small coin*	20 µl	372
1 cm^2*	10 µl	351
2 cm^2*	0,1 ml	379
3 sq cm*	10 mg	398
4 cm sq*	250 mg	318
as small as possible	20 µl	203

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-----	3 mg	142
-----	50 mg	388
-----	60 mg	374
-----	250 mg	171
-----	20 µl	124,314
-----	50 µl	395
-----	0,5 ml	394

*The specific dimensions were not reported

2.2.5 Observation Times and Duration of Application

It is interesting that even though relatively little has been written about the times of observation of the blanching response and the duration of application, these have both varied substantially in reports in the literature. The different combinations of observation times and the duration of application are presented in table 2.2.

Some workers have explained why they have used a particular duration of contact between the corticosteroid and the skin. Shorter application periods have been found to be more discriminating than longer application periods (172,206,248,397). The 6 hour application period has been reported to provide better differentiation between formulated preparations than has a 12 hour application (384) and, especially in the unoccluded mode, is more akin to the usual 3 times a day clinical application of topical corticosteroids (172,343,381). A 5 hour application period has also been reported to give better differentiation than a 16 hour application period (146). Significant differences were also reported when sites were left occluded for 4 - 6 hours, but not for 16 - 20 hours (248). It would appear as though the longer application periods allow more corticosteroid to penetrate the skin, thereby masking the release characteristics of the vehicle.

The observation times and the number of observations have been discussed in the literature to a somewhat greater extent. Opinions on the best time to commence readings after the removal of the residual corticosteroid or vehicle are varied. It has been suggested that the first reading taken 10 minutes after removal of the dressing allows time for erythema produced by the removal of the occlusion to subside and not influence the assessment of pallor (309,370). Other workers have stated

Table 2.2 Combinations of Duration of Application and Observation Times

Duration (hours)	Observation Times (hours after application)	References
2	-----	248
3	14.5,15.5	15
3	3,5,7,12,24,32,48,56	380
3.5	5,6,7,8	252
4	-----	248
4	6	128
4	6,9,24,28	374
5	-----	146
6	-----	373
6	6	337
6	6,7,8,9,10,11,12,13,24,26,28,30,32	370
6	6,7,8,9,12,24,32,48,72,80,96	14,68,101,113,138,194, 195,205-208,309,384,387
6	6,7,9,11,13,24,32,48,56,72,etc., as long as blanching was discernible	380
6	7	184,289,295-297,377
6	7,8,9,10,12,14,16,18,28	243
6	7,8,10,12,14,28,32,48	96
6	7,8,10,12,14,16,18,24,28	302
6	7,9,11,(13),24	400
6	7,9,11,14,16,25,33	304
6	7.5,24,30	250
6	8	305
6	8,10,12,24	237
6	8,24	180,196,385,386
6	8,24,32	144,343,391,397
6	8,24,32,48	172,391,406
6	16,18,20,22	370
6	17	327
6	24	142
6	multiple readings, times not stated	236,272,367,371,383
6.5	8,24,32	381
6.5	8,9.5,24,32	381
8	8,24,32	402
8	8,24,32,48	382
8	8.25,24	95
8	10,14,24	224
about 8	9-20	253
12	14,16,19,21	140
12	24	396
15	16	135
15	17	124
16	-----	245,248
16	16	133,169,401
16	16.3,17	325
16	16.5	199
16	17	203,372
16	18	233,398
16	18,24	136

(continued over page)

16	19	379
16-18	1-2 hours later	112
16-20	16-20	408
16-24	1 hour later	93
16	within 2 hours, generally 30 minutes	351
16	standard observations	146
16	when effects of hydration had worn off	403
about 16	about 16	392
about 17	about 17,20	407
18	18	369,394
18	19,20	15
18	19,20,21,24,30,42	96
18	20	172
19	-----	373
20	-----	171,248
20	23-24	247
20	23,44	314
20-22	24	378
21-24	1,3,6,9,24 hours later	375
24	24.25	404
24	24.5	399

that the marginal erythema was still present after 20 minutes, but had subsided after 1 hour (325). Another reason for allowing time between the removal of dressings and the assessment of pallor is to prevent over-reading due to the whitish appearance of hydrated skin (184). Hydration of the stratum corneum has been reported to return to normal between 30 minutes (169,361) and 1 hour (135,184,203,325) after removal of the occlusive dressings. Tissot and Osmundsen (396) seemed to allow 12 hours after removal of the dressings for the skin to revert to its normal state of hydration.

The large number of reports that have appeared in the literature in which conclusions of comparative blanching assays have been drawn from a single reading (see table 2.2) is disturbing and has recently been a topic of debate in the literature (409-413). Three objections to the use of a single reading (409) are discussed in this section to illustrate the importance of multiple observations.

Firstly, the placebo blanching response elicited by some of the vehicles into which corticosteroids have been incorporated has been noted on several occasions (15,96,208,211,370,381-383,402,411,414,415). This response has generally been found to decline rapidly and is negligible

within a short while after the removal of the occlusive dressing and residual preparation (15,96,208,370,381,383,415), although the response sometimes produces a second small peak during the trial (15,381,383).

Secondly, occlusion of the site of application, either by the vehicle or the occlusive dressing, may also produce pallor (382) and influence the assessment of the degree of blanching. Gibson and Kirsch (412) have claimed that the possible error attributable to the placebo effect would be of similar magnitude for different preparations being tested. This, however, would only be true if the same vehicle was being used, as different vehicles have been shown to elicit different placebo responses (414,415). It has further been reported that scores recorded for sites which had been left blank tended to be higher at the early and late stages of the trial, when the blanching responses of the other sites were weak (15). It was suggested that this is as a consequence of the reader subjectively scaling each site against all the other sites on the volunteer when assigning the scores.

The third, and probably most important point, is that one reading only allows a comparison of blanching responses at that particular time and does not allow the construction of a response-time profile (15). Multiple readings allow the interpretation of onset of action, duration of action (234,343,367,384,397), time of maximum blanching intensity (343), response-time profiles (361), area under the curve (AUC) values and summed percentage of the total possible score (%TPS) values (144, 367), and are essential to obtain a correct interpretation of this assay, particularly if two different corticosteroids are being compared. The AUC is a biological response-time parameter (14) and is generally a well accepted measure of bioavailability (363).

It has often been found that a corticosteroid formulation which produces relatively high blanching values in the early part of the trial can show a rapid decline in blanching at later times and *vice versa* (14,15,68,96, 113,172,204,207,234,236,237,243,367,375,382,397). Considering that the profiles of two topical corticosteroid formulations may therefore be coincident at certain times while deviating substantially at others, the use of a single point assessment may result in an invalid conclusion (411). Gibson and co-workers (304,412) have agreed that relative

differences in blanching activity between different corticosteroid preparations can only be accurately predicted if the full blanching profile of each preparation is known, and further state that where this is the case the interpretation of relative activities obtained from a single reading will suffice. This however will only be true for the same corticosteroid incorporated into exactly the same vehicle. It is well known that the vehicle may alter the rate of release as well as the extent of release, and it is not always possible to predict the changes that may occur (194, section 1.6).

Reports have also appeared in the literature where multiple observations were made, but conclusions were drawn from the results of only one observation time (224,380). In one of these reports (380), responses were observed until blanching was no longer discernible (more than 72 hours), but only the results of the 12 hour reading were used because the "number of reactions was greatest then". Considering that the researchers went to the trouble of recording the degree of blanching on a 0 - 3 scale on at least ten occasions, it would have been more valuable to have compared the blanching intensities elicited by the preparations over more than one observation time. Whilst it is not clear from the report whether the blanching profiles were indeed plotted, and whether the conclusions drawn from the 12 hour reading and complete blanching profiles were the same, it is worth noting that two of the preparations tested were Synalar cream and ointment, which have been shown elsewhere to have a slower onset, but longer duration of action than some of the other preparations tested in this experiment (68,96,204,367).

Taking cognizance of the effect of the vehicle on the release characteristics of corticosteroids, it would seem reasonable to conclude that, whilst not ideal, a single observation may be acceptable for the screening of novel molecules in alcoholic solution, but results of trials on formulated products in which only one observation was made should be interpreted with caution. The corticosteroid/skin contact time has also been found to influence the results of comparative blanching responses (section 2.2.5) and this factor should also be carefully considered when a single observation time is employed.

2.2.6 Grading the Intensity of Pallor

One of the main difficulties of the human blanching assay is the subjectivity of assessing the degree of pallor with the naked eye (391). Comments on this have included that subtle differences in blanching are difficult to assess (171), that attempting to increase the precision of the assay by grading or ranking the responses is not practical (135) and that attempting to estimate and assign numerical value to the degree of pallor may lead to conclusions which are not strictly valid (416). There are several reports in the literature in which the blanching responses were recorded only as absent or present (15,93,95,112,133,135,144,169, 325,343,378,394,401,402,407,408). This 2 point system however has serious limitations and its use should be restricted either to experiments aimed at ascertaining the minimum concentration of corticosteroid required to induce blanching or to experiments in which the corticosteroids are expected to elicit very weak responses (section 2.4.3). In the latter case, the more refined scoring systems automatically revert to the 2 point system, or maybe to the 3 point 0 - 2 scoring scale used in some experiments (15,337,404), as only the most potent formulations applied to the most corticosteroid-sensitive volunteers produce scores of 4 at the peak times of 9 - 12 hours (384).

Clanachan *et al.* (15) found that a 0 - 3 scoring system allowed distinctions to be made between blanching intensities that were not evident with a 0 - 1 scoring system and it has been stated that experienced readers are able to make quantitative distinctions without difficulty (247). Several workers have used the 0 - 3 system (15,23,96,172,184,196, 199,233,245,247,250,289,314,369,374,375,379,380,382,385,386,391,392,397-399) and a half-point interval has been included (373). The scoring system has been refined further to a 0 - 4 system (16,96,243,252,327) and a 0 - 4 system with half-point ratings (14,68,101,113,138,140,194,195,205-208,215,223,228,296,305,309,367,370,377,384,387).

Comparison of the size of the areas of blanching has also been investigated as a means of assessing corticosteroid responses. In an experiment in which 0,02 ml of corticosteroid-containing solutions were applied under occlusion to circular areas of the same size delineated by pressing a coin on the arm (in other words not mechanically delineated), it

was found that the intensity of blanching and the size of the blanched area gave similar potency rankings, although the measure of intensity of blanching was considered as slightly preferable (392).

In another experiment in which the results of corticosteroid responses were compared by intensity and area of blanching, comparisons of the preparations by intensity of brightness produced statistically significant differences, whereas comparisons of the areas of blanching were not statistically different (250). Interestingly the areas were larger for the preparations that elicited lower blanching scores. This experiment was performed using the same corticosteroid in different bases and it would be interesting to know whether the lower blanching scores were possibly as a result of the vehicle in some way affecting the distribution of the corticosteroid molecules over a larger area within the dermis. Depression of cortisol levels was also measured in this experiment and indicated lower systemic absorption from the preparations that elicited lower blanching responses. This may indicate a greater reservoir in the stratum corneum from the preparations inducing a larger area of pallor, with subsequent slower absorption and less cortisol depression, although this seems unlikely as the differences in the areas were small (0,33 cm^2 for creams and 0,12 cm^2 for ointments). Another observation has been that the size of the area of blanching appears to be concentration dependent, but this was not extensively investigated (408).

The human eye is capable of performing as an effective rating device, but its reliability suffers whenever comparisons are separated by distance and time (417). This should not present a problem when grading the intensity of pallor compared to normal adjacent skin, but may pose a problem in paired comparisons (section 2.4.3.2) if the sites are not adjacent. Barry and Woodford (384) assessed pallor by observing the response for 1 minute before allocating scores to allow the eyes to "seek out and identify" the less intense sites.

The lighting conditions and positioning of the arms may influence the assessment of pallor. Whilst a few workers have described the lighting conditions under which pallor was assessed in some detail (140,380,384), most of those who have commented on lighting have merely stated that the conditions were constant or standard (15,184,305,370,375,377,383,385,

386). It would appear as though the best conditions are obtained with the use of fluorescent light (140,380,384,397) with no oblique light source from a window or incandescent side lamp (397), although some workers have performed readings under good natural light (325,337) and in diffuse daylight near a window or at the bedside in the case of bedridden patients (23). The use of a "standard fluorescent-light box" into which the arms could be inserted inferred no advantage and has been reported to give "spurious pallor" (384). Whilst the ideal situation would be for all readings in a trial to be performed under exactly the same lighting conditions, this may not be possible where two or more persons are making independent observations. However, provided that the lighting conditions are adequate and remain standard for each observer, the overall results of comparative blanching responses should not be affected.

With respect to the positioning of the arms it has been suggested that they should be held horizontally or slightly upwards (305,384) to prevent blood vessels enlarging when the arms hang downwards and thereby obscuring pallor assessment (384). When a volunteer has been standing with his arms hanging downwards just prior to the reading, it is useful to allow the arms to rest horizontally for 2 or 3 minutes before assessing the degree of blanching.

The number of observers that should be employed in blanching trials is discussed in section 3.3.

2.2.7 Nonvisual Assessment of Vasoconstriction

One of the criticisms of the blanching assay is the inherent subjectivity of assessing the degree of pallor with the naked eye.

Thune (322-324,418,419) has suggested the use of piezoelectric or photoelectric plethysmographic recording of skin pulses, which does not assess the degree of pallor, but estimates the size of an organ (in this case the skin) according to the state of fullness of its blood vessels (420). A reduction in blood flow has been noted after the application of betamethasone 17-valerate (322,418), but not after the application of the placebo base (322). This method has also demonstrated a greater reduction in blood flow following the application of betamethasone 17-val-

erate ointment when compared to hydrocortisone acetate ointment (419). A decrease in blood flow has also been noted after the application of fluocinolone acetonide cream and fluclorolone acetonide ointment, as well as a greater decrease in blood flow after the application of hydrocortisone acetate ointment when applied to stripped skin as opposed to normal skin (323). Interestingly, an alcoholic solution of betamethasone 17-valerate induced distinct blanching on normal skin, but no significant reduction in the plethysmographic recordings (324). The author was unable to explain this, but offered that it may be related to the depth of penetration and rate of clearance of the corticosteroid molecule from the skin.

The ability of this technique to measure blood flow in diseased skin may offer some advantage over the visual assessment of blanching. The vasoconstrictive action of corticosteroids is said to have little effect in the treatment of skin disorders (section 1.1.1), although the blood vessels in psoriatic skin are congested (323).

Amantea *et al.* (421) attempted to measure corticosteroid-induced vascular changes by laser Doppler velocimetry (LDV). Despite the visual appearance of blanching, the LDV voltage output after the application of fluocinonide cream was not significantly different from the same skin site prior to corticosteroid application. In a further experiment they found that the expected inhibitory effect of prior corticosteroid application on the vasodilative effect of methyl nicotinate was not observed with fluocinonide cream or ointment. Bisgaard *et al.* (422) have, however, been successful by combining LDV with induced reactive hyperaemia attained by arterial occlusion with a blood pressure cuff on the upper arm. The reactive hyperaemia was significantly reduced by prior application of budesonide, but not by the placebo vehicle. They further found a rank order of potency of clobetasol 17-propionate > budesonide > hydrocortisone 17-butyrate > hydrocortisone. These authors did not assess the degree of blanching and although the rank order correlates with other published results a direct comparison would have been useful.

Attempts have also been made to measure temperature changes of corticosteroid-treated skin. Somerma (375) and Kiraly and Soos (388) found that the differences between normal and corticosteroid-treated skin were too

small to be accurately measured by contact thermography. Aiache *et al.* (423) have, on the other hand, found a decrease in skin temperature on the site where the corticosteroid was applied, but there appeared to be no correlation between the temperature change and degree of blanching.

Trikam and Morton (424) investigated the usefulness of measuring the density values of blanching sites on the negatives of black and white film used to photograph blanching responses. The results obtained for the five commercial preparations tested correlated well with published results of comparative blanching and clinical efficacy, but these workers unfortunately did not assess the blanching visually during the experiment. They further found that whilst the microphotometric method produced reliable blanching profiles and has the advantage of a permanent record of the trial, the method is time-consuming and unsuitable for trials requiring the examination of a large number of negatives.

Several workers have investigated and used spectrophotometric techniques to assess the intensity of blanching. The physics and explanations of the spectrophotometric reflectance of light by the skin are not within the purview of this work, but are described by Dawson *et al.* (425).

The first report on the objective measurement of the blanching response appears to be that of Reid and Brookes (259) who used a spectrophotometric technique. Preparations containing triamcinolone acetonide and fluocinolone acetonide elicited similar blanching responses and both elicited more blanching than hydrocortisone alcohol. The similarity between fluocinolone acetonide and triamcinolone acetonide was found in a clinical trial, where fluocinolone acetonide was also superior to hydrocortisone alcohol, but triamcinolone acetonide and hydrocortisone alcohol produced similar results. The objectively measured blanching response therefore correlated with the clinical response, except in the comparison of triamcinolone acetonide and hydrocortisone alcohol. The lack of predictability of the clinical efficacy of triamcinolone acetonide from results of blanching trials has been noted elsewhere (426).

Dawson *et al.* (425) compared the objective and subjective assessment of the intensity of blanching induced by four corticosteroid creams and found that the correlation of the two methods of assessment was good.

The correlation coefficient was 0,87 with individual values ranging from 0,51 to 0,92. It is not clear from the report how familiar the observers were with the assessment of corticosteroid-induced blanching. Good correlation has also been reported by this group of workers in other experiments in which they performed objective and subjective assessments (16) and in comparisons of their objective assessments with subjective assessments performed in other laboratories (427,428). Other workers have also found good correlation between the visual and spectrophotometric methods of assessment, either by simultaneous assessment (388) or by comparisons with results from other laboratories (429,430). There have been other reports in the literature (269,276,304,344,431-434) in which spectrophotometric techniques appeared to have been successfully employed, but these do not allow comparisons with visual assessment because the corticosteroids or formulations were not necessarily the same as those reported elsewhere.

The instrumental methods of assessment of the degree of pallor generally have the disadvantages of being expensive, cumbersome and time-consuming (179,361). These methods also do not have the ability to compensate for skin imperfections and may cause spurious pallor if they apply even a slight weight to the skin (179).

In view of the good correlation that exists between the subjective and currently available objective assessment techniques, it can be concluded that if the aim of an experiment is to ascertain the relative blanching responses of topically applied corticosteroids, the objective instrumental methods offer no advantage over the subjective methods (427). While successful applications of these objective methods have been reported, no single approach has yet been sufficiently well validated such that it competes with the subjective assessment used in the blanching assay (17).

2.2.8 Correlation of the Blanching Assay with Clinical Trials

The ultimate test of any drug is the efficacy of the drug in a formulated product administered under therapeutic conditions. Clinical trials are expensive and tedious to perform, thus necessitating the use of bioassays that can reliably predict the efficacy of the product (325).

Although there is no irrefutable evidence that blanching and anti-inflammatory efficacy are directly related, strong correlations in activity have been recorded, and the blanching assay can be regarded as a very valuable method for rapid preclinical assessment of novel compounds or formulations (370).

Bagatell and Augustine (351) compared the blanching abilities of four novel corticosteroids and betamethasone 17-valerate in alcoholic solution to their clinical efficacies in the same cream base. The rank order of these corticosteroids was the same in blanching and clinical trials, with one exception which the authors felt was due to the low solubility of the corticosteroid in the cream base. Burdick (172) found that fluocinonide produced more intense blanching than fluocinolone acetonide in the alcoholic blanching assay, but when incorporated into a cream base they elicited similar results in the blanching assay and clinical trials. The vehicle used was expected to promote effective release of fluocinolone acetonide and poor release of fluocinonide. Fluocinonide in FAPG has further been found to be superior to betamethasone 17-valerate cream in both the blanching assay and clinical situation (406). The equivalent therapeutic effectiveness of diflorasone diacetate and fluocinonide creams in a clinical trial confirmed the predictive data obtained in the blanching assay, although diflorasone diacetate elicited a superior response in the alcoholic blanching assay (373).

Varying concentrations of betamethasone 17,21-dipropionate and betamethasone 17-valerate incorporated into an identical vehicle indicated that the 17,21-dipropionate ester elicited a superior response to the 17-valerate ester (379). Results of a clinical trial showed that the commercially available preparations, which contained either 0,05% of 17,21-dipropionate or 0,1% of the 17-valerate, were equally efficacious, which appears to be the same as the comparative blanching abilities of these preparations. Results of a second clinical trial, however, indicated that the dipropionate was superior to the valerate. This discrepancy may arise from the fact that 10 patients were used in the first clinical trial and 60 patients in the second. The system of evaluation also appeared to be more refined in the second clinical trial. The authors made no comment on this or on the predictability of clinical efficacy from the blanching responses.

Leer (59) has found that the rank order of the blanching abilities of betamethasone 17,21-dipropionate incorporated into two ointments and one cream base was similar to the rank order of the results of a clinical trial. Correlation was also found in creams containing either triamcinolone acetonide or desonide, which elicited similar blanching responses and gave similar results in a clinical trial (95). Correlation was similarly found in an experiment undertaken to compare the blanching and therapeutic abilities of betamethasone esters to each other and to fluocinolone acetonide (135), and in another experiment Dioderm (containing 0,1% hydrocortisone) was superior to 1% hydrocortisone cream B.P.C. in a blanching assay and a clinical trial (403).

Crijns *et al.* (399) found a fair correlation between blanching responses and the treatment of contact allergic dermatitis in the seven corticosteroid preparations they tested. A notable exception was Diprosone cream which rated poorly in the blanching assay but well in the alleviation of inflammation.

The most comprehensive published study of the comparison of blanching and clinical efficacy is that of Cornell and Stoughton (398). In this comparison of blanching ability and the treatment of plaque psoriasis, correlation occurred in 20 of the 23 preparations tested. The corticosteroid-containing creams, ointments or gels were applied without occlusion in the clinical studies and the blanching assays. Gibson (426) has suggested that the lack of correlation in the three instances may be due to the blanching having only been assessed at one reading time, thus not allowing the use of AUC values in the comparisons.

Some interesting points have emerged from a study of the results of four of the preparations studied in experiments performed by Gibson *et al.* (304). In one of the experiments reported, the AUC value for Betnovate ointment was statistically significantly superior to the AUC value for Eumovate ointment, whereas these two preparations elicited similar responses at the 7 hour reading. This was, however, not the same in the second blanching study reported in the same paper, where Betnovate elicited a statistically significantly lower blanching response than Eumovate at the 7 hour reading, but the AUC values were similar. Although the authors point out that in the first study the maximum possible score

at each reading time was 17 whereas in the second study it was 12, it is not clear from the methodology why this is so and no explanation is offered in the discussion why different results were obtained in the two studies. The authors concluded from their clinical study that "technically", Betnovate ointment was more potent than Eumovate ointment and that this was predicted by the AUC measurement of the blanching assay. They however appear to contradict this by stating that "from a practical point of view, there seems to be little clinically relevant difference between Betnovate and Eumovate in the treatment of plaque psoriasis" (the basis of the clinical trial) as shown by the similarity in results obtained after 4 weeks of therapy. In the same report, a 1 in 5 dilution of Dermovate ointment in white soft paraffin elicited a similar blanching response to undiluted Dermovate ointment at the 7 hour reading, but the former was significantly less active in the AUC analysis. The undiluted Dermovate ointment was found to be superior throughout the 4 weeks of the clinical study. It is also stated in this paper that the 7 hour and peak readings predict, with reasonable accuracy, differences in blanching potency of diluted products, but that the 7 hour reading is more easily obtainable and therefore preferable to the peak reading. However, in the comparison of two of the Dermovate dilutions (1 in 5 and 1 in 10), the 1 in 5 dilution elicited less blanching at the 7 hour reading, but more blanching at the other readings, a cross-over having occurred between 7 and 9 hours after application. The authors concluded that the AUC values allow more accurate predictions of relative clinical effects between corticosteroid preparations than do the values obtained at the 7 hour readings, and that comparative AUC values do allow reasonably accurate predictions to be made.

It is interesting to note that the prevailing disease being treated during the clinical trial, as well as the duration of treatment, can influence the overall correlation; psoriasis has been said to provide a more discriminating test than eczema (435). Bagatell (436) 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 the treatment of various other skin disorders and in the blanching assay. Allenby and Sparkes (92) similarly 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.

Sefton *et al.* (400) found little evidence of correlation between results of blanching assays and clinical trials in comparisons of hydrocortisone 17-valerate with several other corticosteroid preparations. Although hydrocortisone 17-valerate ointment elicited similar blanching responses to fluocinolone acetonide ointment and triamcinolone acetonide ointment, it was superior to triamcinolone acetonide in the treatment of both psoriasis and atopic dermatitis after 7 days of treatment, but equivalent after 14 days. In a clinical trial comparing hydrocortisone 17-valerate with fluocinolone acetonide in the treatment of atopic dermatitis, the response was similar for the duration of the experiment, whereas in the treatment of psoriasis differences were not found until 14 days of treatment. Hydrocortisone 17-valerate was, however, more effective after 3 weeks of treatment.

Selection of corticosteroids for clinical use will involve considerations not dealt with in blanching assays. Aspects of the toxicology of the drug such as excessive effects at normal dosage, overdosage, side effects, idiosyncrasies, hypersensitivities and drug interactions require the relevant investigations (367,370). However, irrespective of the arguments raised concerning the correlation between blanching and clinical efficacy, there is no doubt that blanching is an indication of absorption and bioavailability, since it can only occur when the corticosteroid reaches the relevant receptors, which lie in the skin below the stratum corneum (234,325).

2.3 METHODOLOGY EMPLOYED IN THIS STUDY

The general methodology of the blanching assay as normally performed in our laboratories (244,361) was utilized in this study. Aspects that were altered to meet the specific needs of the experiments reported in this thesis are discussed in the relevant chapters.

Healthy male or female Caucasian subjects were selected for each trial from a panel of pharmacy student and staff volunteers known to show a response to a standard preparation (Betnovate cream) applied under occlusion for 6 hours. The intensity of pallor for the screening was noted

once, not less than 1 hour after removal of the occlusive dressing. Several researchers select volunteers without reference to corticosteroid sensitivity (section 2.2.1). In our laboratories, however, the volunteers are selected so that a range of responses will be observed in the trial, unless the corticosteroid preparation to be tested is expected to elicit a very poor response, in which case strong blanchers are recruited for that trial. It has been noticed that volunteers who show a very good response to preparations that elicit a high degree of blanching are "poor discriminators", in other words it is difficult to differentiate between differences in blanching intensities at adjacent sites. This is especially true at the peak blanching times between 9 and 14 hours after application. The inclusion of average and poor responders allows the differentiation between responses that may not be noticed in the good blanchers.

Volunteers were not included in a trial if they had used any form of systemic medication or had used topical corticosteroids for at least 6 weeks prior to the investigations. At the initial screening mentioned above, the arms were checked for scars and blemishes which may interfere with the assessment of blanching. Volunteers presenting such scars or blemishes were not included in any trials.

All volunteers were aware of the purpose of the investigations and the methodology employed. They were also assured that only corticosteroids of known safety were to be applied to their arms and that they could withdraw from any experiment or the blanching panel without in any way prejudicing their academic standing.

Semi-solid preparations were applied to the forearms by means of disposable plastic syringes which were filled immediately prior to use in order to minimize any possible interaction between the corticosteroid and the matrix of the syringe barrel. The needles were of constant size (25G 5/8") and were cut to 5 mm to facilitate extrusion of the preparations. Lotions were applied by means of a micropipette. The first 1 cm of the semi-solid preparations was extruded from each tube and discarded in case of any interaction between the closure and the formulation. In the case of lotions the container was thoroughly shaken before use. It is important that sufficient corticosteroid be applied to the skin to

produce a blanching response that is readily discernible so as to facilitate observation and scoring of the responses, as well as comparisons of responses at adjacent sites. On the other hand if an excess of corticosteroid is applied a plateau may be reached (see section 1.3.3) which may mask the inherent potency of one corticosteroid molecule compared with another, or may mask superior release characteristics of one base over another. Experience in our laboratories has shown that the application of four stripes (7 mm) of semi-solid preparations (equivalent to approximately 3,2 mg) or 5 µl of lotion (equivalent to approximately 4 mg) produces a sufficiently strong blanching response whilst apparently not inducing maximum blanching. The preparations were applied to twelve discrete 7 mm x 7 mm sites on the flexor aspect of each forearm. The demarcating labels were left in place for the duration of the application period.

Four different application patterns were used in each trial. The preparations were not applied in the same pattern on both forearms of a volunteer and the random allocation of the application charts containing the pattern, as well as coding of the preparations were performed by two different persons involved in the trial to prevent the unconscious recognition of a pattern by the observers during the assessment of pallor (397). The codes were broken after the completion of the trial, except for the trials reported in Chapters 4 and 5, in which Betnovate cream was always included as a standard preparation. The use of four different application charts, together with not applying more than four preparations in a trial, ensured that no preparation was ever applied to the same area on the forearms of all the volunteers in any one trial. Blanching responses have been found to vary on different areas of the forearm (see Chapter 6), and the comparison of the same preparation on the same area of the forearms of all the volunteers to other preparations on another area of the forearms of all the volunteers may lead to erroneous conclusions of comparative bioavailability.

The 12 sites were demarcated by applying six self-adhesive labels, from which two 7 mm x 7 mm holes had been punched, onto each forearm (figure 2.1). The use of small demarcating labels and occluding strips, as opposed to one continuous strip, ensures that the preparations do not spread unevenly under the occlusive dressing (180,247) and that the en-

Figure 2.1 Arm with demarcating labels and four stripes of preparation

tire occlusive dressing remains in contact with the skin, thereby maintaining standard conditions of humidity and temperature (203,325). The areas adjacent to the wrists and forearms were avoided, unless the subject had short forearms (section 2.2.3). Each preparation was spread evenly with a different glass rod, or the tip of the micropipette in the case of lotions, prior to the occlusion or protection of the sites. In trials in which both the occluded and unoccluded application modes were used, all the sites on one forearm of each volunteer were occluded while the sites on the other arm were left unoccluded. The writing arm of the volunteer was occluded, as the occlusive dressings are less cumbersome than the protective guards. Occlusion was attained by means of six individual strips (70 mm x 25 mm) of non-porous plastic tape (Blenderm surgical tape; Hypoallergenic, 25 mm, No.1525, 3M Medical Products Division, St. Paul, MN 55101, United States of America) placed over the self-adhesive labels (Chevron; 25 mm x 38 mm, Waltons Stationery Co. (Pty.) Ltd., Johannesburg, South Africa) (figure 2.2) and the sites that

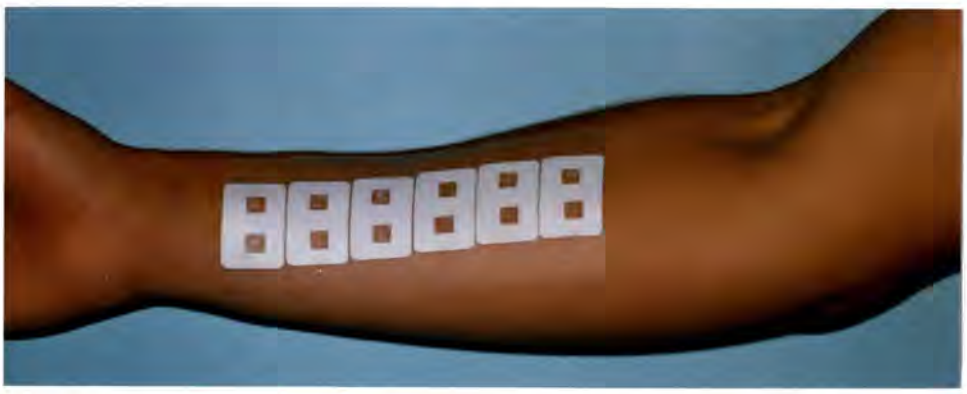


Figure 2.2 Occluding tape applied over demarcating labels

were left unoccluded were protected by plastic coverings designed so as to allow a free flow of air and held in place by Micropore surgical tape (12 mm, No.1530, 3M Medical Products Division, Johannesburg, South Africa) (figure 2.3). As all volunteers in a trial are processed sequentially, it is not possible for only one investigator to perform the entire application procedure in one hour (see below). Three experienced investigators were therefore employed during each trial, but individual procedures were carried out by the same person for any one trial. In the trials reported in Chapter 3 it was ensured that the same investigator performed the above procedures for all three trials. All investigators in our laboratories are competent to perform all procedures, and the current author was actively involved throughout each trial reported in this thesis.

Figure 2.3 Plastic covering used to protect the unoccluded application sites

Volunteers presented themselves for the application of the preparations at given times between 7.30 a.m. and 8.30 a.m., to give an average application time of 8.00 a.m. All volunteers partaking in a trial were processed sequentially in order to minimize any possible effects of environmental variables such as ambient temperature and humidity. Each volunteer recorded the time at which the application procedure was completed so that the protective coverings, occluding tape, demarcating labels and residual corticosteroid preparations could be removed 6 hours later. The volunteers were requested to remove the demarcating labels and occlusive tape slowly so as to avoid stripping of the skin and to minimize erythema. The residual steroid was then removed by gentle washing with soap and warm water and the arms were patted dry with a towel. The use of 70% aqueous ethanol instead of soap and water has been

found to offer no advantage and produced occasional transient pallor (384). In the trials reported in Chapter 3, one of the investigators removed the demarcating labels and residual corticosteroid preparations, whereas in the other trials reported in this thesis this procedure was carried out by the volunteers. Minimal erythema was noted in very few cases in any of the trials.

Blanching responses were assessed independently by three experienced observers at 7, 8, 9, 10, 12, 14, 16, 18, 28 and 32 hours after application which allowed statistical analyses at a number of times over an extended period and the construction of blanching profiles. The results recorded by the three observers were pooled for the calculation of the %TPS values, as well as for the statistical analyses. 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 2.4 and 2.5 depict typical 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 their normal daily activities (385), 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 (68,215,296,325,367,377, 384). The blanching response has been reported to fade quickly and erratically when volunteers become hot or when their arms come into contact with hot water (384).

2.4 EVALUATION OF RESULTS

The methods of evaluation and interpretation of the data obtained from blanching assays differ to some extent amongst the workers in the field. Most researchers draw conclusions from a study of the blanching

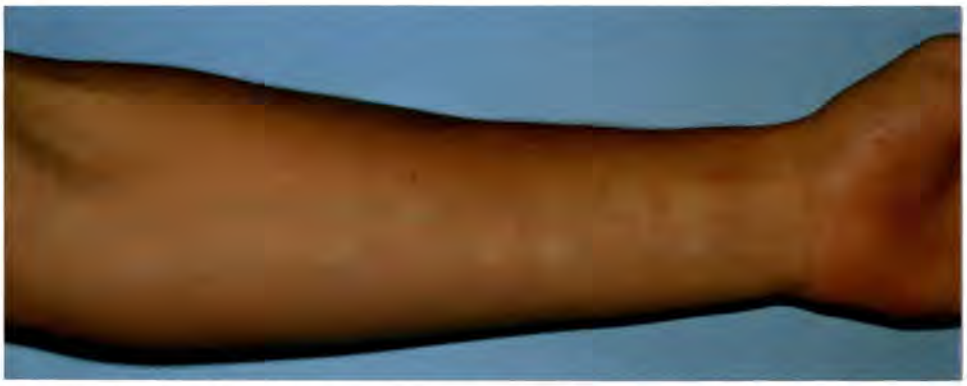


Figure 2.4 Blanching response at 10 hours after application

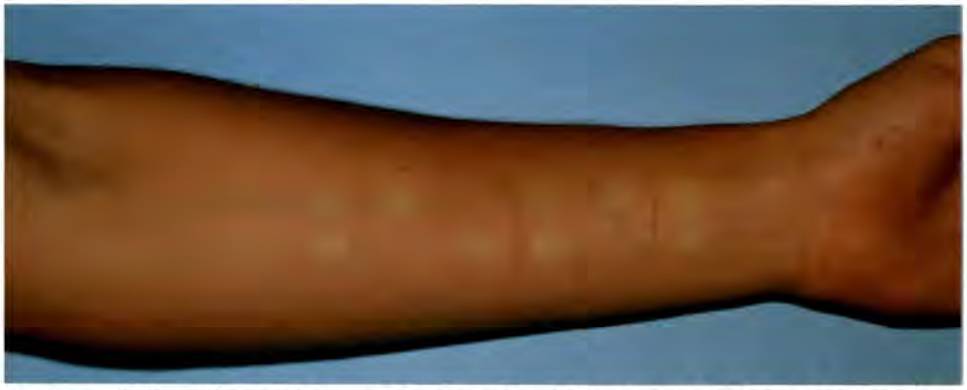


Figure 2.5 Blanching response at 12 hours after application

profiles, AUC values and one or more forms of statistical analysis. The statistical methods that were utilized in the studies presented in this thesis have been previously described (236,244,361,391,437,438).

2.4.1 Calculation of the Percentage Total Possible Score (%TPS)

The %TPS was calculated as follows:

Total possible score (TPS) = B.O.S.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 = (AS/TPS).100

where AS = the actual score attained for the particular preparation.

The %TPS values are used to plot the blanching profiles and to calculate the area under the curve values.

2.4.2 Determination of the Area Under the Curve (AUC) Values

The trapezoidal rule was used to calculate the AUC values. The parameters used to obtain this value were %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

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

where R_1 = response at time_1

R_2 = response at time_2

t = time between R_1 and R_2.

Magnus (438) and Coldman *et al.* (382) 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 small blanching responses usually found at the final stages of the trials, the corrected AUC determination would not be employed in this study. Consequently not all AUC values reflect total blanching to infinite time (302).

2.4.3 Statistical Evaluation of Results

Three methods of statistical analysis (391) were previously used in our laboratories to evaluate the results of blanching studies (244). One of these was an analysis of the number of sites exhibiting blanching, in other words a YES/NO analysis. This method of performing chi-squared analysis on the number of sites that exhibited blanching (YES) and the number of sites at which no blanching could be seen (NO) is, however, an insensitive method as it gives no indication of the degree of observed blanching (361) and is in effect an analysis using a simple 2 point (0 - 1) scoring system. It may be useful in experiments in which corticosteroids eliciting only a weak blanching response are studied (391,439) or in which the minimum effective concentration is being ascertained, and it was therefore decided not to utilize this method of analysis in this study. The methods of statistical analysis that were used are discussed below.

2.4.3.1 Intensity of Blanching (Graded Response Analysis)

In this method of analysis chi-squared values are obtained by comparing the intensity of observed blanching for two preparations by using the 0 - 4 scoring system described previously. Since a 5 point (0 - 4) scoring system is used, a 2 x 5 contingency table is obtained for each of the preparation comparisons. Chi-squared values greater than 9,49 signify real differences based on the 95% level of significance and assuming 4 degrees of freedom (440).

One of the factors to be considered in using this analysis is that the assumption is made that the expected frequencies are not "too small" (441). This vague term has generally been interpreted as meaning that all expected frequencies in the table should be greater than 5 for the chi-squared test to be valid (441,442). Some authors have, however, argued that this rule is unnecessarily restrictive and that chi-squared analysis may be used in cases with expected frequencies in excess of 0,5 in the smallest cell (443-445).

It has been suggested (442,446) that values in adjacent cells be combined in cases where individual cells have an expected value of less than 5, provided that this is appropriate (446). In blanching studies this may be done by, for example, combining the numbers of 3's and 4's observed at a particular reading time for the two preparations being compared. It may be necessary to combine the numbers of 2's, 3's and 4's in cases where blanching is fairly weak. The combining of values in adjacent cells however effectively decreases the sensitivity of the scoring system from a 5 point scale to a 4, 3 or in some cases even a 2 point scale. In experiments assessing the blanching abilities of corticosteroids that elicit a moderately potent or better response, this practice can generally be avoided by employing a sufficient number of subjects and not testing too many different corticosteroid preparations in the same trial. Experimenters will have to weigh the value of this controversial adjustment against a reduction in the sensitivity of the scoring system used.

One of the aspects of the assessment of the blanching assay reported in this thesis necessitated the analysis of the blanching responses of two

preparations on individual volunteers (see section 3.4). This allowed a study of the difference of interpretation of data obtained by combining values in adjacent cells and by analysing the data on a 0 - 5 scoring system. The results of this are discussed in section 3.4.

2.4.3.2 Paired Comparison of Adjacent Application Sites

This method involves a direct comparison of the observed blanching or absence thereof at different application sites. The observers record that one site exhibits more blanching than the other, both sites exhibit equal blanching or that no blanching is present at either site. The procedure of McNemar (440) is employed to obtain chi-squared values. Values of greater than 3,84 signify real differences based on the 95% level of significance and assuming 1 degree of freedom (440).

This method of statistical analysis is more sensitive than the previously described method in that smaller variations of blanching at adjacent sites may be recorded as being different (391). For example the blanching responses exhibited at two adjacent sites may both be allocated a score of 3 on the 5 point scoring system, but one of these sites may be recorded as exhibiting a greater intensity of blanching in the paired comparison. The paired comparison is therefore in effect dividing the 5 point scoring system into as many subdivisions as the observer believes he is able to distinguish when comparing blanching responses at adjacent sites. Some workers do indeed score at half-point intervals (section 2.2.6), but a problem could be encountered if one preparation elicits very slightly more blanching than the other. The situation does arise where the AUC values for two preparations are similar, the graded response analysis indicates that the frequency of scores is similar (statistically non-significant differences), the blanching profiles lie very close together and yet the chi-squared values for the paired comparisons indicate significant differences. This form of statistical analysis should therefore not be used in isolation for the interpretation of results of blanching trials.

It has been suggested that the paired comparison technique is particularly helpful to investigators having limited or infrequent experience with the blanching assay (391), where consistent grading of

scores may pose a problem.

2.4.4 Interpretation of Results

Conclusions from the results of trials performed in this study were drawn after examination of the blanching profiles, AUC values and both forms of statistical analysis. The profiles and AUC values indicate the relative blanching abilities of two or more preparations quite clearly, but do not indicate statistical differences. The blanching profiles also provide an indication of the rate of onset and duration of action of a preparation, as well as indicating the time at which maximum blanching was recorded.

From the statistical point of view it is frequently noted that the results of the two forms of analysis are not congruent. Significant differences were found more often in the comparisons of adjacent sites (see section 2.4.3.2). When, in any particular trial, significant differences were found in the paired comparison analysis, but few or none were found in the graded response analysis, the preparations being compared generally could not be regarded as being statistically significantly different. In cases where significant differences were found in the graded response analysis, the comparisons of adjacent sites invariably also indicates statistically significant differences.

The situation may also arise where the AUC values for two preparations are similar, but the graded response analysis indicates significant differences. This may occur where one of the preparations exhibits more 2's and 3's but fewer 4's than the other preparation. This happens infrequently, but it should be borne in mind that in these cases the presence of statistically significant differences does not automatically allow the conclusion to be drawn that the preparations elicited statistically significant different degrees of blanching.

2.4.5 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 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 (244). This allowed the required data for one trial to be generated by a competent operator in approximately twelve hours. Whilst the introduction of this programme saved a great deal of time, it had two major deficiencies. Firstly, data were generated individually for each time interval which required manual collation of these data and separate computer programmes for the statistical analyses and the calculation of AUC values. Secondly, an error in entering the recorded blanching score or the result of the comparison of two sites caused the operator to have to re-enter all the data for that particular time interval.

A new software package has since been compiled (271) and is currently in use in our laboratories. The time taken to enter the raw data has not been reduced, but the data from each trial is now all stored on separate floppy disks. The calculation of chi-squared and AUC values has been incorporated into the programme and is therefore performed automatically. It is also possible to correct erroneously entered data.

The programme also allows the evaluation of data from individual volunteers and/or observers or any combinations of volunteers and/or observers. It further allows the evaluation of data from the two arms (one usually occluded and the other unoccluded) to be analysed separately or together. Both of these facilities were used for the generation of the data utilized for the assessment of the blanching assay reported in Chapter 3.

CHAPTER 3

ASSESSMENT OF SOME VARIABLES OF THE BLANCHING ASSAY

It is evident from the review in Chapter 2 that even though the blanching assay is extensively used to assess the potencies of topical corticosteroids, there appears to be little consensus on how best to perform the assay. It was decided to investigate certain variables of the assay in an attempt to ascertain the extent to which they may influence the results of comparative bioavailability studies on topical corticosteroids. The variables investigated in this study were not the technical aspects of the assay such as the amount of preparation applied or the duration of application, but mainly the influence of volunteers and observers on comparative bioavailability studies.

The investigation consisted of three identical trials performed at 8 week intervals. Twenty healthy Caucasian subjects were selected from a panel of volunteers known to consistently demonstrate a response to a standard preparation (Betnovate cream), and with the intention that the panel would represent a range of responses (see section 2.3). As one of the objectives of the study was to ascertain whether there is a difference between the blanching responses between males and females, the panel consisted of an equal number of males and females. One female subject withdrew from the investigation and the results of one male subject were therefore discarded. All the results reported were thus obtained from the same 18 volunteers.

It was decided for this investigation to use two proprietary corticosteroid preparations that may be expected to elicit different blanching intensities. There were two reasons for not using only one preparation. It was thought that the observers would be more meticulous when grading the intensity of pallor if they were unsure whether different blanching responses would be elicited at different application sites. The use of two preparations that would possibly elicit different degrees of blanching also allowed the comparison of bioavailabilities in the assessment of the variables studied. Whilst different blanching responses could have been induced by using two preparations each containing a different corticosteroid, it was decided that this would introduce an unnecessary

variable into the study.

The corticosteroid chosen for this study was betamethasone 17-valerate. This corticosteroid has been shown to elicit a blanching response when applied in alcoholic solution (15,96,112,124,128,135,234,327,351,367, 369,370,373,375,378,385,386), extemporaneously prepared cream (101,180, 195,245,270,277,302,305,374,397) and ointment (16,245,296,297,303,304, 408,428) formulations, as well as various proprietary creams (14,68,101, 113,122,138,173,184,194,195,206,207,215,223,228,229,233,246,247,302,305, 309,337,367,371,380-382,387,391,398-400,406,424,429,447), ointments (16, 96,184,204,233,237,246,247,295-297, 303,304,309,343,367,371,377,380-382, 393,398,400,427-430,447) and lotions (243,372,379,429). In several reports in which betamethasone 17-valerate preparations were utilized it is not clear whether these were commercially obtained or extemporaneously prepared (23,205,208,234,430,439), whilst another made no mention of whether the preparations were liquid or semi-solid (448). Blanching studies in which preparations containing betamethasone 17-valerate have been compared to other preparations studied in this thesis are reported in Chapter 5.

The two preparations used in this study were Betnovate cream and Celestoderm-V cream, both containing 0,1% betamethasone (as the 17-valerate). Betnovate cream was chosen as it is used as the standard preparation for initial screening in these and other laboratories (see sections 2.2 and 2.3) and has been utilized in a number of trials performed in our laboratories (122,173,224,271,294,302,371,437,438,447). Celestoderm-V cream was chosen as the second preparation because it contains the same concentration of betamethasone 17-valerate as does Betnovate. The blanching abilities of Celestoderm-V and Betnovate creams have previously been compared in both the occluded and unoccluded application modes. A study on these preparations manufactured in South Africa has shown that the blanching elicited by Celestoderm-V is greater than that elicited by Betnovate when assayed in the occluded mode, whilst the two preparations elicited similar blanching responses when applied without occlusion (244,371,437), although Smith (271) has found that Betnovate elicited more blanching than Celestoderm-V in the unoccluded application mode.

Poulsen and Rorsman (380) have studied the blanching intensities of *Celestona valerat* and *Betnovat* creams, preparations which were purchased in Sweden. These preparations both contain 0,1% betamethasone 17-valerate, and although the names of the manufacturers are not reported, it can most likely be assumed that they are the Swedish equivalents of the South African preparations used in this study. Contrary to the abovementioned study performed in our laboratories (244,371), Poulsen and Rorsman (380) found that *Betnovat* elicited more intense blanching than *Celestona valerat* in both modes of application. The discrepancy between these reports may be as a result of the formulations having been designed for the specific climatic conditions encountered in the two countries.

The creams used in this study were manufactured in South Africa and were purchased from a local pharmacy shortly before the first trial. The methodology of the blanching assay used in this study is described in section 2.3. One of the objectives of the investigation was to assess whether different blanching responses, and more importantly different results in the comparative bioavailabilities of the two preparations tested, were obtained from the results of left or right arms. For this reason the same mode of application was used for both arms in all volunteers. The unoccluded mode of application was chosen as this is more akin to the clinical use of topical corticosteroids. The preparations were applied to a total of 12 sites on each arm of the 18 subjects for 6 hours. Each preparation was therefore applied to six sites on each arm.

In an attempt to standardize conditions, all volunteers were required to remain in the building housing our laboratories from the commencement of each trial until after the 18 hour reading, with the exception of attending lectures, laboratory sessions and meals. All meals were standardized.

The raw data and statistical analyses utilized in this chapter are presented in the appendix at the end of the chapter. All conclusions drawn from chi-squared analyses of the graded responses were based on the 95% level of significance. Four degrees of freedom were assumed in all cases except where the degrees of freedom are indicated by super-scripted numerals in the relevant tables. In these cases the frequencies

of the responses were small and the frequencies of adjacent cells were therefore combined (see section 2.4.3.1). The analyses of the paired comparisons of adjacent sites were all based on the 95% level of significance and assuming 1 degree of freedom.

3.1 Comparison of Celestoderm-V Cream with Betnovate Cream (tables 3.1.1-3.1.6)

The comparative blanching responses of Celestoderm-V cream and Betnovate cream were assessed for each of the three trials. The blanching profiles depicted in figure 3.1.1 represent the pooled results recorded by the three observers for both arms of all 18 volunteers. It can be seen from these profiles that Celestoderm-V elicited a more intense blanching response than Betnovate in all three trials. Statistical analyses concurred with this, with non-significant differences found only in trial 2 at 28 hours in the graded response analysis and at 32 hours in the analysis of the comparison of adjacent sites. The magnitudes of the chi-squared values and differences in the AUC values indicate that the largest differences between Celestoderm-V and Betnovate occurred in trials 1 and 3.

The AUC values (see appendix) for Celestoderm-V were almost identical for trials 1 and 2, with trial 3 being slightly lower at 92% of the values of trials 1 and 2. There was greater variation in the case of Betnovate, with trial 2 giving the highest value and trial 3 giving the lowest value; the lowest value being 86% of the highest. Maximum blanching was observed for both preparations at 12 hours after application in all three trials. The %TPS values obtained at the 12 hour and 14 hour readings in trial 3 were similar; the peak blanching response may therefore have occurred at 13 hours after application, a time at which responses were not measured. It should be noted that in our laboratories points on the blanching curves are joined by straight lines; computer assisted smoothing of curves is not employed.

The blanching responses elicited by each preparation over the three trials were also compared statistically. Although the AUC values for Betnovate were similar for trials 1 and 3, statistically significant differences in the graded response analyses were found between all three

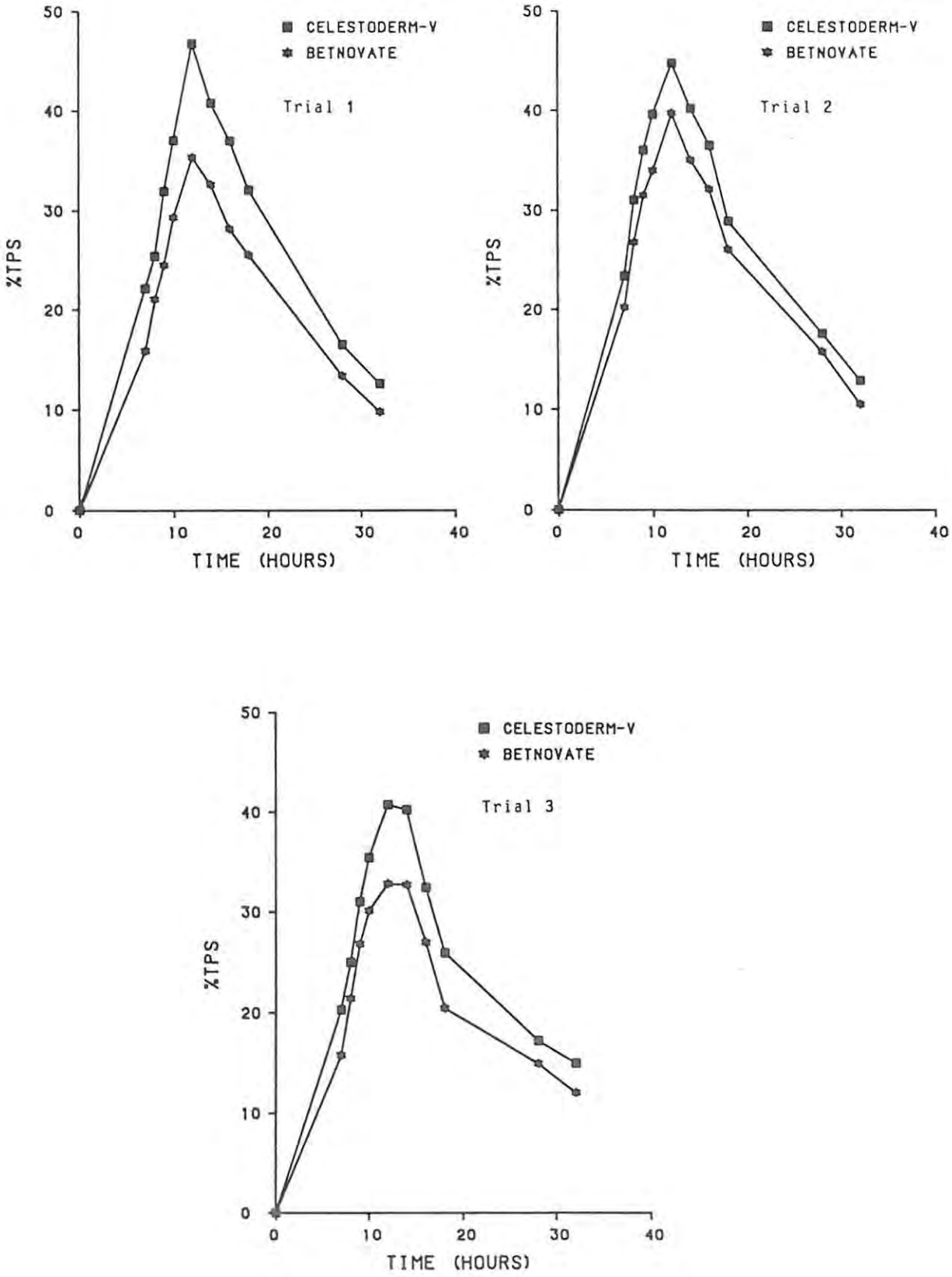


Figure 3.1.1 Blanching profiles of Celestoderm-V cream and Betnovate cream : all observers, all volunteers

trials at most readings. Statistical analysis of the graded responses of Celestoderm-V also indicated significant differences in the blanching responses of this preparation between the three trials. It should be borne in mind that the same two tubes of cream were used over the three trials and the same observers and volunteers were employed for each trial. Possible reasons for the difference in blanching responses could have been different ambient conditions (temperature and relative humidity), inconsistencies of observers, or inconsistent reactions of the volunteers to the corticosteroids over the three trial periods. Each of these possibilities is discussed in the following sections of this chapter.

3.2 Influence of Environmental Conditions on the Blanching Response (tables 3.1.3,3.2.1)

The increased blanching response elicited by corticosteroids when applied under occlusion was discussed in section 1.3.2.2, and the choice of whether the application sites should be occluded or left unoccluded was discussed in section 2.2.2. It can be seen from section 1.3.2.2 that the enhanced blanching is most likely due to increased hydration of the stratum corneum, whilst the influence of the alteration of temperature due to occlusion is unclear. There only appears to be one report in the literature concerning the influence of ambient temperature and relative humidity on the blanching response in the unoccluded mode of application. Burdick (397) commented that examination of data regarding ambient temperatures, season and humidity gave no indication as to the causes of variations observed in a series of 23 trials. Only two reports appear to have discussed the influence of environmental conditions on blanching responses after occluded application. McKenzie (133) has reported that whilst blanching was generally confined to the site of application, in some subjects, particularly in hot humid weather, confluent blanching was found after application of the corticosteroid under occlusion. Zaun and Altmeyer (431) noted more intense placebo and corticosteroid-induced blanching in volunteers who perspired heavily during the occluded application period. It was decided in this study to attempt to ascertain whether there was any correlation between environmental conditions and the blanching responses observed during the three trials reported in this chapter.

Under normal conditions the stratum corneum receives moisture from the fluids which bathe the lower layers of the skin, as well as from sweat glands when they are active (449). However, over major portions of the body the sweat glands are active only above 30°C (449). It has been found in *in vitro* studies that at the relative humidities studied (from 23% to 88% at a temperature of 23°C) water readily diffused through the dermis to the epidermis, but not in sufficient quantities to keep pace with the rate of evaporation that was allowed to occur under the conditions of the experiment (449). However, it was further found that the stratum corneum absorbed water from the environment as the relative humidity increased (449). The water content of the stratum corneum therefore appears to be influenced to a greater extent by sweat glands and the water content of the surrounding air than by diffusion through the stratum corneum, except at relative humidities below 60%, when the stratum corneum becomes increasingly dependent on water diffusion from the surrounding tissue.

The two aspects of the environment that may influence the blanching response are temperature and relative humidity. These are both measured on a continuous basis by the Hydrological Research Unit at Rhodes University and the data utilized for figure 3.2.1 were supplied by them. Whilst the potential disadvantage of not measuring environmental conditions in the laboratories was identified during the planning of this series of trials, it was felt that as this aspect of the study of variables of the blanching assay was intended to be a preliminary investigation, the measurement of outdoor conditions was appropriate. Doors and windows are generally left open in our laboratories and ambient conditions inside and outside are similar. Each trial has, for the purposes of this study, been divided into two separate phases, namely the application period consisting of the first six hours of the trial and the observation period consisting of the remainder of the trial.

Temperatures of less than 60°C have been found not to influence the permeability of the stratum corneum to topical corticosteroids (section 1.3.2.2). The temperature during the first phase of the trial would therefore not influence the permeability of the stratum corneum *per se*. It could, however, influence the degree of hydration of the stratum corneum, as an increase in temperature may increase the hydration of the

stratum corneum due to a possible increase in activity of the sweat glands. If increased temperature did increase hydration due to sweating, this could be expected to play a more significant role if an occlusive dressing was used. These trials were, however, performed without any occlusion.

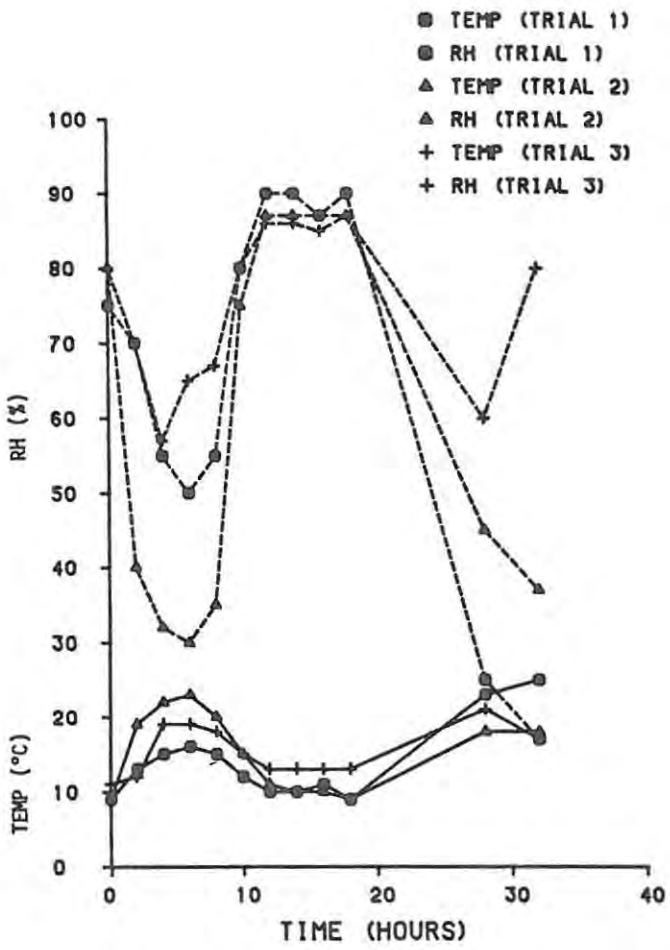


Figure 3.2.1 Temperature and relative humidity during trials 1, 2 and 3

The ambient relative humidity during the application phase of the trial may also influence hydration of the stratum corneum and consequently the percutaneous absorption of the corticosteroid molecule. Relative humidity would almost certainly have more of an affect on hydration of the stratum corneum if the test areas were left unoccluded. It is possible that if more rapid percutaneous absorption occurred due to more active sweat glands or higher relative humidity, the corticosteroid could be cleared from the site of action more rapidly. This would lead to a lower degree of blanching on the hottest or most humid day and *vice versa*.

Ambient temperature during the observation stage of the trial may have an effect on blanching due to temperature dependent changes in surface vasculature brought about by the necessity of the body to maintain a constant core temperature. An increase in ambient temperature leads to vasodilation, whilst a decrease in ambient temperature leads to vasoconstriction (450). These autonomic nervous functions may alter the subjective comparison between the site of blanching and the surrounding untreated skin, although this would most likely not influence results of comparative blanching studies.

Ambient relative humidity during the observation phase of the trial could be expected to have less influence on the blanching response than during the application phase. An elevated relative humidity may continue to facilitate the permeation of corticosteroid molecules already in the stratum corneum, especially in the initial stages of the observation phase. Conversely relative humidities low enough to dehydrate the stratum corneum may retard the migration of corticosteroid molecules present in the stratum corneum after the residual corticosteroid preparation has been removed from the arm. The blanching profile may therefore be sharper in the case of naturally hydrated as opposed to dehydrated stratum corneum, but not as sharp as when the trial is performed in the occluded application mode, where the stratum corneum would probably be hydrated to a larger extent than in the case of unoccluded application under conditions of high ambient relative humidity.

It can be seen from the curves in figure 3.2.1 that the temperature during the application phase on the day of trial 2 was higher than on the day of trial 3, which was higher than on the day of trial 1. If temperature influenced percutaneous absorption due to increased hydration, one might expect the degree of observed blanching for each preparation to follow the same rank order as the temperature. It can be seen from the curves in figure 3.2.1 that temperature differences during the observation phases of the trials were inconsistent over the three trials.

The relative humidity was lowest during trial 2 from 2 - 10 hours after the commencement of the trial, whilst the relative humidity recorded during trial 1 was lower than during trial 3 from 6 - 8 hours after the commencement of the trials with similar humidity having been recorded up

to 6 hours after application. Similar degrees of relative humidity were recorded during all three trials from 10 - 18 hours after the commencement of the trials.

The degree of blanching observed for Betnovate was greater during trial 2 than during trial 1 which was greater than during trial 3, although the difference between trials 1 and 3 was small. In terms of AUC values, the degree of blanching observed for Celestoderm-V was almost identical during trials 1 and 2, and was lowest during trial 3. A comparison of the rank order of blanching between the three trials was neither the same nor opposite to the rank order of the environmental conditions, except to an extent in the case of the opposite rank order of blanching of Betnovate and the relative humidity during the application phase and the initial stages of observation. The observation of lowest relative humidity, highest temperature (during the application phase) and highest degree of blanching however only held true for Betnovate during trial 2.

With respect to the shapes of the blanching profiles, those constructed for both preparations from the results of trial 2 are marginally sharper than trial 1 which are sharper than trial 3, which is opposite to the rank order of relative humidity during the application and initial observation phase. However the difference in relative humidity during this phase between trials 1 and 3 were only notably different at the 6 and 8 hour recordings, and in addition the largest difference in relative humidity was between trial 2 and the other two trials whilst the largest difference in the shapes of the blanching profiles was between trial 3 and the other two trials.

It would therefore appear from the above that environmental conditions could possibly have some influence on the blanching response. However, the lack of correlation in the blanching responses and environmental conditions discussed above does not indicate any direct relationship. It is possible that larger differences in environmental conditions may influence blanching, but this is difficult to measure. Firstly, the trials would have to be conducted under conditions where extremes in temperature and relative humidity could be artificially induced. Secondly, it would have to be assumed that similar blanching would always be obtained in a particular panel of volunteers under the same environmental condi-

tions, if one were to conclude that the different conditions were causing the different blanching responses. As different blanching responses were noted in these three trials and the differences were probably not attributable to environmental conditions, it would possibly be fallacious to assume that similar blanching would always be obtained in a particular panel of volunteers under the same environmental conditions.

3.3 Observer-Dependent Variations in Blanching Responses (tables 3.3.1-3.3.8)

Whilst the methodology of the human skin blanching assay is generally well accepted, the subjective nature of visual assessment of the intensity of pallor has been criticized by some researchers. Various methods of non-visual assessment of vasoconstriction were discussed in section 2.2.7 and it was concluded that in experiments performed to ascertain the relative blanching responses of topically applied corticosteroids, the objective instrumental methods offer no advantage over the subjective methods (427). Subjective assessment should, however, be performed with care, and training of observers is essential (179).

In most reports, observations of pallor appear to be made by the researchers themselves, although there have been reports in which some observations were made by the volunteers (96,237). Gruvstad and Bengtsson (96) correlated the readings made by the volunteers with those of the investigator and found that there was a tendency for the volunteers to assign lower scores than the investigator. It was found that these differences did not, however, influence the results of the investigation. The authors suggest that this tendency of the volunteers to assign lower scores may have been due to the fact that the investigator was more trained to estimate pallor. Burdick (397) has however reported that inexperienced readers almost always over-read, whilst with experienced readers the tendency to over-read is greater when only a few areas of faint blanching are present. He has further pointed out that the most common sources of error for the inexperienced reader are vasomotor mottling, scars and irregularities in pigmentation. A comparison of AUC values obtained in our laboratories from scores assigned by an inexperienced observer and the combined scores of three experienced observers indicated that the inexperienced observer assigned lower scores than the

pooled results of the experienced observers (451). This assessment was performed for two trials and it was found that the differences between the AUC values calculated from the scores recorded by the individual observer were smaller than the pooled AUC values calculated from the scores recorded by the experienced observers in the second trial. It was further found that the blanching profiles plotted from the results obtained by the inexperienced observer in his second trial were smoother than for the first trial. It was also found that the rank order of the AUC values of the different preparations was the same in the second trial for the inexperienced observer and the pooled results of the experienced observers, which was not the case in the first trial. Preliminary results therefore indicate that the experience gained by the above observer in two trials was sufficient to include him as the third experienced observer in subsequent trials. This training procedure may, however, take somewhat longer in laboratories where observations are not made at several reading times over an extended period.

There does not appear to be any comment in the literature on how many observers should be employed in blanching trials. Reports have included the use of one (96,124,136,140,184,233,237,247,314,384), two (93,95,112, 172,224,337,343,381,382,385,386,389,397,399,401,402,404), three (122, 236,295-297,304,369,376,377,383,447), four (15) and five (15) observers. Some of these reports (295-297,304,377,382) have mentioned that the results of individual observers were sufficiently similar to allow the results to be pooled, but no further details were given, whilst other authors have reported using the averaged readings of two or three observers to analyse the data (236,383,391,399). Another system that has been reported is the employment of two observers to independently assess whether blanching was absent or present, but only those sites at which both observers noted blanching were recorded as positive, whilst the sites at which only one or neither observer noted blanching were recorded as negative (401). It should be noted that whilst "pooling" or "averaging" the results recorded by more than one observer will not influence the calculation of %TPS and AUC values, the terms pooling and averaging are not synonymous from the point of view of chi-squared analyses. The calculation of chi-squared values utilizes the actual frequencies recorded, as well as the total number of observations made. The chi-squared values obtained from pooled results and averaged results

will therefore not be the same in most cases.

In order to ascertain to what extent the variation in blanching responses recorded for each preparation was a function of the observers, the profiles of each preparation were plotted for individual observers for trials 1, 2 and 3. At the time of conducting this series of three trials, observer 1 had been making observations in blanching assays for approximately 5 years, and observers 2 and 3 had been making observations in blanching assays for approximately 10 years. It can be seen from the profiles in figure 3.3.1 that in trial 1, observer 1 recorded a greater degree of blanching for Celestoderm-V at 12, 16 and 18 hours after application, when compared to trials 2 and 3, whilst at the 28 and 32 hour readings the responses were lower in trial 1 than trials 2 and 3. The remainder of the responses were generally fairly similar. The responses recorded by observer 1 for Betnovate cream appeared to be less consistent, with cross-overs occurring at several places in the blanching profiles. The AUC values obtained from the readings of this observer indicate that Celestoderm-V elicited a greater degree of blanching in trial 1 than in trial 2, and a greater degree of blanching in trial 2 than in trial 3. The AUC values obtained for Betnovate from the results of this observer indicate a greater degree of blanching in trial 2 than in trials 1 and 3. Similar AUC values were obtained in trials 1 and 3.

The graded responses assigned to both preparations by each observer were analysed statistically to ascertain whether the differences for each preparation were statistically significant between trials. Chi-squared analyses indicate significant differences at several readings recorded by observer 1 when the scores for Celestoderm-V are compared between the three trials. The significant differences were, however, scattered over the readings and seldom occurred at consecutive reading times. It is therefore not possible to conclude with certainty that blanching elicited by Celestoderm-V was significantly different over the three trials, as recorded by observer 1. The superior blanching elicited by Betnovate in trial 2 was significantly greater than in trial 1 from 8 - 12 hours, and than in trial 3 from 8 - 10 hours. This does indicate that a significantly greater degree of blanching was noted by observer 1 for Betnovate in trial 2 over the peak blanching times.

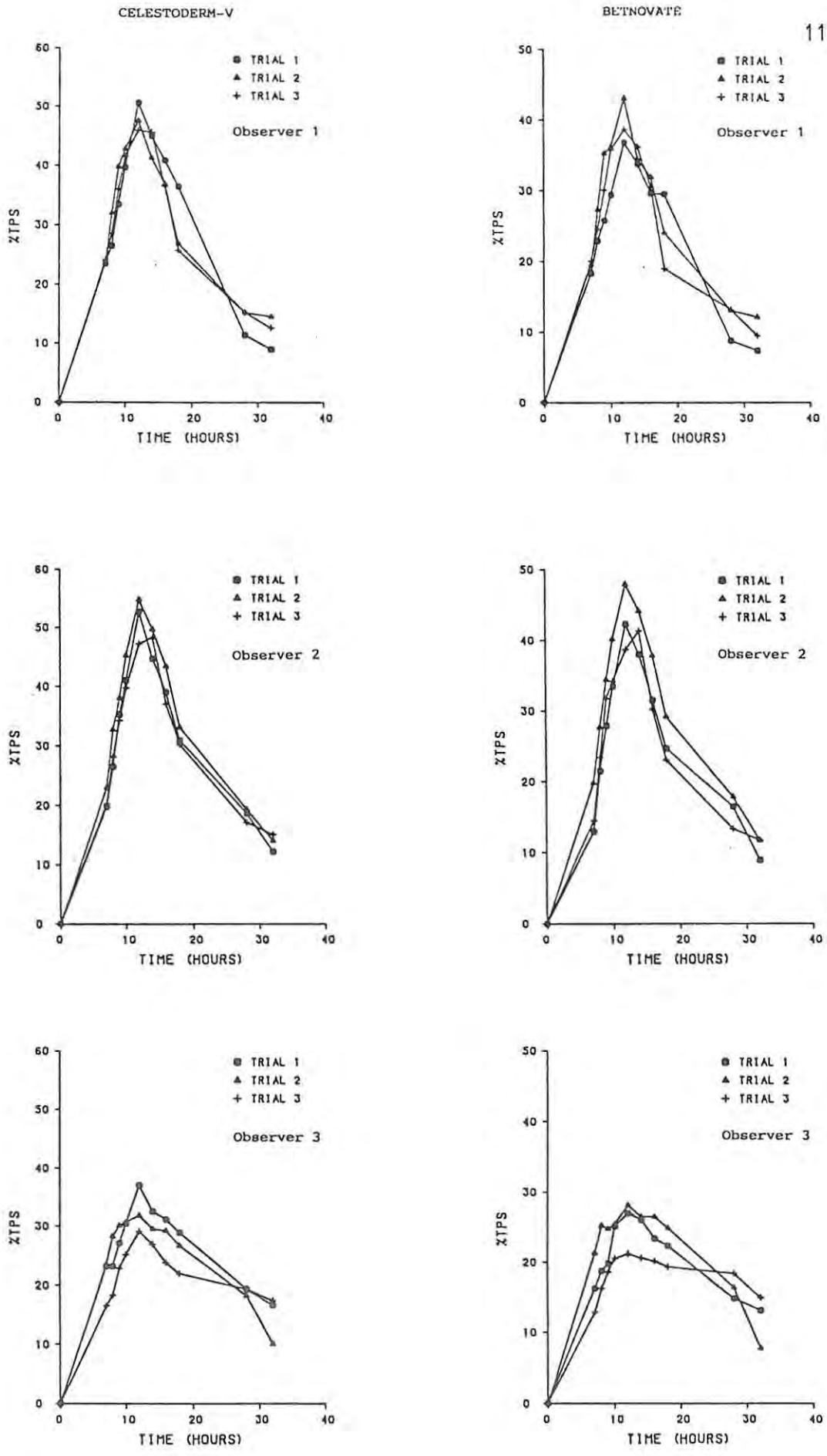


Figure 3.3.1 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents one preparation and one observer over three trials

A study of the profiles of blanching depicted in figure 3.3.1 indicates that observer 2 noted the highest degree of blanching by Celestoderm-V in trial 2. The maximum blanching responses in trials 1 and 2 were noted earlier than in trial 3. Cross-overs can be seen at three points in the profiles for trials 1 and 3. The AUC values indicate that blanching was greatest for Celestoderm-V in trial 2, and larger for trial 1 than trial 3. The shapes of the profiles for Betnovate were similar to those for Celestoderm-V except that the difference in the peak responses between trials 1 and 2 was greater for Betnovate than for Celestoderm-V. The results obtained from observer 2 also indicate that Betnovate exhibited a longer duration of action in trial 2 than in trials 1 and 3. The AUC values indicate that, as in the case of Celestoderm-V, this observer noted the greatest degree of blanching for Betnovate in trial 2, and similar blanching in trials 1 and 3.

Some significant differences were found for observer 2 in the comparison of Celestoderm-V over the three trials. It can be concluded from the statistical analyses that the blanching response of Celestoderm-V was significantly greater in trial 2 than in trial 3, from 8 - 16 hours after application, and in trial 2 compared to trial 1 from 8 - 16 hours after application, with the exception of the 12 hour (peak) reading. The blanching response of Betnovate was statistically significantly greater in trial 2 than in trial 1 from 7 - 18 hours after application, and in trial 2 than in trial 3 at most of the readings.

The degree of blanching recorded by observer 3 for both preparations in all three trials was substantially lower than for observers 1 and 2. The highest blanching recorded by observer 3 for Celestoderm-V occurred in trial 1, followed by trials 2 and 3, respectively. This is evident from the AUC values and the blanching profiles. A cross-over of the profiles occurred between trials 1 and 2 close to the beginning of the trials, and a much less intense decline can be seen in the curve for trial 3 than for trials 1 and 2. The profiles constructed from the scores assigned to Betnovate by observer 3 indicate that similar blanching was noted at the 10, 12 and 14 hour readings in trials 1 and 2. More blanching was, however recorded during trial 2 at all the readings other than the 32 hour reading. The shape of the blanching profile constructed for Betnovate in trial 3 was quite different to any of the other pro-

files depicted in figure 3.3.1. The normal increase in blanching was seen from 7 - 12 hours after application, after which a plateau (with only a very slight decrease) was observed until 28 hours, followed by a decline to the 32 hour reading. This is most likely due to the low blanching noted by observer 3 in this trial. It is worth noting that although observer 3 had been making observations in blanching trials for approximately 10 years, he had not been involved in blanching trials for approximately 2 years prior to this series of trials. This may account for the low blanching scores and differently shaped profiles obtained from the results of this observer. Observers 1 and 2 had, on the other hand, been involved in several trials each year prior to this study.

Few statistical differences were noted in the responses recorded by observer 3 for Celestoderm-V in the comparison between trials 1 and 2. The blanching was, however, significantly lower in trial 3 than in trials 1 and 2 at all but two readings in each of the comparisons. The comparison of the blanching elicited by Betnovate was similar in trials 1 and 2. The blanching elicited in trial 3 was statistically significantly lower than in trial 1 from 12 - 28 hours after application, and lower in trial 3 than in trial 2 at all reading times, except 28 hours after application.

The variations in blanching responses recorded by the three observers in each trial are depicted graphically in figure 3.3.2. It can be seen that the highest blanching scores were assigned by observer 2 at most readings in all three trials, whilst the lowest blanching scores were recorded by observer 3 at most of the readings in all three trials. The scores recorded by observer 1 lay between those of observers 2 and 3, but were generally closer to those recorded by observer 2. Statistical analyses of the graded responses confirm that the differences were significant at the majority of reading times. It is interesting that although the AUC values obtained by observers 1 and 2 for Celestoderm-V were similar (especially in trials 1 and 3), and the profiles indicate that the %TPS values are similar, the differences are nonetheless statistically significant.

It would be unreasonable to expect three observers scoring independently to assign the same scores to the blanching response elicited by a prepa-

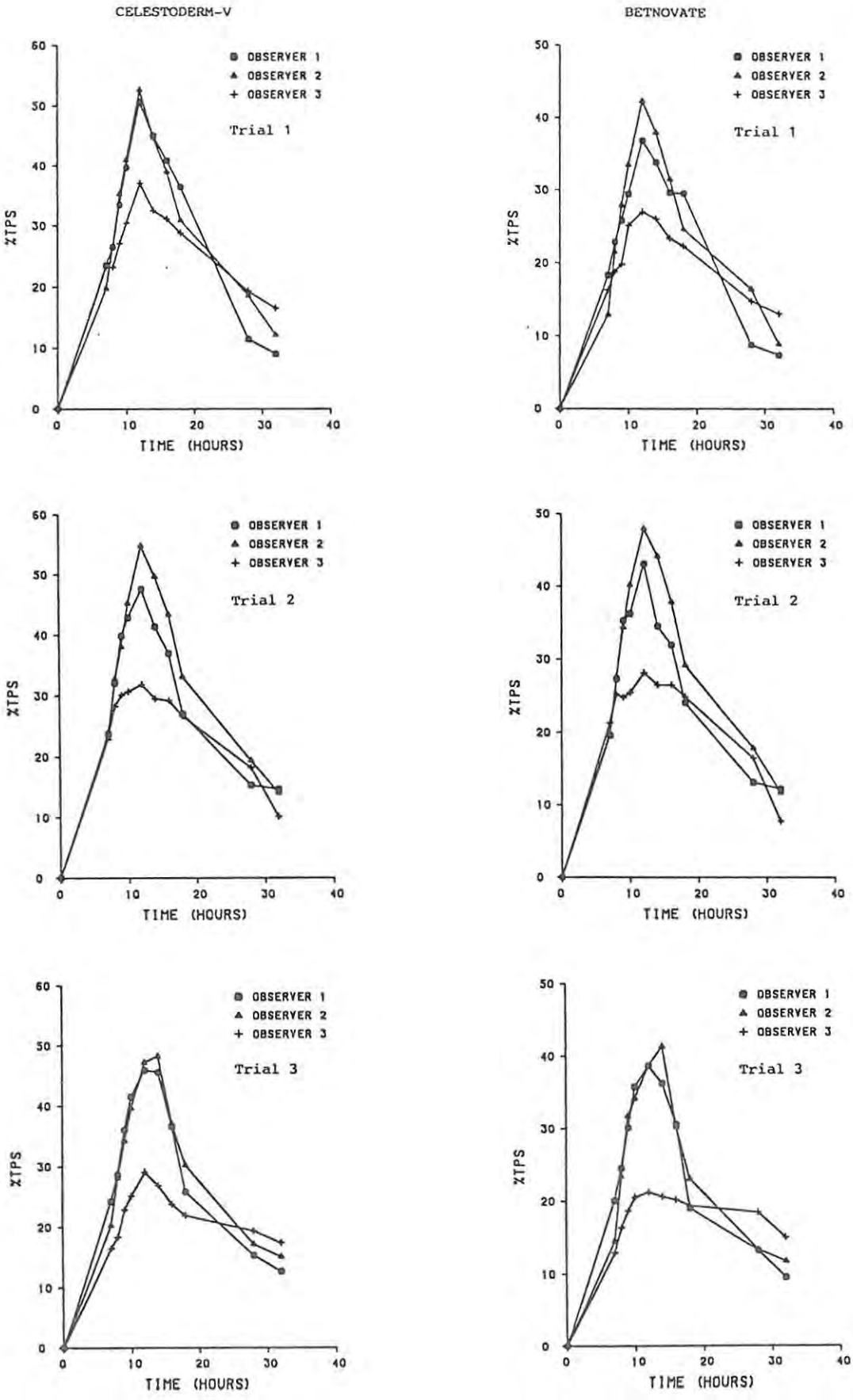


Figure 3.3.2 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents one preparation and three observers for each trial

ration in any single trial. It may, however, be reasonable to expect the rank order of the responses of individual preparations between three trials to be consistent for two or more observers. It can be seen from table 3.1 that, based on AUC values and graded response analyses, this was not the case in this series of three trials. It can also be seen from the table that all three scorers recorded the lowest degree of blanching for Celestoderm-V in trial 3 and the highest degree of blanching for Betnovate in trial 2.

Table 3.1 SUMMARY OF THE COMPARISON OF THE BLANCHING RESPONSES OF EACH PREPARATION BETWEEN TRIALS individual observers, all volunteers

	Celestoderm-V	Betnovate
Observer 1	Trial 1 > Trial 2 > Trial 3	Trial 2 > Trial 3 ≡ Trial 1
Observer 2	Trial 2 > Trial 1 > Trial 3	Trial 2 > Trial 1 > Trial 3
Observer 3	Trial 1 > Trial 2 > Trial 3	Trial 2 > Trial 1 > Trial 3

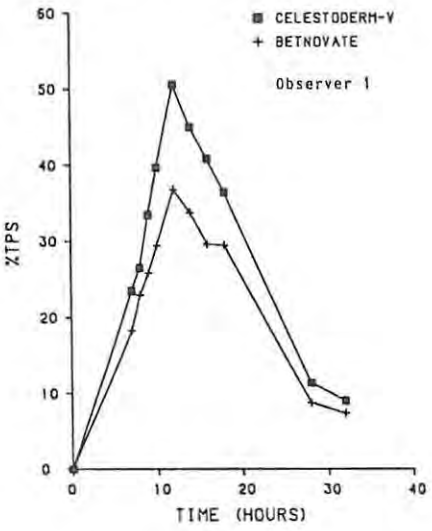
The inconsistent rank order seen in table 3.1 may have contributed to the smaller difference between Celestoderm-V and Betnovate in trial 2 compared to trials 1 and 3 (section 3.1). A further contributing factor could have been inconsistent blanching responses in the volunteers; this is discussed in section 3.4. The lack of consistency noted in table 3.1 may be a cause for concern, but a much more important practical consideration is whether the results of comparative bioavailability between the two preparations were the same for individual observers as those drawn from the pooled scores of the three observers. This was therefore investigated.

It is evident from the blanching profiles in figure 3.3.3 that the same rank order of Celestoderm-V and Betnovate was obtained from the results of each observer, and that this rank order was the same as that obtained from the pooled results.

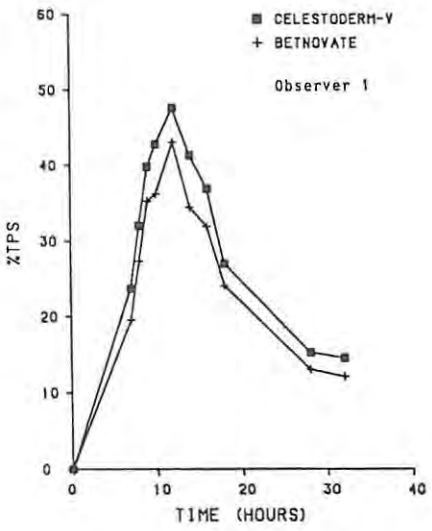
Statistical analyses of the results for trial 1 show that significant differences in favour of Celestoderm-V were found by each of the observers at most readings. In trial 2, however, few statistically significant differences were found when the graded response results recorded by the individual observers were analysed separately. One significant dif-

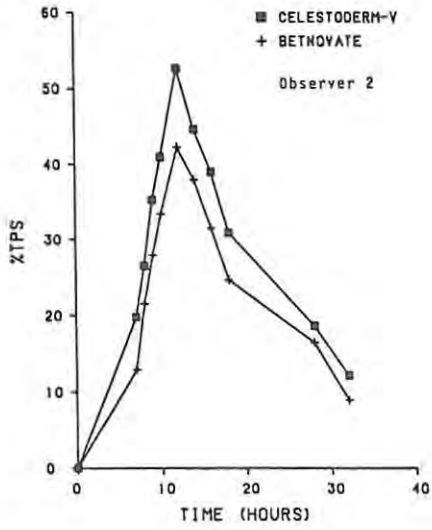
Figure 3.3.3 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for one observer and one trial (9 sets of profiles)

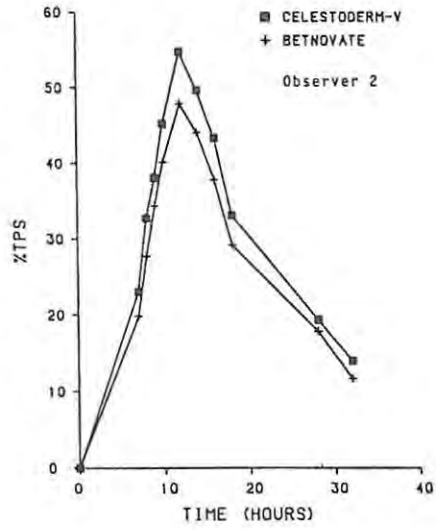
TRIAL 1

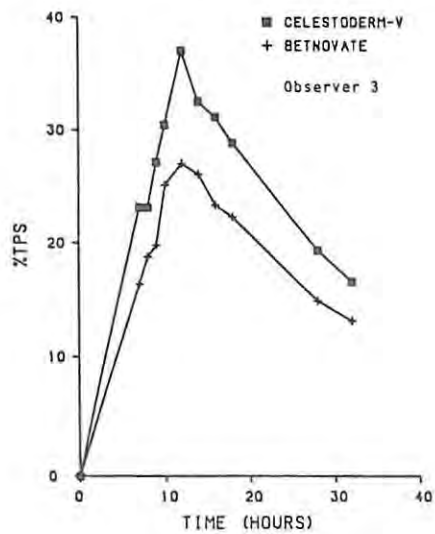


TRIAL 2









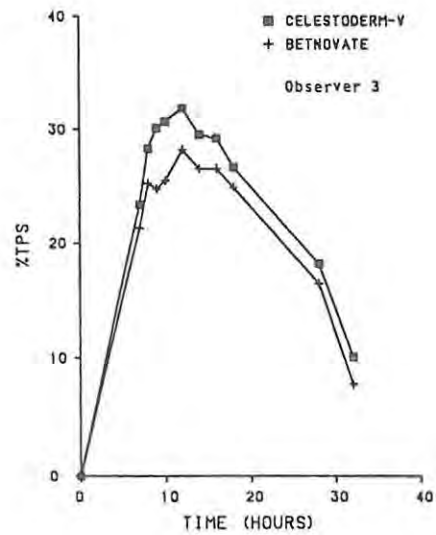
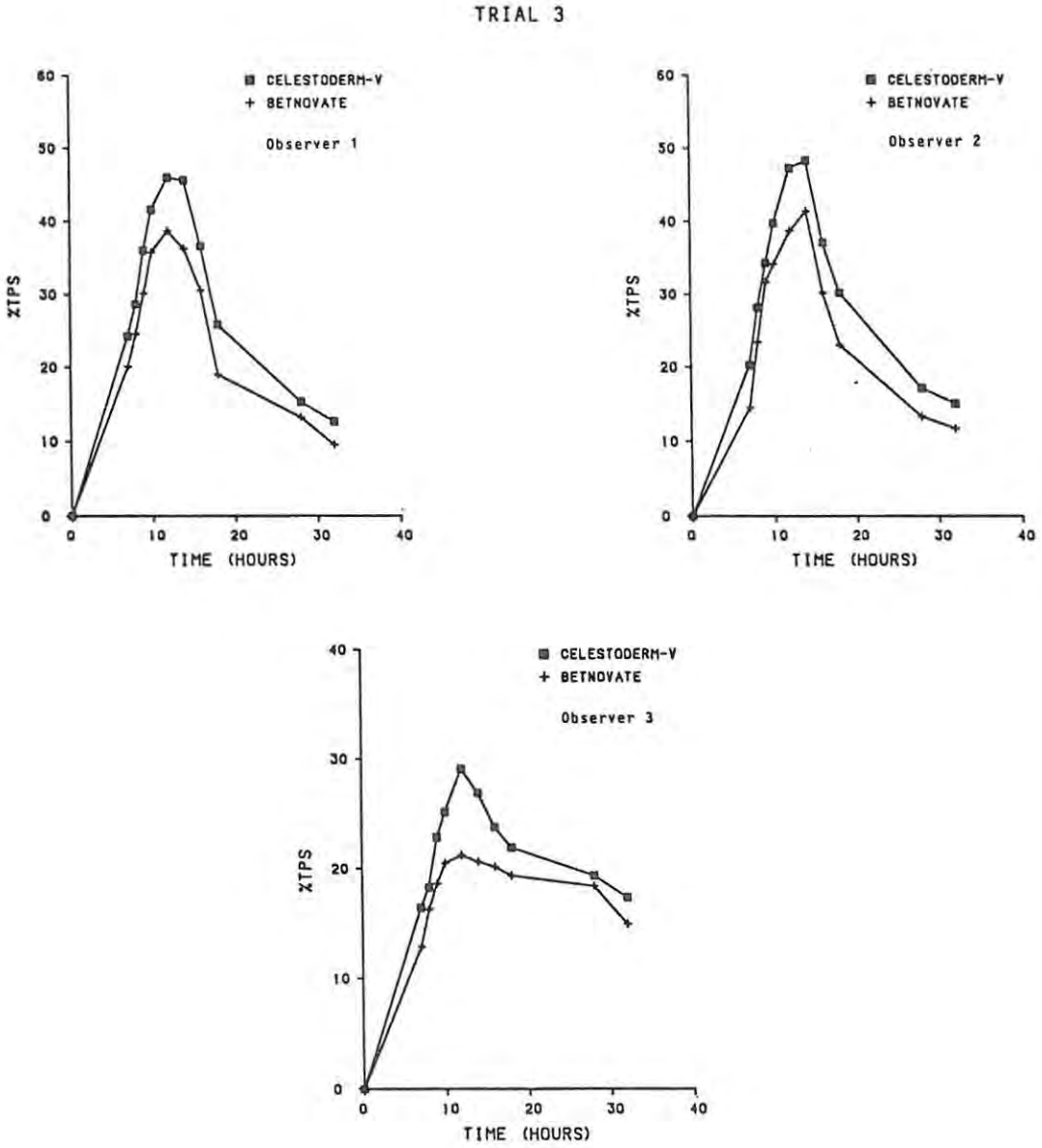


Figure 3.3.3 (continued)



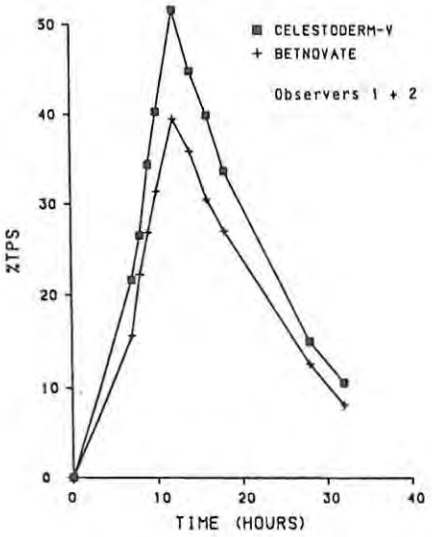
ference was found in the results from observer 1 (14 hours), four in the results from observer 2 (12 - 18 hours) and five in the results from observer 3 (9, 10, 14, 16 and 18 hours). Significant differences were found in the results of the comparisons of adjacent sites by all three observers at most readings. In trial 3, significant differences were found in the graded response analysis by observer 1 at 14, 16 and 18 hours, by observer 2 at all except the 9, 28 and 32 hour readings, and by observer 3 from 9 - 16 hours after application. Significant differences were found in the analysis of the paired comparisons of all three observers at most readings.

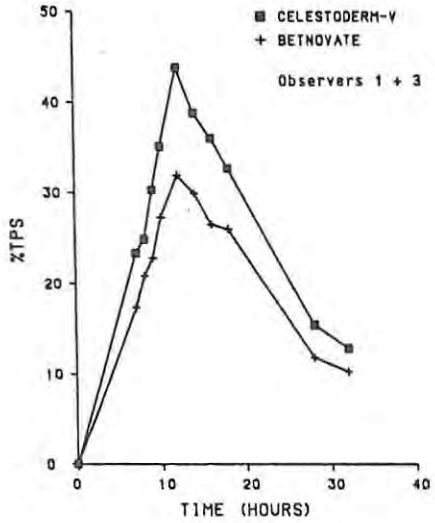
It is evident from the above that the blanching response elicited by Celestoderm-V in trial 1 was found by all three observers to be statistically significantly superior to that elicited by Betnovate. It was discussed in section 2.4.4 that conclusions should be drawn with caution in cases where results of the two forms of statistical analysis are not congruent. Examples of this are seen in the results obtained from the analysis of the results of individual observers in trials 2 and 3. Whilst significant differences were found in the comparisons of adjacent sites, the situation was less clear in the analysis of the graded responses. Unequivocal superiority of Celestoderm-V over Betnovate could not have been concluded from the results of observer 1 in trial 2 or trial 3. In the results obtained by observer 2 in trial 2, Celestoderm-V was significantly superior to Betnovate from 12 - 18 hours after application, whilst in trial 3 the blanching elicited by Celestoderm-V could be regarded as superior to that elicited by Betnovate, as non-significant differences were found only at 9, 28 and 32 hours after application in the graded response analysis and at 28 hours in the analysis of the comparisons of adjacent sites. A similar situation occurred for observer 3 where in trial 2, Celestoderm-V elicited a statistically superior response to Betnovate from 14 - 18 hours after application, whilst in trial 3 significant differences were found from 9 - 16 hours after application. If the assumption is accepted that the conclusions drawn from the pooled observations of the three observers reported in section 3.1 can be used as an accurate standard, then it appears as though results obtained by one observer may not always be reliable in terms of statistically significant differences, although the same rank order of the two preparations was obtained by all three observers in each trial. It was therefore decided to ascertain whether pooling the results recorded by two observers would provide the same general conclusions that were obtained from the pooled results of three observers.

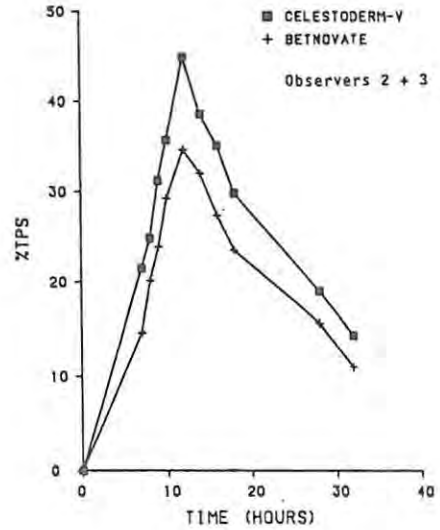
The scores obtained by the individual observers were pooled in the following way: observer 1 + observer 2; observer 1 + observer 3; observer 2 + observer 3. The shapes of the blanching profiles (figure 3.3.4) obtained from these combinations were generally similar to those obtained from the results of individual observers and from the pooled results of all three observers.

Figure 3.3.4 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for combinations of two observers (9 sets of profiles)

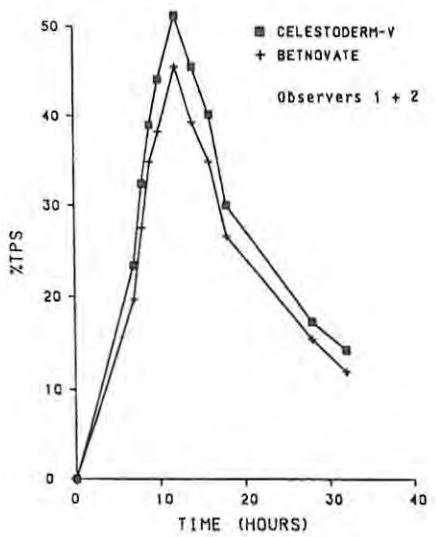
TRIAL 1

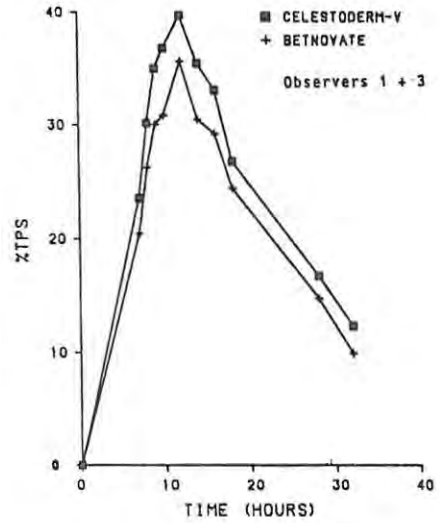






TRIAL 2





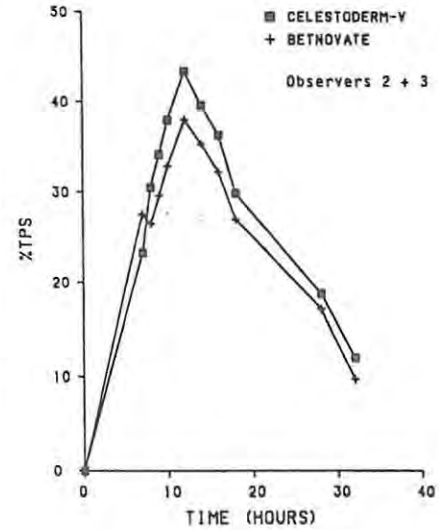
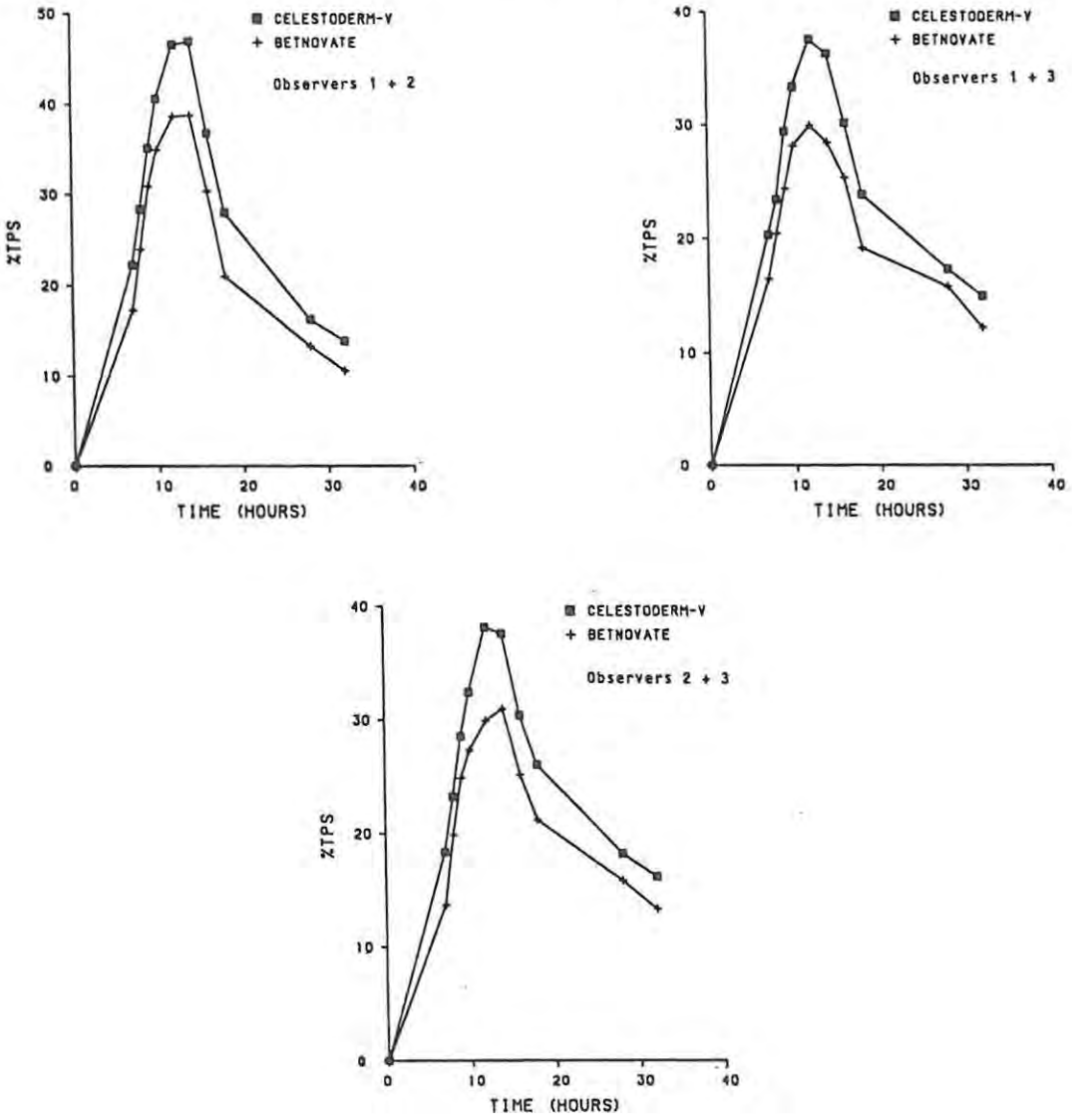


Figure 3.3.4 (continued)

TRIAL 3



Statistical analyses indicate that, as expected, significant differences between Celestoderm-V and Betnovate were found in both forms of analysis for all three combinations of observers in trial 1. It was further found that statistically significant differences were found at most readings in trials 2 and 3 when the recorded blanching responses were pooled for two observers.

In the graded response analysis of the combined results of observers 1 and 2, non-significant differences were found only at the 9, 28 and 32 hour readings in trial 2, whilst in trial 3 significant differences were found at all readings. In the analysis of the combined observations of

observer 2 and 3, non-significant differences were found at the 7, 28 and 32 hour readings in trial 2, and at the 28 hour reading in trial 3. Fewer significant differences were, however, found in the combined results of observers 1 and 3. In trial 2 significant differences were found at the 10, 14, 16 and 18 hour readings, whilst in trial 3 significant differences were found at all except the 8 and 28 hour readings. Very few non-significant differences were found in the analysis of the paired comparisons of adjacent sites. Not more than one non-significant difference was found in any of the combinations of observers in any of the trials. Those non-significant differences that were found occurred at either the 28 hour or 32 hour readings. It can therefore be concluded that similar results of comparative bioavailability were noted from the pooled results of any two or all three observers.

An important practical consideration that arises from this is the number of observers required to produce a reliable conclusion in a comparative bioavailability study. From a statistical point of view, sufficient data will be generated by one observer if a sufficient number of volunteers are employed. It would appear from the results presented above that one observer would be sufficient to produce reliable results in cases where the blanching responses elicited by the different preparations are either almost identical or very different. In cases where statistically significant differences are not noted throughout the trial and where the chi-squared values are close to the cut-off point, the conclusion drawn from the results of one observer may be different to that drawn from the pooled results of two or more observers. As it is not possible to know this before the trial, it is recommended that a minimum of two observers be employed in the human skin blanching assay.

3.4 Volunteer-Dependent Variations in Blanching Responses (tables 3.4.1-3.4.19)

It is well accepted in the natural, medical and pharmaceutical sciences that, due to inherent biological variability, repeated measurements of the same response do not necessarily produce the same results (452,453). In blanching studies the application of corticosteroids to human skin does not induce pallor in all individuals (135,250). Wallace *et al.* (404) reported that 53% of their 84 subjects responded to the applica-

tion of 2,5% hydrocortisone ointment. It is further known that sensitivity to topical corticosteroids, and the resultant blanching response in sensitive subjects, differs between volunteers. This also applies to the percutaneous penetration of these drugs (454). Several observations regarding interpersonal and intrapersonal variations of percutaneous absorption and the blanching response have appeared in the literature.

Studies measuring the urinary excretion of radiolabelled hydrocortisone have shown variations between subjects after the administration of the drug to intact (6,168,178) and stripped (168) skin. Variations were also found within individual subjects over a range of experiments (168). It was noted that the intrapersonal variation over four experiments was not as great as the interpersonal variation, indicating that there are marked differences between individuals in the efficiency and character of the skin barrier (168). It is known that the thickness of the stratum corneum from the same region of the body of different subjects differs, with the number of cell layers ranging from 12 to 30 (161). Exposure to UV rays from the sun has been found to thicken the horny layer, particularly in darkly pigmented persons, and this increases the barrier properties of the skin (161). It has further been noted that, in general, fair skinned, blue-eyed Celtic persons have thinner horny layers and thus more permeable skin (161).

Interpersonal variability in the blanching response has been observed by several researchers in this field. The mean blanching response of Betnovate cream in ten trials performed over a 3,5 year period has been reported to vary between individuals, with the lowest score being 68% of the highest score (384). Feather *et al.* (427) have also reported different blanching responses between individuals. It is interesting to note that deviations of individual responses from the mean responses for each of the preparations tested appeared to be smaller for the more potent preparations than for the weaker preparations, although this was not a definite trend. It was further found that the coefficient of variation was reduced with an increase in the number of volunteers. Repetitions of a blanching assay, performed with several corticosteroids to establish the minimum effective concentrations required to induce pallor, indicated that different results were obtained in different subjects (112). It has also been reported that variability in activity of sweat glands

from one subject to another was found to lead to large variations in blanching responses when occlusion was used with ointments, creams and lotions (247).

Variations in the time taken to exhibit peak blanching responses have also been reported. Whilst the peak blanching response is usually noted between 9 and 14 hours after application of the corticosteroid, peak times of between 24 and 32 hours have been reported in isolated cases (384,397). In one of these experiments (397) the rate of onset of action to the response of intradermal corticosteroid was the same in all subjects, as was the duration of action, indicating that the abovementioned delayed response was due to different barrier properties of apparently normal strata corneum. Other reports have mentioned that blanching was different between individuals, but did not give further details (68,135,237).

The apparent lack of statistical differences between preparations in some trials has been partly attributed to the variation between volunteers; with most preparations the total score at all time periods attained by the most sensitive subject was approximately twice that produced by the least sensitive subject (68).

It would be interesting to ascertain to what extent the abovementioned variations in the blanching response are due to percutaneous penetration or individual sensitivity to the pharmacologic response of the corticosteroids. A study of this would require simultaneous observation of the blanching response and quantitative measurement of the amount of corticosteroid that has penetrated the stratum corneum and reached the epidermis or dermis.

It was established in section 3.2 that environmental conditions probably had very little influence on the blanching responses observed in the three trials reported in this chapter. In section 3.3 it was established that the observers did not record the same rank order of blanching for each preparation between trials, although the rank order between the preparations was consistent for all three observers over the three trials. Whilst this may have been partly responsible for the smaller difference between the two preparations in trial 2, another contributing

factor may have been inconsistencies within each volunteer over the three trials.

The blanching responses exhibited by each volunteer were calculated from the pooled scores of the three observers. The AUC values for each preparation were obtained from these results to establish in which of the three trials each of the preparations exhibited the greater degree of blanching, in an attempt to explain the smaller difference between Celestoderm-V and Betnovate in trial 2. A summary of the comparisons of the blanching elicited by each preparation in the three trials (in terms of AUC values) is presented in table 3.2. It can be seen from the table that Celestoderm-V elicited the greatest degree of blanching in trial 2 in six volunteers, and the second greatest degree of blanching in trial 2 in another six volunteers. Betnovate, on the other hand, elicited the greatest degree of blanching in trial 2 in nine volunteers and the second greatest degree of blanching in trial 2 in five other volunteers. Celestoderm-V therefore elicited the greatest or second greatest degree of blanching in 12 volunteers, whilst Betnovate elicited the greatest or second greatest degree of blanching in 14 volunteers. Although this indicates that Betnovate elicited a greater degree of blanching more often than Celestoderm-V in trial 2, it does not provide conclusive evidence that this was the major cause of the smaller difference between the two preparations in this trial.

In the discussion of the results recorded by individual observers (section 3.3), the graded responses assigned to both preparations by each observer were analysed to ascertain whether the differences for each preparation were statistically significant between trials. A similar analysis was performed for this section on the results observed in individual volunteers. The pooled results of the three observers were used to determine the significant differences in blanching responses for each preparation in each volunteer between the three trials to ascertain whether, for example, Celestoderm-V elicited a greater degree of blanching in volunteer 1 during trial 1 compared with trial 2 compared with trial 3, *etc*. A study of the overall analyses indicates that differences were found between the trials at approximately half the observation times. The largest number of statistically significant differences was found in the comparison between trials 1 and 2, followed by trials 2 and

Table 3.2 SUMMARY OF THE COMPARISON OF THE BLANCHING RESPONSES (IN TERMS OF AUC VALUES) OF EACH PREPARATION BETWEEN TRIALS individual volunteers, all observers

Volunteer	Celestoderm-V Trial numbers	Betnovate Trial numbers
1	2 > 3 > 1	2 > 1 > 3
2	1 > 3 > 2	2 > 3 > 1
3	1 > 3 > 2	1 > 3 > 2
4	3 > 2 > 1	3 > 2 > 1
5	1 > 2 > 3	3 > 2 > 1
6	2 > 1 > 3	2 > 3 > 1
7	2 > 3 > 1	2 > 3 > 1
8	2 = 3 > 1	2 > 3 > 1
9	2 > 3 > 1	2 > 3 > 1
10	3 > 1 > 2	1 > 3 > 2
11	3 > 2 > 1	2 > 3 > 1
12	1 > 2 > 3	1 > 2 > 3
13	1 > 2 > 3	1 > 2 > 3
14	1 > 3 > 2	2 > 1 > 3
15	1 > 3 > 2	1 > 3 > 2
16	1 > 3 > 2	3 > 1 > 2
17	2 > 1 > 3	2 > 3 > 1
18	1 > 2 > 3	1 > 2 > 3

3, with the smallest number of statistically significant differences found in the comparison between trials 1 and 3. It was further found that there were fewer differences in the comparison of Betnovate over the three trials than in the comparison of Celestoderm-V over the three trials. Little can be gleaned from these analyses as to why the difference between the two preparations tested was smaller in trial 2 than in trials 1 and 3. It therefore appears as though this phenomenon is due to a combination of the observers not recording the same rank order between trials (section 3.3) and the volunteers reacting differently to the corticosteroids between trials, although the reasons for the intrapersonal variability are elusive.

It is interesting to note from the %TPS values and blanching profiles in figure 3.4.1 that in some volunteers the maximum blanching responses exhibited for any one of the preparations in some trials was almost twice that exhibited for the same preparation in the same volunteer in other

trials. (It should be noted that the maximum values on the y-axes are not the same in all cases, but have been expanded or contracted to accommodate the various maximum %TPS values. It is felt that amplification of the curves in which weak blanching responses are depicted allows a clearer picture of the blanching responses over the duration of the trials.) Personal details and a medical history were obtained from each volunteer before the commencement of the study, and all volunteers were asked before each trial whether anything in their medical status had changed. Three of the volunteers (13, 14 and 18) were taking an oral contraceptive during the trials, but the shapes of the blanching profiles constructed for these volunteers were normal. The female volunteers were required to provide details of their menstrual cycles; only volunteer 17 had an irregular cycle. The blanching exhibited by this volunteer was generally lower than most of the other volunteers, although it was not consistently the lowest of the panel. It is naturally not possible to draw any conclusions from this with respect to the influence of the menstrual cycle on the blanching response. It would be interesting to perform trials with half of the volunteers using an oral contraceptive, as well as trials with half of the volunteers having normal menstrual cycles and half presenting with irregular cycles or other menstrual/hormonal irregularities. This may provide information on whether fluctuations in circulating female hormone levels influence the vascular tone and reactivity of blood vessels to topically applied corticosteroids, and may further provide guidelines as to whether female volunteers should be excluded from blanching panels unless they have normal menstrual cycles.

The female volunteers were not involved in sporting activities, and were regarded as being unfit. It was therefore not possible to make any inferences relating to the possible influence of physical fitness on the blanching responses. In the group of male volunteers, all but one (volunteer 2) partook in regular strenuous exercise and were therefore physically fit. This also therefore did not allow comparisons of fitness within the group of male volunteers. A study of the personal details and medical histories did not allow a pattern to be identified for those volunteers who showed relatively consistent blanching between trials, nor for those who showed very different blanching responses between trials. A comparison of the blanching responses between males and fe-

Figure 3.4.1 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents one preparation and one volunteer over three trials (36 sets of profiles)

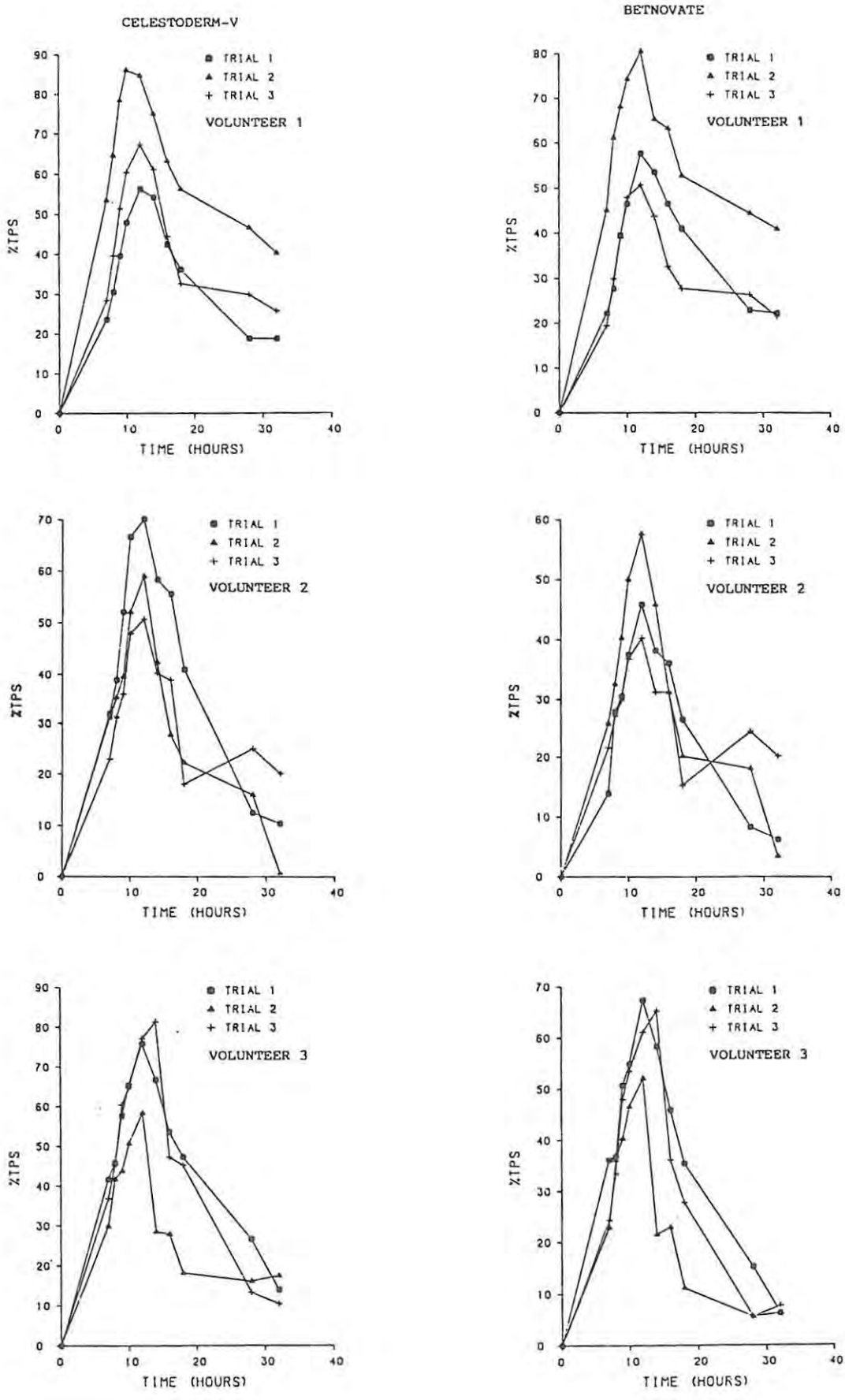
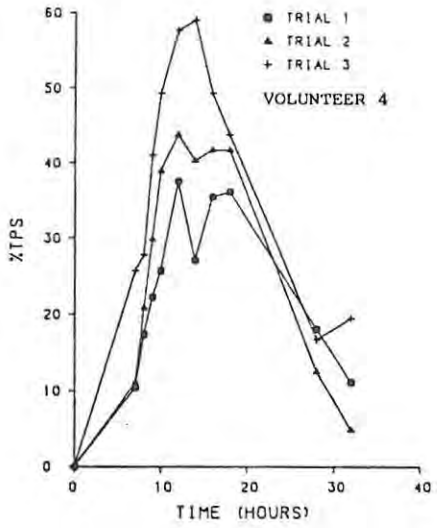
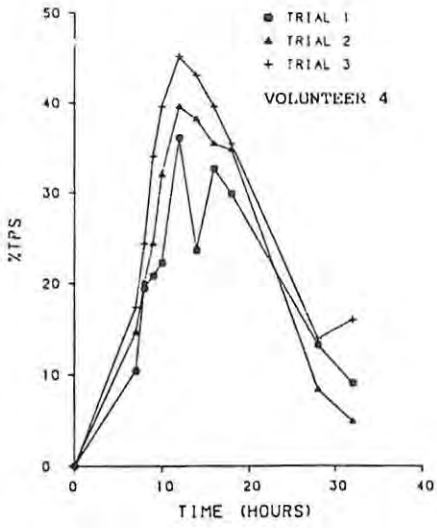


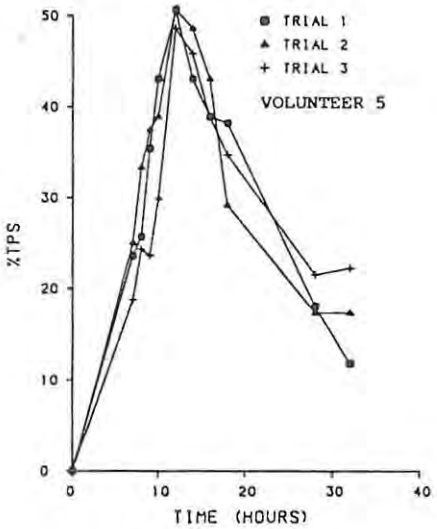
Figure 3.4.1 (continued)

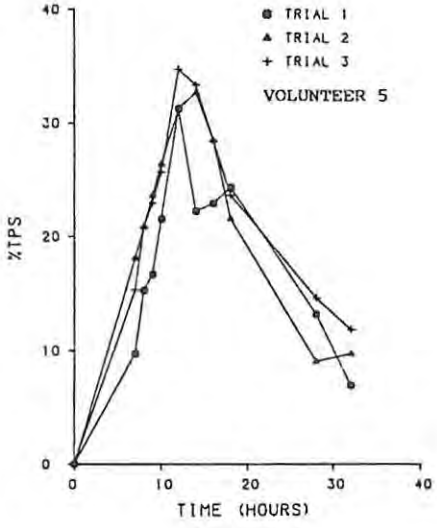
CELESTODERM-V

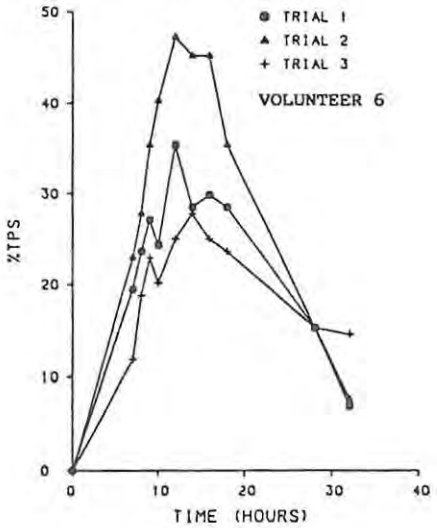


BETNOVATE









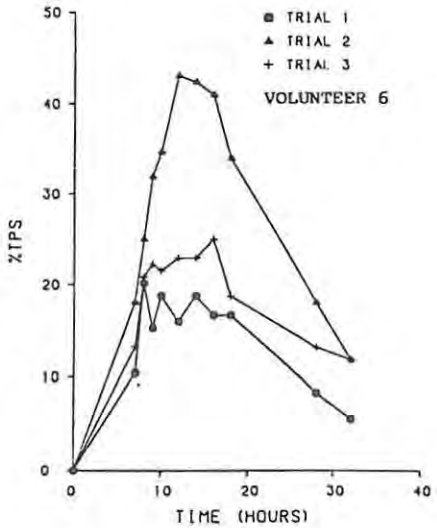
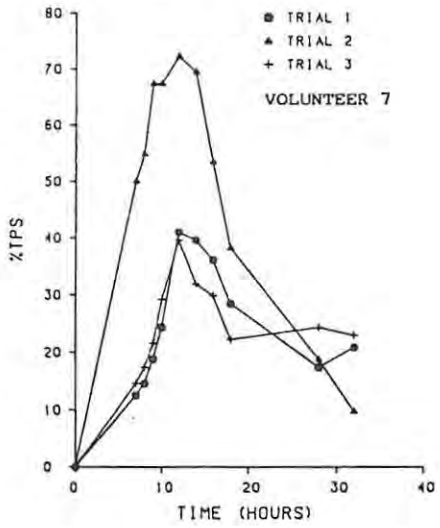
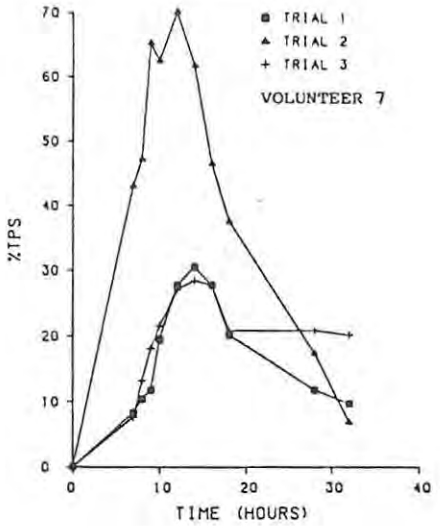


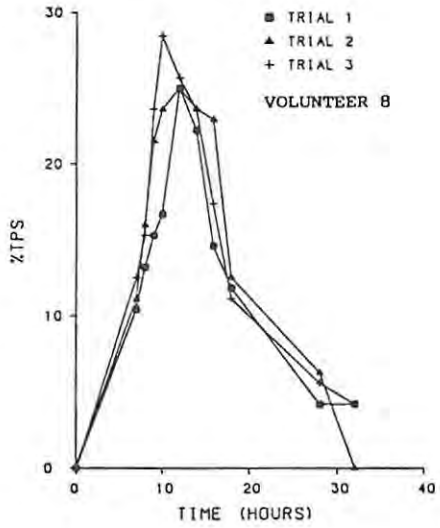
Figure 3.4.1 (continued)

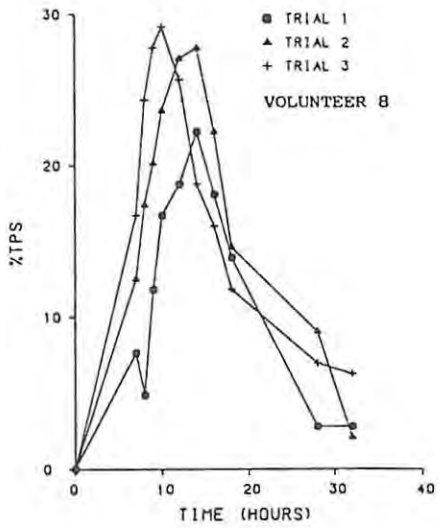
CELESTODERM-V

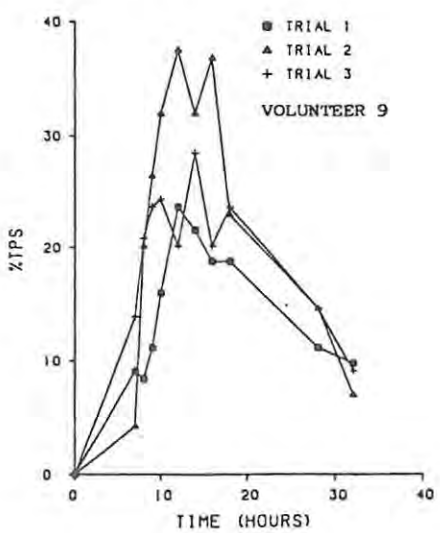


BETNOVATE









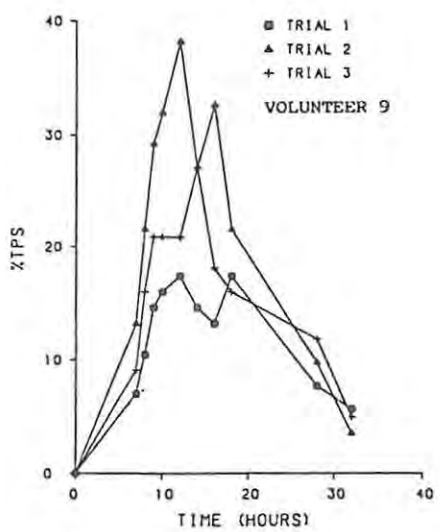
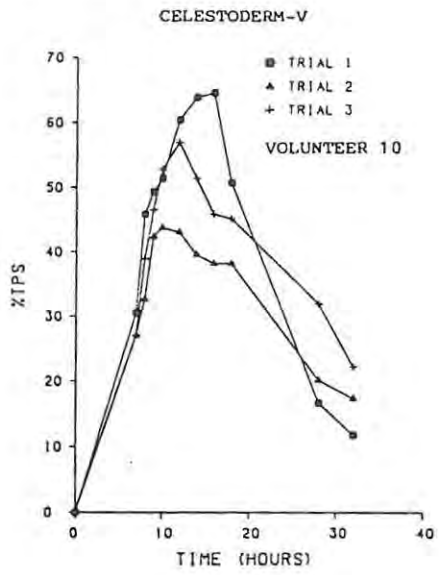
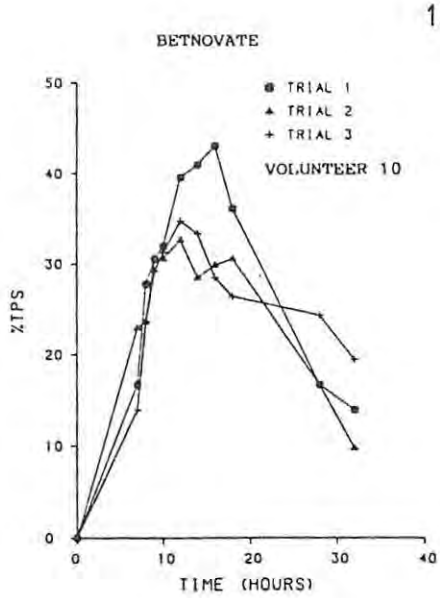
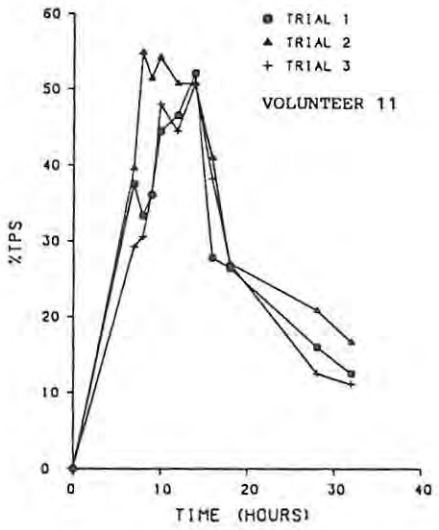
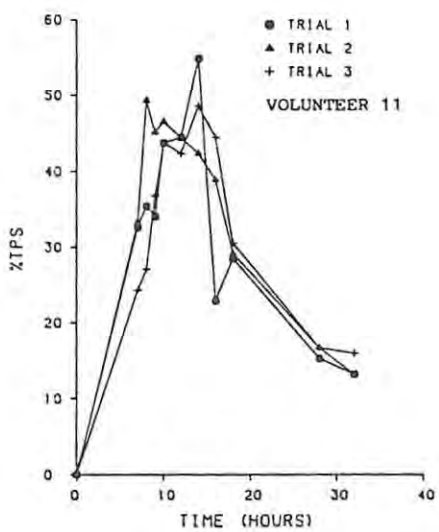


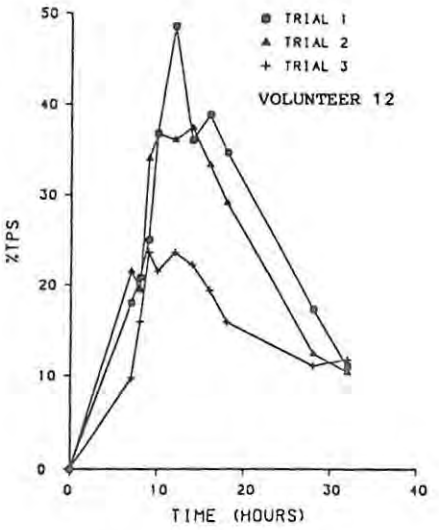
Figure 3.4.1 (continued)











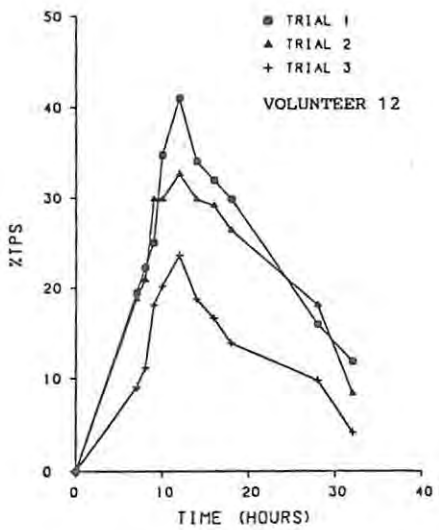
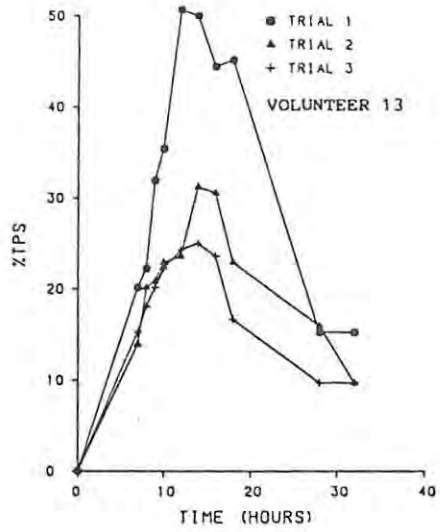
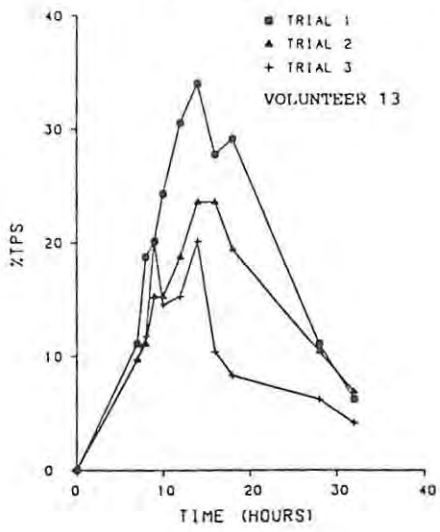


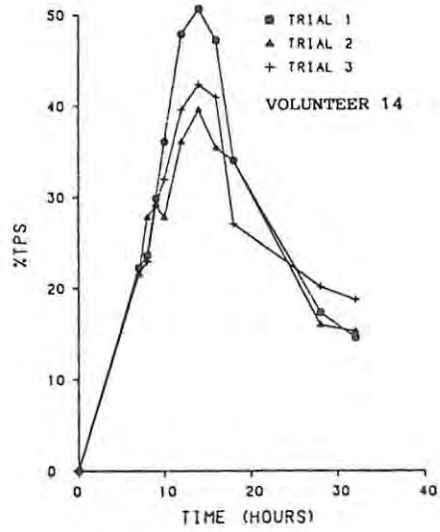
Figure 3.4.1 (continued)

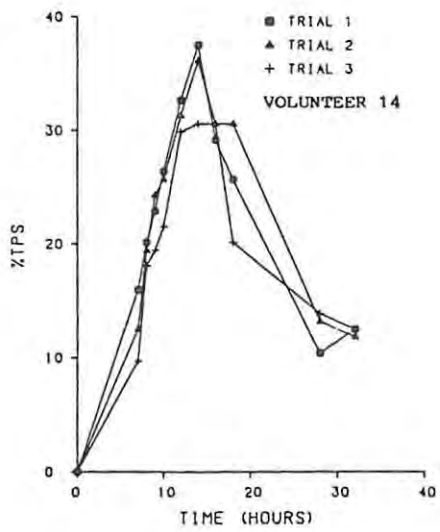
CELESTODERM-V

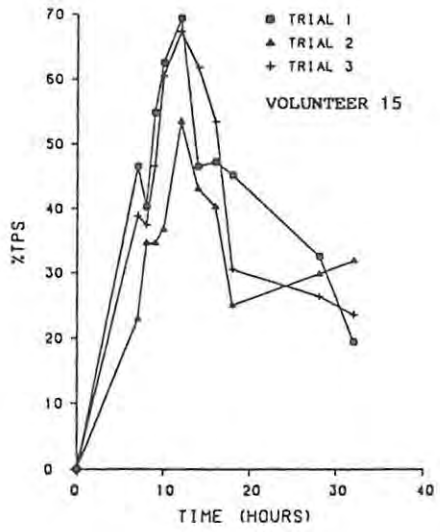


BETNOVATE









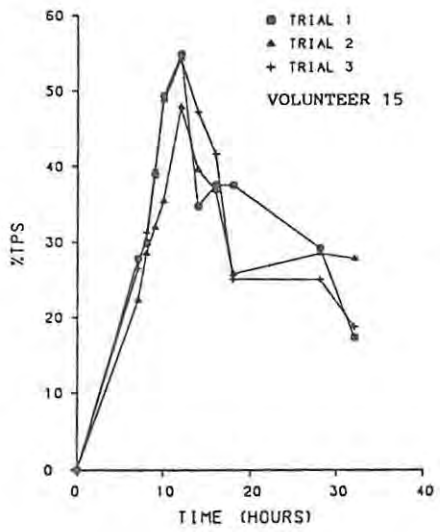
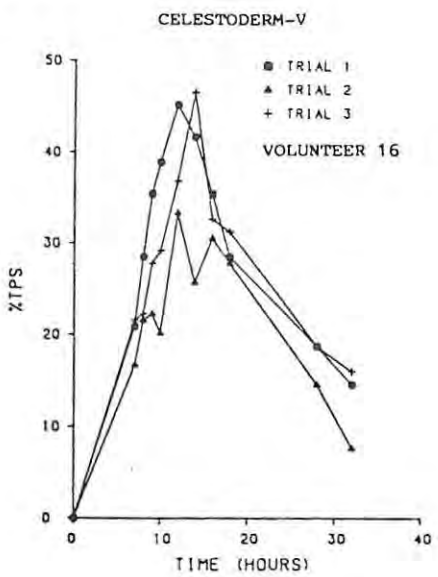
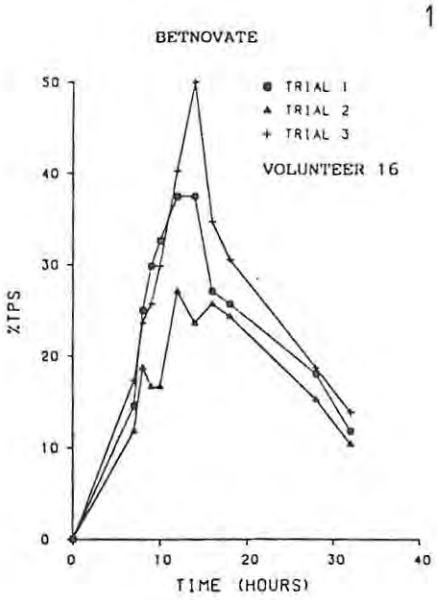
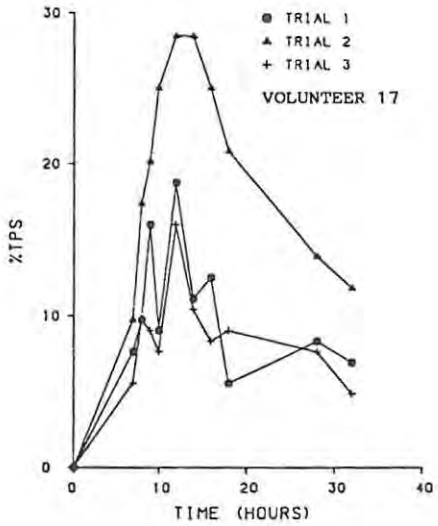
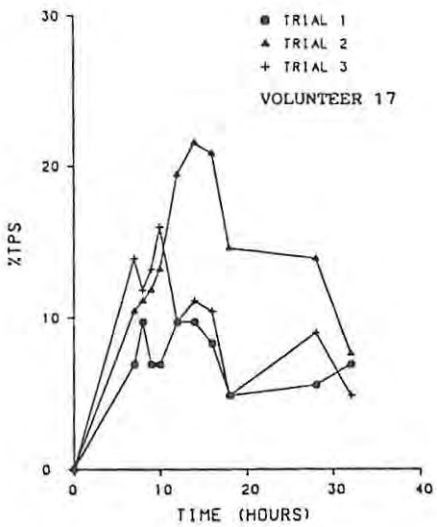


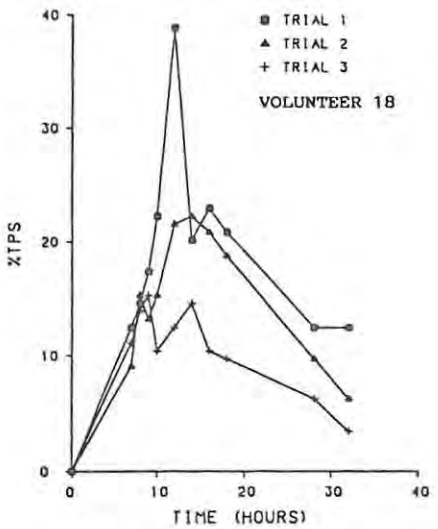
Figure 3.4.1 (continued)

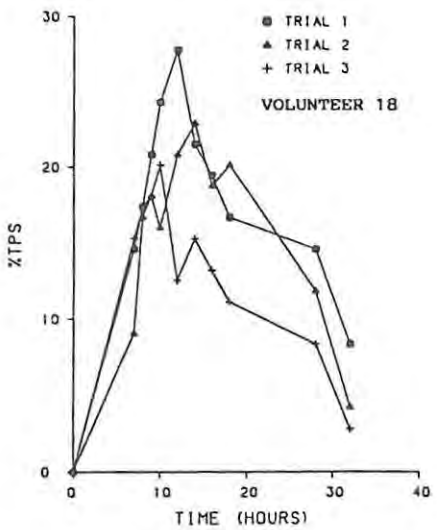












males is discussed in section 3.5.

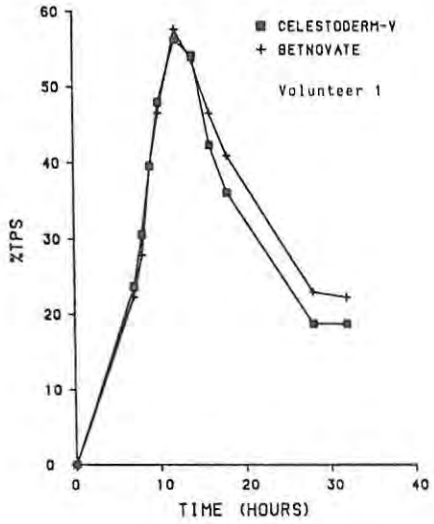
The comparative blanching abilities of Celestoderm-V and Betnovate were analysed in each trial for each volunteer to ascertain whether the results of comparative bioavailability were consistent. The blanching profiles are presented in figures 3.4.2a (trial 1), 3.4.2b (trial 2) and 3.4.2c (trial 3).

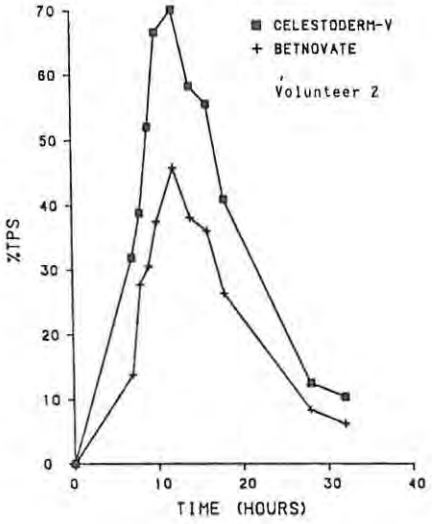
A summary of whether differences occurred between the blanching elicited by Betnovate and Celestoderm-V in individual volunteers is presented in table 3.3. Decisions were made by studying the chi-squared values, AUC values and blanching profiles for each volunteer. It can be seen from table 3.3 that in trials 1 and 3 the number of volunteers in whom different or similar results were found were approximately the same. In trial 2, however, the blanching responses of the two preparations were similar in 15 of the 18 volunteers. It should be noted that the criteria used for drawing the conclusions were not applied in precisely the same way as for the pooled results. For example, volunteer 6 in trial 3 would have been reported as exhibiting similar blanching activities for both preparations, but mention would have been made of the longer duration of action of Celestoderm-V compared to Betnovate. Celestoderm-V in volunteer 9 in trial 3 would normally have been reported as exhibiting significantly different blanching to Betnovate in the latter part of the

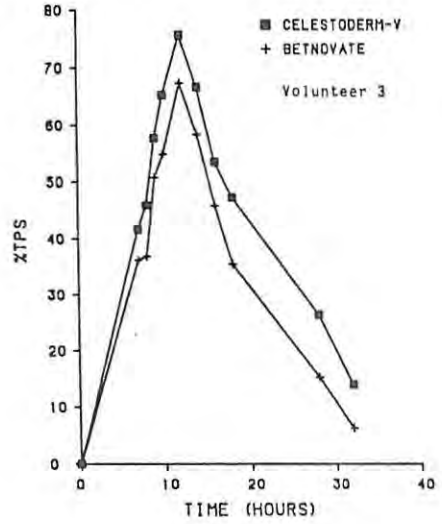
Table 3.3 VOLUNTEERS ARRANGED ACCORDING TO WHETHER THE RESULTS OF BLANCHING INDICATED DIFFERENCES OR SIMILARITIES IN COMPARATIVE BIOAVAILABILITY

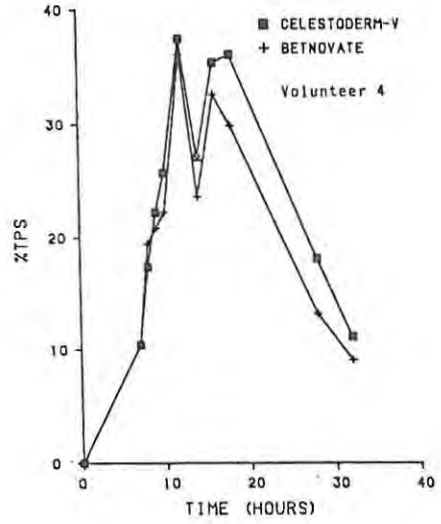
Celestoderm-V > Betnovate		Celestoderm-V ≡ Betnovate	
Trial 1	Volunteers 2, 5, 6, 7, 10, 13, 14, 15	Trial 1	Volunteers 1, 3, 4, 8, 9, 11, 12, 16, 17, 18
Trial 2	Volunteers 5, 10, 17	Trial 2	Volunteers 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18
Trial 3	Volunteers 1, 2, 3, 5, 7, 10, 13, 14, 15	Trial 3	Volunteers 4, 6, 8, 9, 11, 12, 16, 17, 18

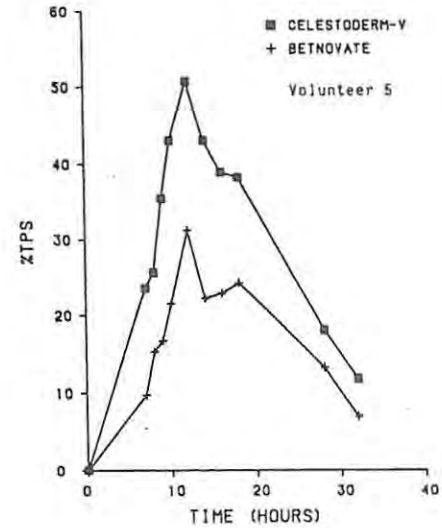
Figure 3.4.2a Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for trial 1 for one volunteer (18 sets of profiles)











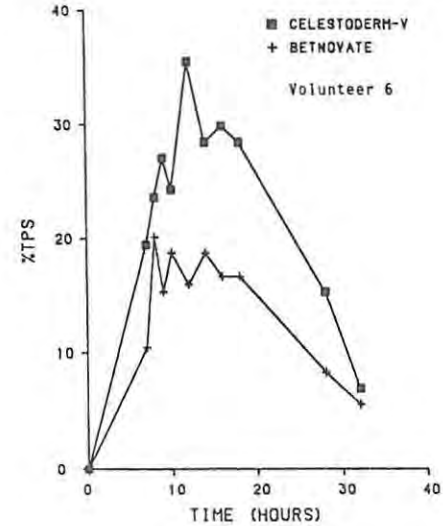
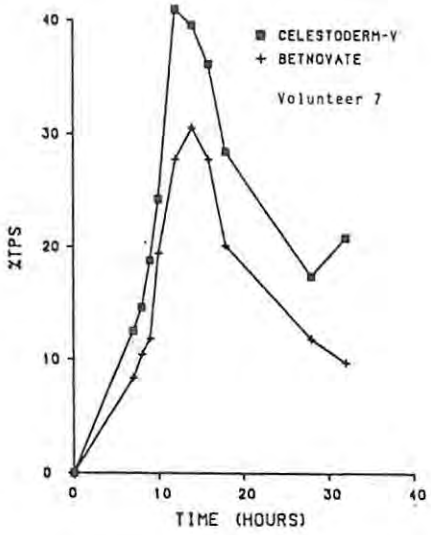
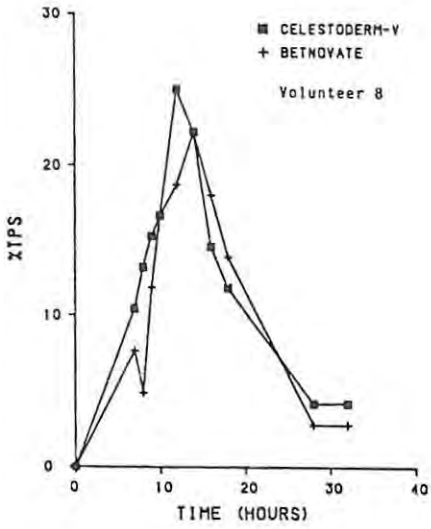
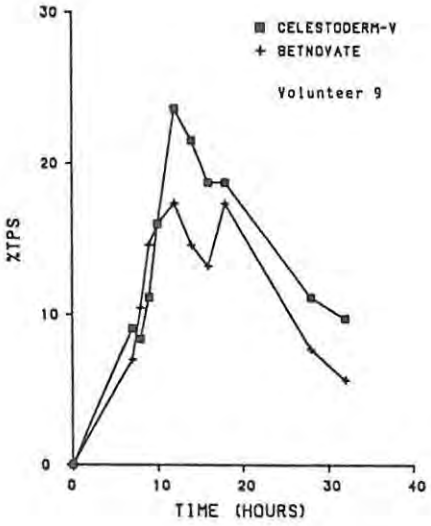
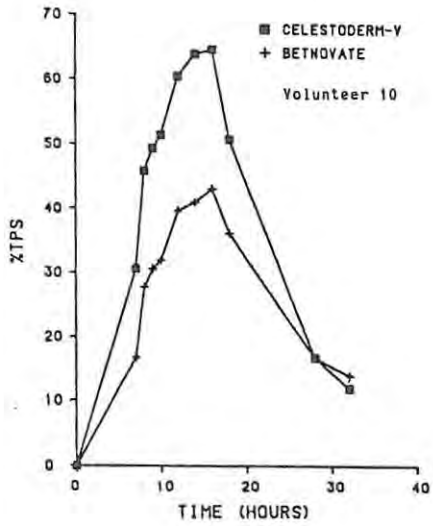


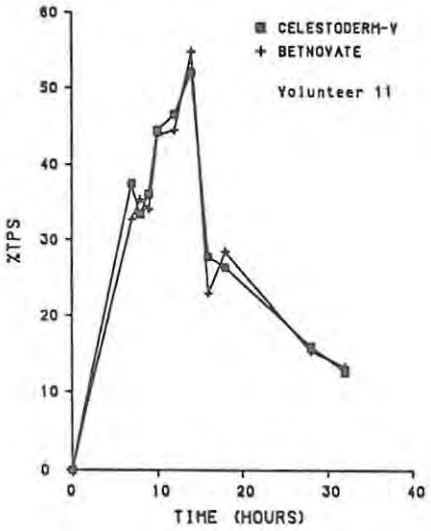
Figure 3.4.2a (continued)

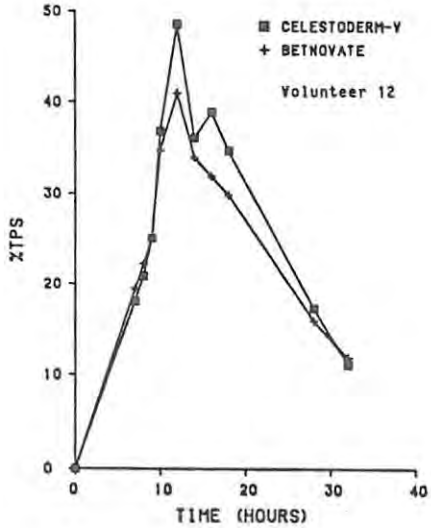












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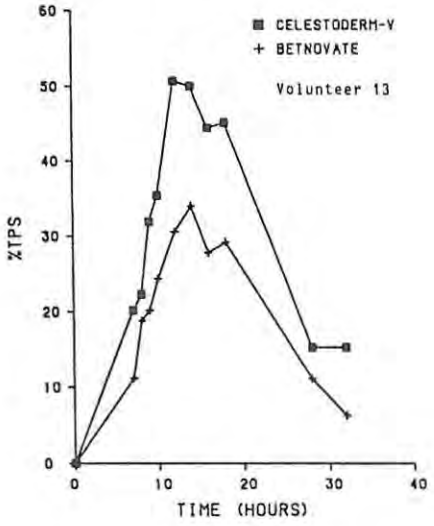
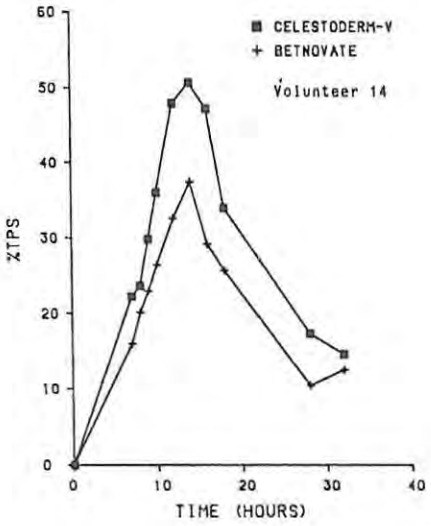
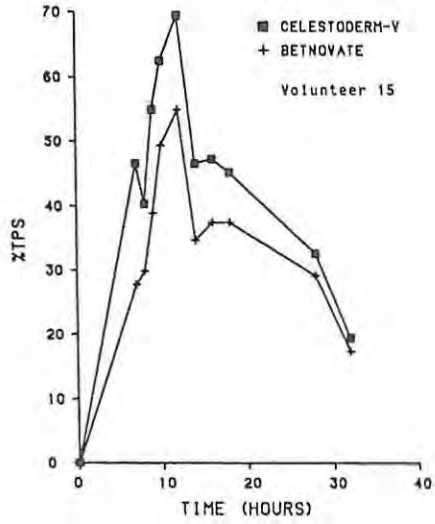
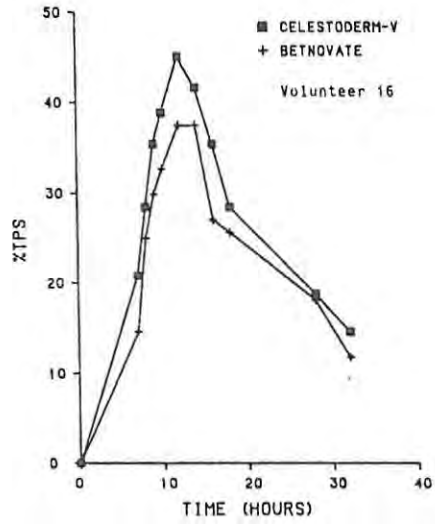
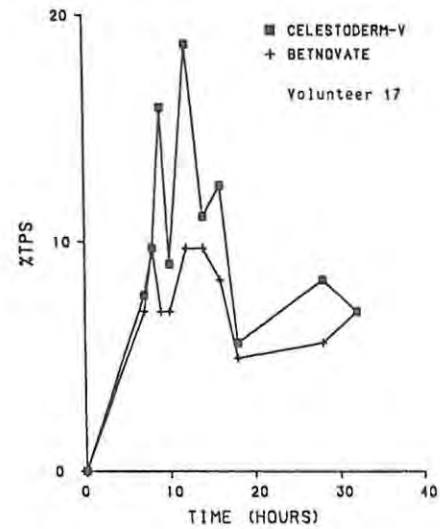










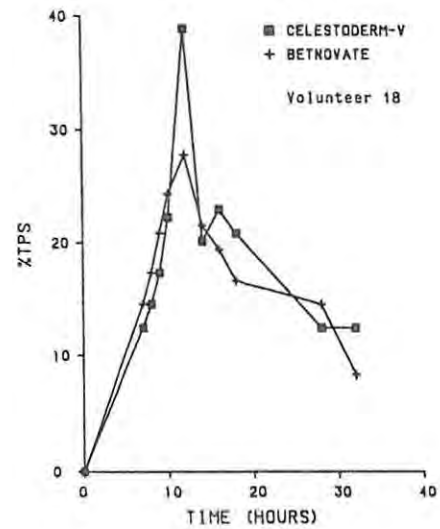
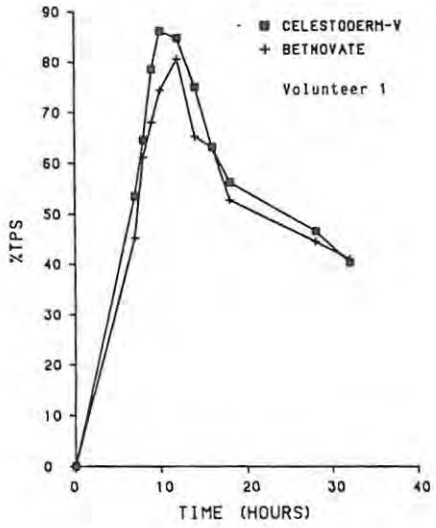
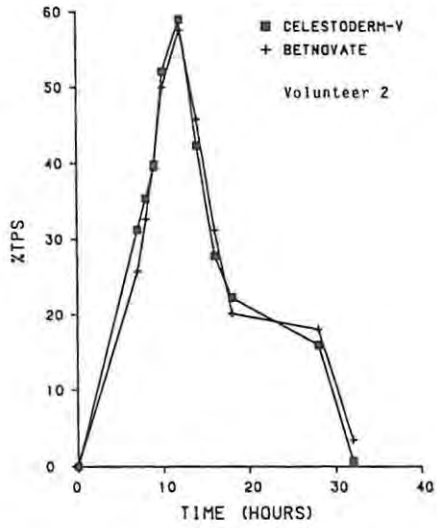
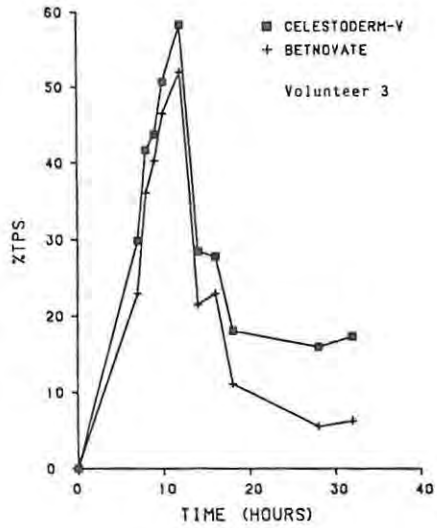
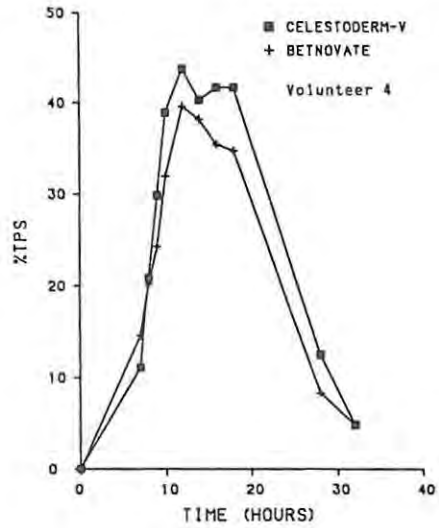


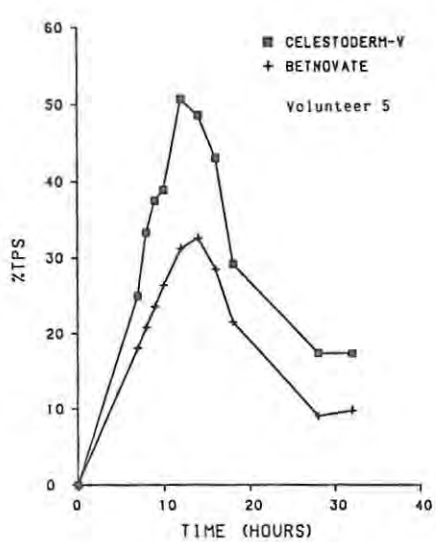
Figure 3.4.2b Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for trial 2 for one volunteer (18 sets of profiles)











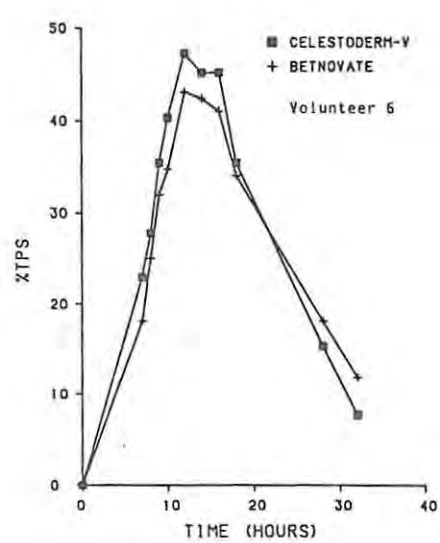
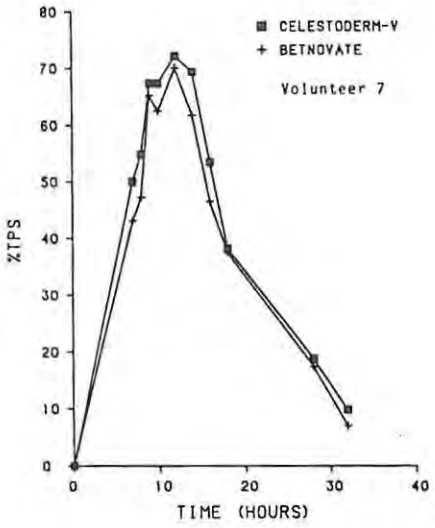
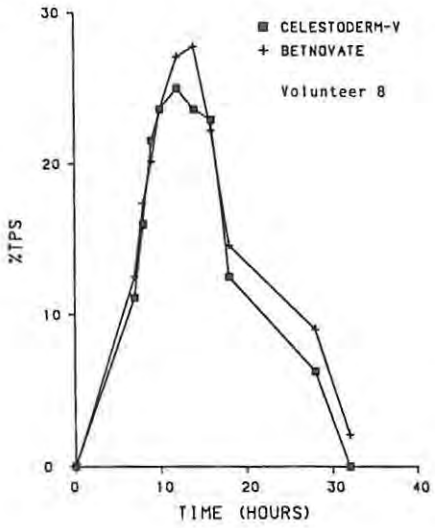
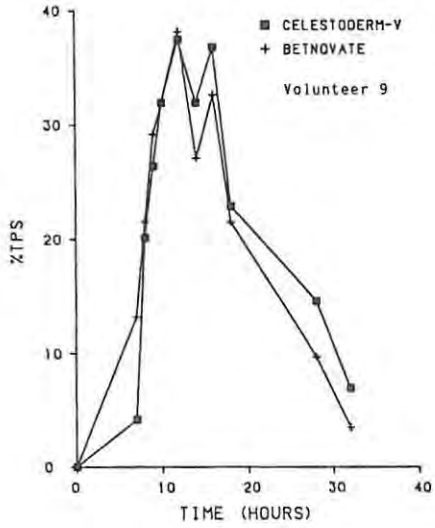
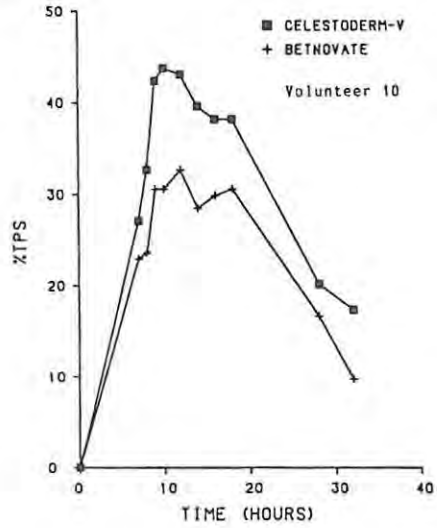


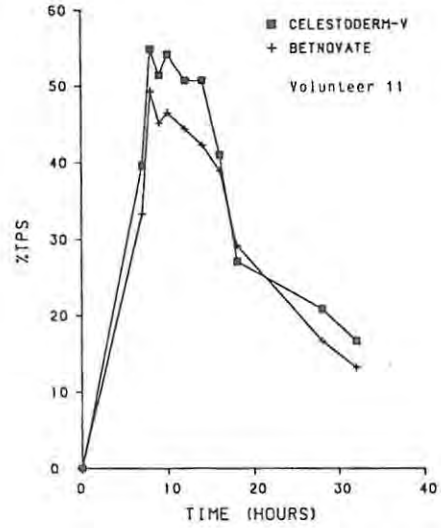
Figure 3.4.2b (continued)











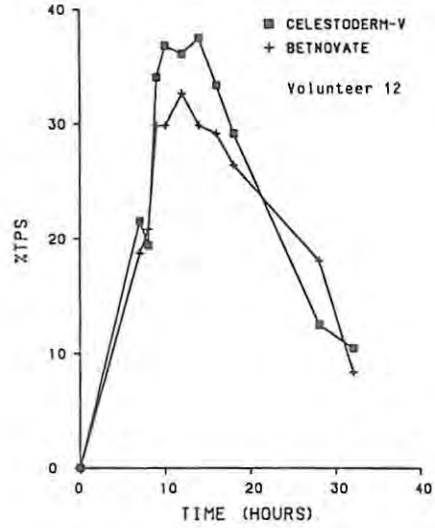
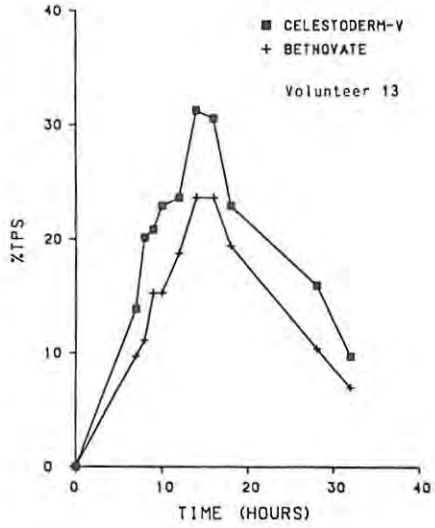
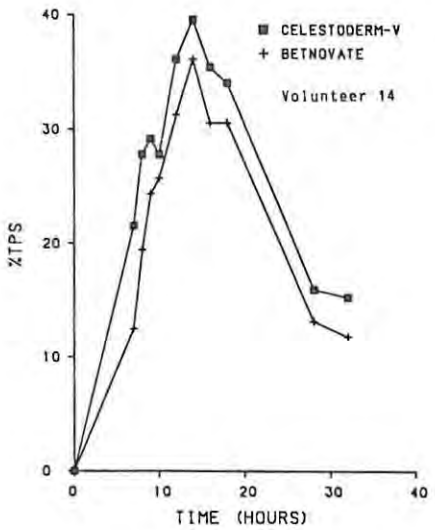
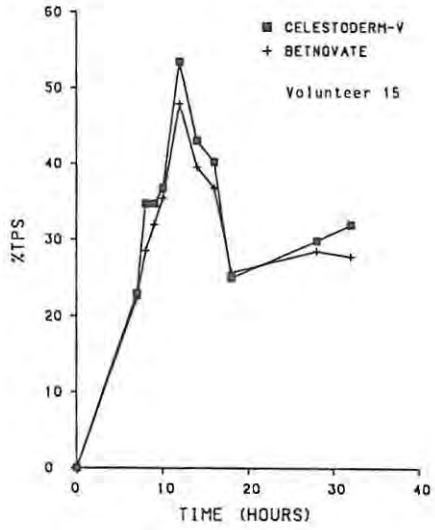
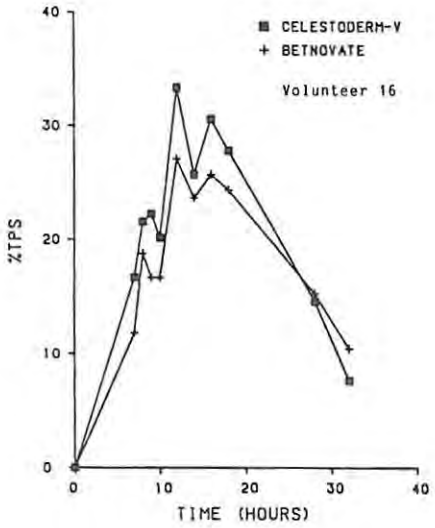


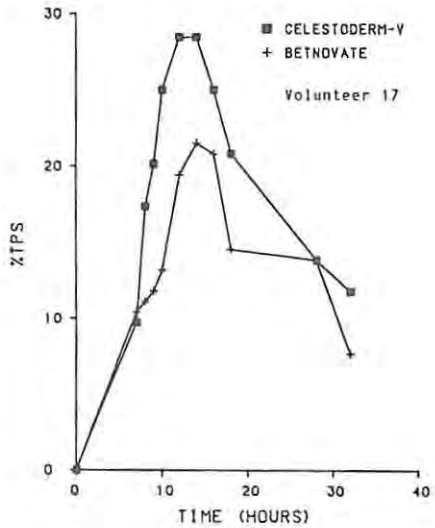
Figure 3.4.2b (continued)











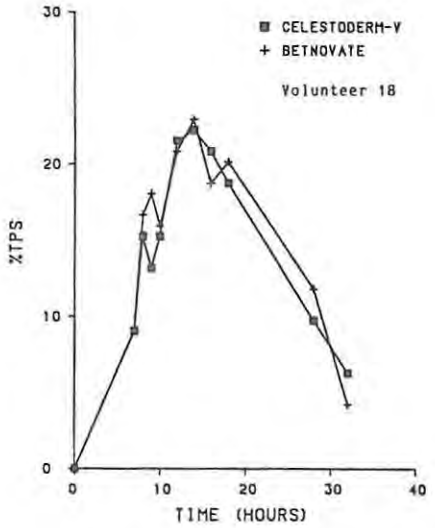


Figure 3.4.2c Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for trial 3 for one volunteer (18 sets of profiles)

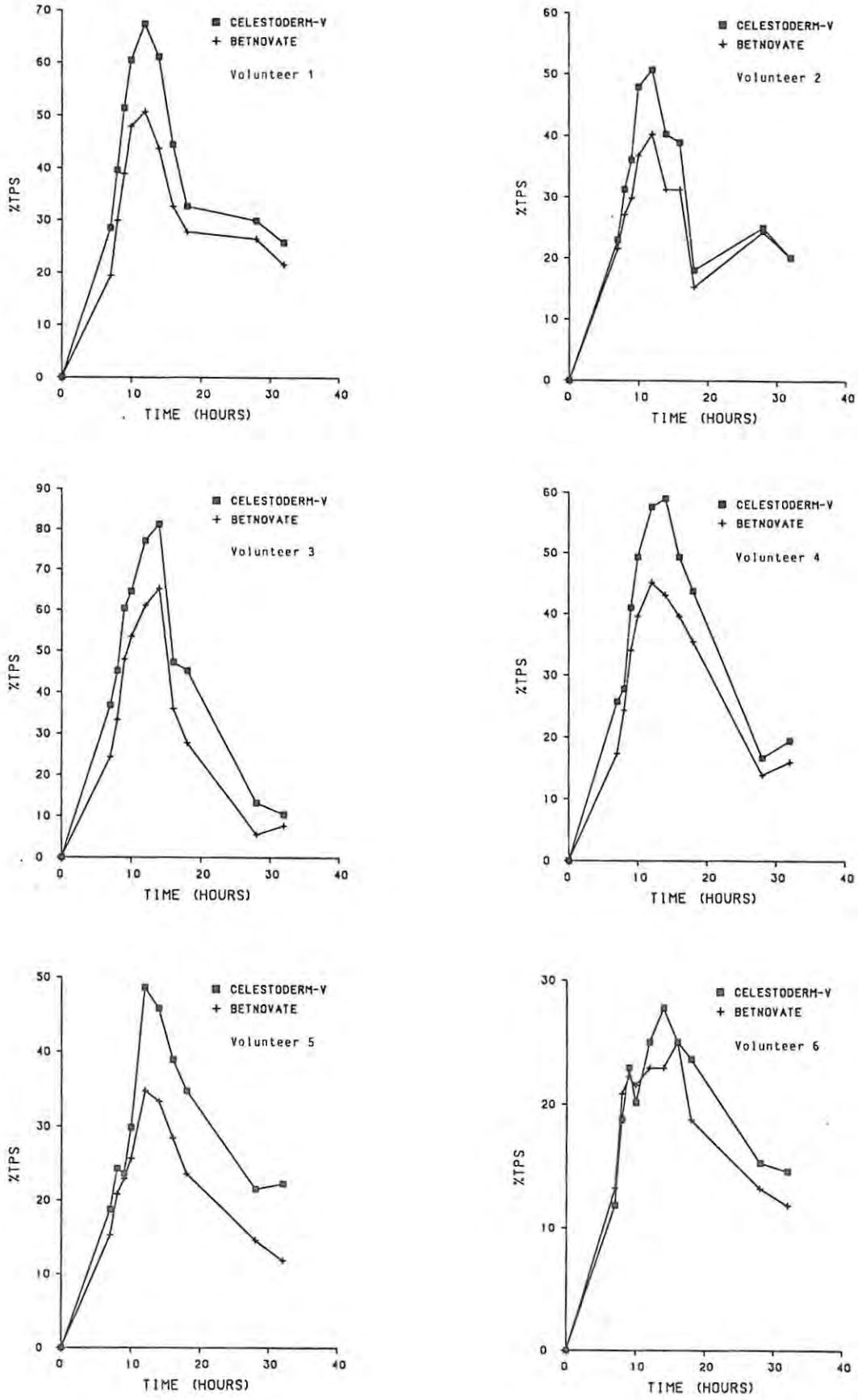
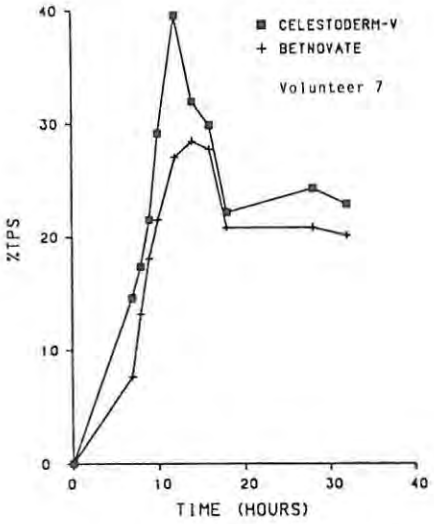
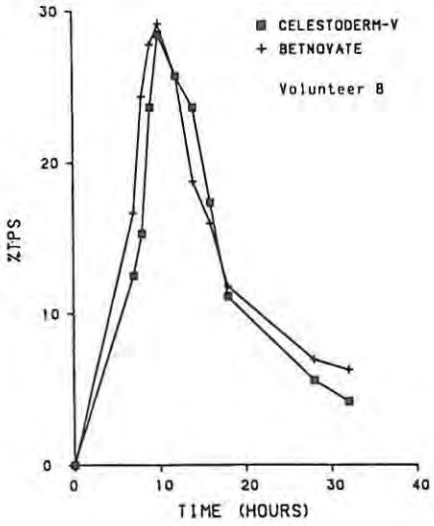
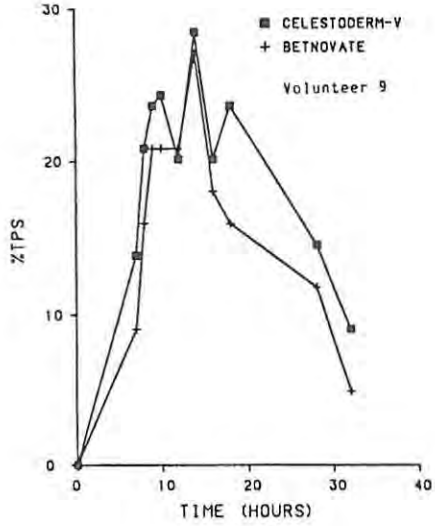
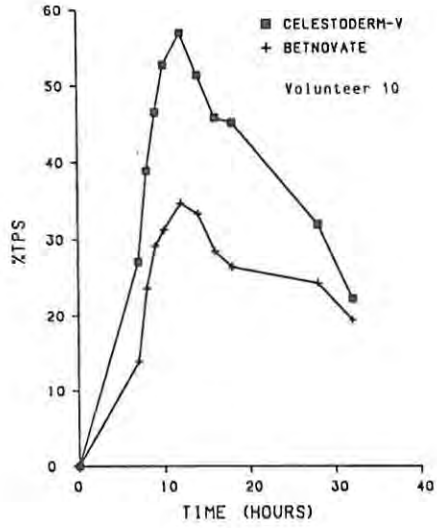


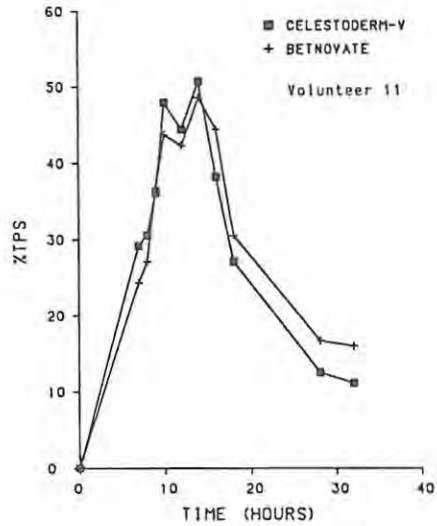
Figure 3.4.2c (continued)











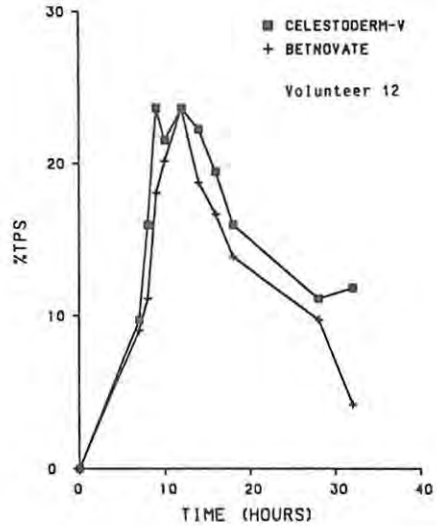
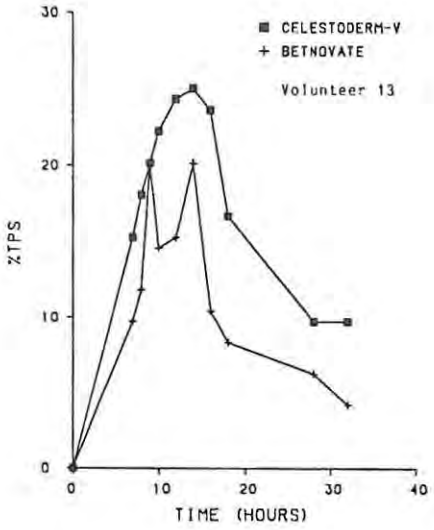
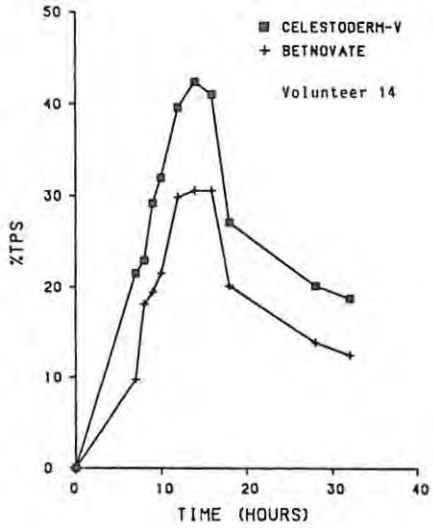
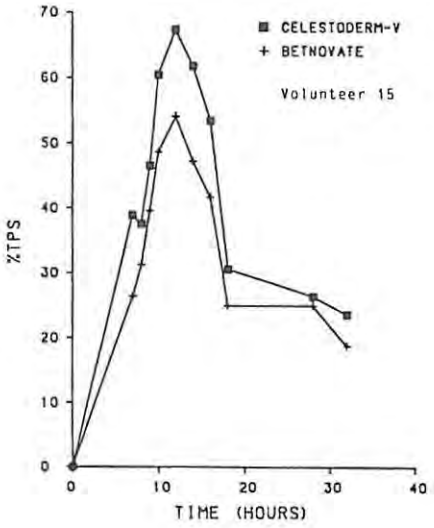
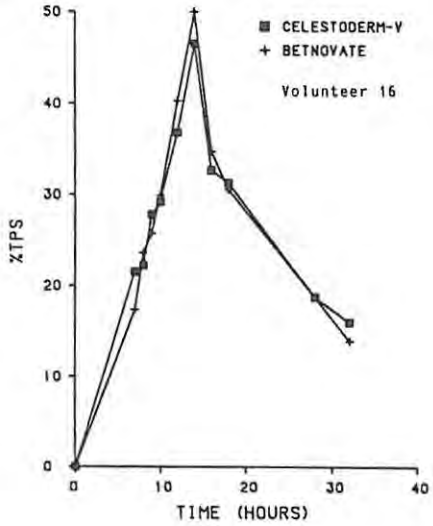


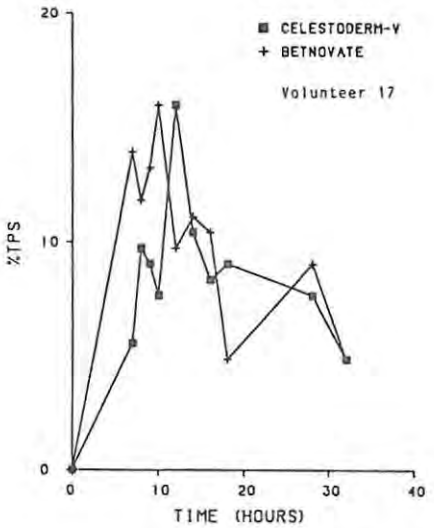
Figure 3.4.2c (continued)

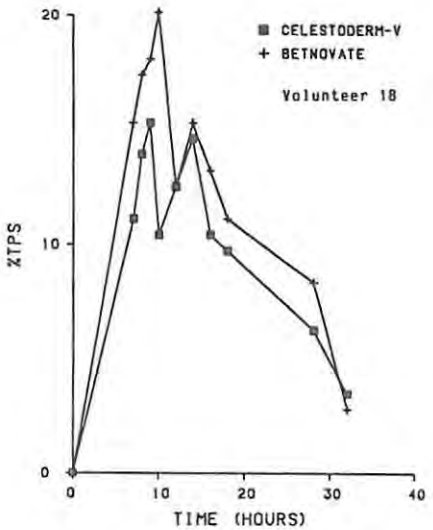












trial, and considering the relatively large difference in AUC values would most likely have been regarded as presenting more intense blanching. However, when due consideration is given to the aim of this part of this study, a detailed description of each pair of blanching profiles would be of no value.

It can be seen from the blanching profiles presented in figure 3.4.2 that in most, but not all volunteers, Celestoderm-V elicited a greater degree of blanching than Betnovate. In trial 1, volunteer 1 produced a reverse rank order; in trial 2, volunteers 8 and 18 produced reverse rank orders and in trial 3, volunteers 8, 11, 17 and 18 produced reverse rank orders.

The chi-squared values of the graded responses and the comparisons of adjacent sites were also studied. It should be remembered when studying tables of chi-squared values that the frequencies used were substantially lower than those used for any pooled results previously discussed, and especially for those of all volunteers and all observers. The data were analysed both by combining frequencies in adjacent cells and by using the actual scores without taking the small expected frequencies into consideration (see section 2.4.3.1). It was found that significant differences occurred more often when results were combined, although the conclusions of comparative bioavailability would have been the same irrespective of which tables were used. The discussion that follows is based on tables in which adjacent cells were combined to produce adequate expected frequencies.

In the graded response analyses of trial 1, overall significant differences between Celestoderm-V and Betnovate were found in six volunteers. Volunteer number 6 gave five significant and five non-significant chi-squared values, interspersed over the different time intervals. In all but three volunteers there were both significant and non-significant values, but a propensity of one of these led to the overall conclusion for the individual volunteers. It is worth noting that in all but three volunteers, conclusions of statistical analysis were similar for both the graded response and comparison of adjacent sites analyses. Significant differences were found for volunteers 6 and 7 in the analysis of the comparisons of adjacent sites but not the graded response analysis,

while for volunteer 15 significant differences were found in the graded response analysis but not the analysis of the comparison of adjacent sites.

It can be seen from the analyses of the comparisons of adjacent sites that significant differences were found in seven volunteers. In the case of volunteer 1 where Betnovate was greater than Celestoderm-V, only one significant difference was observed. In all volunteers the statistical analyses were generally consistent over all time intervals, that is to say the majority of chi-squared values for any individual volunteer were either significantly different, or not significantly different, as the case may be. The least consistent case was volunteer 15 where four observation times produced significant differences and six observation times produced non-significant differences. There were also a few cases in this trial where the frequencies of greater than to less than observations were opposite to the conclusion of rank order. The chi-squared values in all of these cases were statistically non-significant and were consistent with the overall observations in these particular volunteers.

Although there were significant differences noted in the graded response analyses in trial 2 at several observation times over the 18 volunteers, conclusions of statistically significant superiority between the preparations could only be drawn in volunteers 5 and 10. Volunteers 1, 2, 6, 8, 15 and 18 showed no significant differences, whereas the remainder of the volunteers had significant differences scattered over the trial.

Fewer statistically significant differences between Celestoderm-V and Betnovate were found in this trial than in trials 1 and 3 in the comparisons of adjacent sites. Overall significant differences were found only for volunteers 1, 3, 5 and 14. In general it was found that the frequencies of observed superior blanching responses were greater for Celestoderm-V with the exceptions of volunteers 8, 12 and 18. Volunteers 8 and 18 were those in whom Betnovate was found to elicit a superior response. Interestingly, in volunteer 12 paired comparisons favoured Betnovate at 8 of the 10 observations. However, none of the differences was statistically significant. In all the other volunteers where the number of paired comparisons indicated a reversal of rank order, the chi-squared values did not indicate statistically significant differences.

Only volunteer 5 showed significant differences in both forms of analysis; in the other volunteers (1, 3 and 14) in trial 2 where statistical superiority was concluded, this was found only in the paired comparisons of adjacent sites.

The statistical analyses for trial 3 were somewhat less clear-cut than for trials 1 and 2. There were fewer cases where all chi-squared values for a particular volunteer in either the graded response or paired comparison analyses were significant or non-significant, as the case may be. There were also more instances where chi-squared values at four or five observation times for a particular volunteer differed in terms of significance *versus* non-significance for that volunteer. An example is the graded response analysis for volunteer 3 where significant differences were noted at five observation times and non-significant differences were noted at five observation times. General conclusions were therefore more difficult to draw. Taking this into account, it can be seen that when considering both forms of statistical analysis, significant differences between Celestoderm-V and Betnovate were found in volunteers 1, 3, 5, 10 and 14. All of these were in favour of Celestoderm-V. In the volunteers in whom Betnovate elicited superior blanching only a small number of significant differences were found in both forms of analysis. In the graded response analysis significant differences were found in volunteer 8 at 8 hours after application, in volunteer 17 at 7, 10 and 12 hours after application, and in volunteer 18 at 10 hours after application. In the analysis of paired comparisons of adjacent sites, significant differences were found in favour of Betnovate in volunteer 17 at 7 and 10 hours after application, and in volunteer 18 at 10 hours after application.

It can be seen from the profiles of pooled results (figure 3.1.1 in section 3.1) that normal blanching profiles were obtained in all three trials. The blanching profiles usually demonstrate this shape although the peak may not always be as pronounced (as seen in trial 3), a trough or shoulder may be present, some curves may be narrower or broader than the "normal" curve and a slight increase in blanching has occasionally been noted at the end of the trial, or the last two readings may be equal. Generally, however, the curves indicate a rapid incline to the peak, followed by a less rapid decline. It is also usually found that

curves for different preparations tested in the same trial exhibit similar shapes; however, different rates of onset and different durations of action of blanching will mean that the curves will not always be parallel or coincident and cross-overs near the beginning and/or towards the end of the trial are not uncommon.

As expected, the blanching profiles obtained from the results of individual volunteers are generally less smooth, exhibiting several peaks, troughs and shoulders. In some cases the troughs or peaks coincide for both preparations on the same volunteer, whereas in others they appear with no apparent pattern. An example of the former instance is volunteer 4 in trial 1. This is an idiosyncratic response of the particular volunteer at that time. It is hard to explain why the observed blanching at 14 hours for this volunteer was so much lower in this trial. It is highly unlikely that the corticosteroid response was different at this time and is probable that the apparent blanching was lower due to a transient alteration in surface vasculature, resulting in lower blanching being observed and recorded by the observers.

The change in surface vasculature may be caused by a number of environmental factors which may have affected the volunteers in which this phenomenon was observed. The volunteer may have exposed the arms to a sudden change in temperature whilst, for example, moving from one building to another, the volunteer may have run between buildings or may have run up or down a flight of stairs. It is also possible that the volunteers' biorhythms influenced the blanching response, especially at the midnight (16 hour) or 2 a.m. (18 hour) readings. It unfortunately does not seem possible to study these hypotheses under controlled conditions. The subjective nature of the assay and the possibility of different reactions of individuals to the same stimuli or conditions would preclude this. This explanation for simultaneous troughs in the profiles, however, does not suffice for cases where a trough occurs in the curve of only one of the preparations (for example volunteer 13 in trial 3). There does not seem to be a ready explanation for this occurrence other than attributing it to the subjective nature of the assay.

The troughs noted in the blanching profiles should also be seen in terms of the size of the trough, that is to say the difference between the

%TPS value in the trough and at the two adjacent peaks. The troughs observed in volunteer 4 in trial 1 and volunteer 2 in trial 3 are quite large, whereas those seen in volunteer 6 in trial 1 and volunteer 18 in trial 2 are smaller and less significant. It should, however, be borne in mind that the aim of these trials is normally to study comparative bioavailabilities of topical corticosteroids. If only one preparation exhibits a sudden transient decrease in blanching it may influence the AUC value and statistical analyses to an extent that conclusions may be inaccurate, but if this depression is small or concordant for all the preparations being tested it should not influence the accuracy of the final conclusions of comparative bioavailabilities.

The small number of volunteers in whom significant differences were found in trial 2 deserves comment. It will be recalled that the difference between the two preparations in trial 2 was smaller than the differences between the preparations in trials 1 and 3 (section 3.1). Similarly, the difference in the AUC values for the pooled results of trial 2 are smaller than for trials 1 and 3, and although statistically significant differences were found, the chi-squared values were smaller in trial 2 than in trials 1 and 3. This clearly demonstrates the importance of pooled results, which were therefore studied further.

It was decided at this stage to pool the results obtained from individual volunteers in different ways. It was firstly decided to ascertain whether the good blanchers were more likely to show significant differences, or alternatively similarities, in comparative blanching responses of the two preparations, in other words to ascertain whether good blanchers were better discriminators than poorer blanchers. The volunteers were divided into two equal groups for each trial according to AUC values. The AUC values for each preparation for each volunteer were arranged in descending order. It was, however, noticed that the volunteers representing the nine highest AUC values did not always correspond for both preparations. For example in trial 1, volunteers 5 and 13 were in the highest nine AUC values for Celestoderm-V but not for Betnovate, whilst volunteers 12 and 16 were in the top nine AUC values for Betnovate but not for Celestoderm-V. It was therefore decided to calculate the average AUC values for the two preparations for each volunteer and group the volunteers according to these values. It can be seen from

table 3.4 that the groups of volunteers arranged according to average AUC values were similar to those arranged according to the AUC values of the individual preparations. The volunteer numbers presented on the extreme right of table 3.4 represent those volunteers who differed between the groups arranged according to average AUC values and the groups arranged according to the AUC values of the individual preparations. It is felt that in view of the similarities of the compositions of the different groups, valid results would be obtained from the groups arranged according to average AUC values. The groups of volunteers arranged in

Table 3.4 VOLUNTEERS GROUPED ACCORDING TO AREA UNDER THE CURVE VALUES

		Volunteer numbers										
Trial 1												
Group 1	(Celestoderm-V)	1	2	3	10	11		13	15		5	14
	(Betnovate)	1	2	3	10	11	12		15	16		14
	(Average)	1	2	3	10	11	12	13	15	16		
Group 2	(Celestoderm-V)	4		6	7	8	9		17	18	16	12
	(Betnovate)	4	5	6	7	8	9		17	18	13	
	(Average)	4	5	6	7	8	9	14	17	18		
Trial 2												
Group 1	(Celestoderm-V)	1	2		5	6	7	10	11	15	3	
	(Betnovate)	1	2	4		6	7	10	11	15	12	
	(Average)	1	2	4	5	6	7	10	11	15		
Group 2	(Celestoderm-V)		8	9	12	13	14	16	17	18	4	
	(Betnovate)	3	8	9		13	14	16	17	18	5	
	(Average)	3	8	9	12	13	14	16	17	18		
Trial 3												
Group 1	(Celestoderm-V)	1	2	3	4	5	10	11	15		14	
	(Betnovate)	1	2	3	4	5	10	11	15	16		
	(Average)	1	2	3	4	5	10	11	15	16		
Group 2	(Celestoderm-V)	6	7	8	9	12	13		17	18	16	
	(Betnovate)	6	7	8	9	12	13	14	17	18		
	(Average)	6	7	8	9	12	13	14	17	18		

descending order of AUC values are shown in table 3.5. Group 1 represents those nine volunteers in whom the highest averaged AUC values were found, and group 2 represents the nine volunteers in whom the lowest averaged AUC values were found. Both groups are arranged in descending order of AUC values. It is interesting to note that the rank order of the volunteers differed over the three trials. The %TPS, AUC and chi-

Table 3.5 VOLUNTEERS ARRANGED IN DESCENDING ORDER OF AUC VALUES

Trial 1				Trial 2				Trial 3			
Group 1		Group 2		Group 1		Group 2		Group 1		Group 2	
3*	(1123)**	14	(720)	1	(1613)	14	(690)	15	(1005)	14	(653)
15	(1106)	5	(687)	7	(1145)	12	(674)	1	(982)	7	(633)
1	(960)	4	(638)	11	(920)	3	(657)	3	(975)	6	(525)
10	(950)	7	(584)	15	(886)	16	(569)	10	(918)	9	(480)
2	(841)	6	(511)	6	(807)	9	(565)	4	(890)	12	(406)
11	(824)	18	(503)	10	(795)	13	(497)	11	(812)	8	(397)
12	(739)	9	(377)	2	(779)	17	(466)	2	(760)	13	(377)
13	(729)	8	(313)	4	(703)	18	(421)	16	(759)	18	(306)
16	(727)	17	(226)	5	(699)	8	(406)	5	(721)	17	(254)

* Volunteer number

** AUC value

squared values for Celestoderm-V *versus* Betnovate were analysed for group 1 and group 2 in each trial. It can be seen from the %TPS values and profiles (figure 3.4.3) that the time of maximum blanching was similar for both groups of volunteers. In trial 3, group 2 peaked slightly later for Celestoderm-V and Betnovate (between 12 and 14 hours), while all the other peak times were at 12 hours. The curves for group 2 were slightly broader than for group 1 and the curves for group 2 lay somewhat closer together (in trials 2 and 3). Although the differences in AUC values between Celestoderm-V and Betnovate in group 1 were greater than in group 2, the percentage differences were quite similar, with the smallest percentage difference having occurred in trial 2.

The chi-squared analyses for group 1 showed distinct differences between Celestoderm-V and Betnovate in all three trials, with very few statistically non-significant differences. The differences between the two preparations for group 2 were less clear. Trial 1 showed only one statistically non-significant difference indicating that Celestoderm-V was clearly superior to Betnovate. In trial 2, statistically significant differences were found at all reading times other than 7, 28 and 32 hours in the paired comparisons, but more non-significant than significant differences were found in the graded response analysis. Considering this and the similarity in AUC values, it can be concluded that the two preparations elicited similar blanching responses in this group. Similarly, in trial 3 the chi-squared analyses and AUC values do not allow a definite conclusion of the superiority of one preparation over the other although the rank order was the same for each group in all trials.

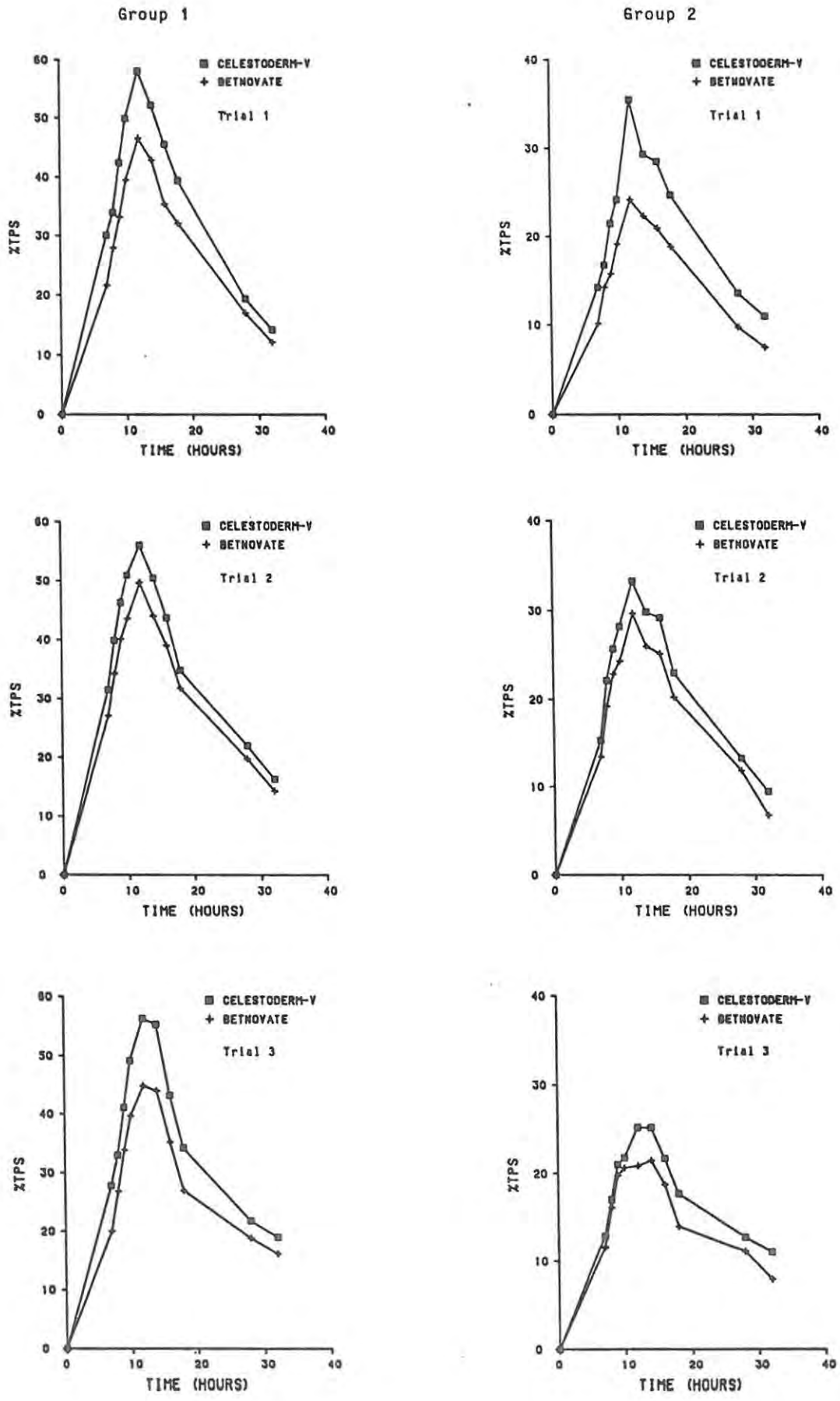


Figure 3.4.3 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for group 1 (nine volunteers with highest AUC values) or group 2 (nine volunteers with lowest AUC values) for one trial

The results of individual volunteers were grouped in another way for each trial, namely those volunteers who showed similar blanching between the two preparations (group A) and those where differences were found (group B). The groups were comprised of volunteers showing different or similar responses to the two preparations, as indicated in table 3.3. It was expected that those volunteers in whom differences were found would show differences when their results were pooled. The main aim of this re-grouping was therefore to ascertain whether differences would be found between the two preparations when the results of volunteers who individually showed no differences were pooled.

As was expected, the results of those volunteers in whom differences were found when the results were analysed individually indicated differences when the results were pooled (figure 3.4.4). Marked differences were found in AUC values, and chi-squared analyses showed statistically significant differences in most cases. Similar blanching responses between the two preparations were found in the analyses of the pooled results of volunteers where no differences were found on individual analysis (group A). The differences in AUC values were small and generally the chi-squared values indicated few significant differences. A notable exception was the paired comparison response in trial 2, where significant differences were found at all but the last reading time. The similarity in AUC values, closeness of the curves, as well as the graded response analysis, however, do not allow an unquestionable conclusion of superiority of Celestoderm-V over Betnovate.

It was interesting to note which of the volunteers in the two groups in table 3.3 (volunteers arranged according to whether the results of blanching indicated differences or similarities in comparative bioavailability) were placed in group 1 or group 2 in table 3.5. In trial 1, the groups showing either similar (group A) or different (group B) blanching were comprised equally of volunteers from group 1 and group 2, in other words four of the eight volunteers who individually showed differences were from group 1 and four were from group 2. The small number of volunteers showing differences in trial 2 does not allow this comparison to be meaningfully made for this trial. In trial 3, it was found that six of the nine volunteers who showed differences (group B) were from group 1 and only three from group 2, and of those volunteers where the two

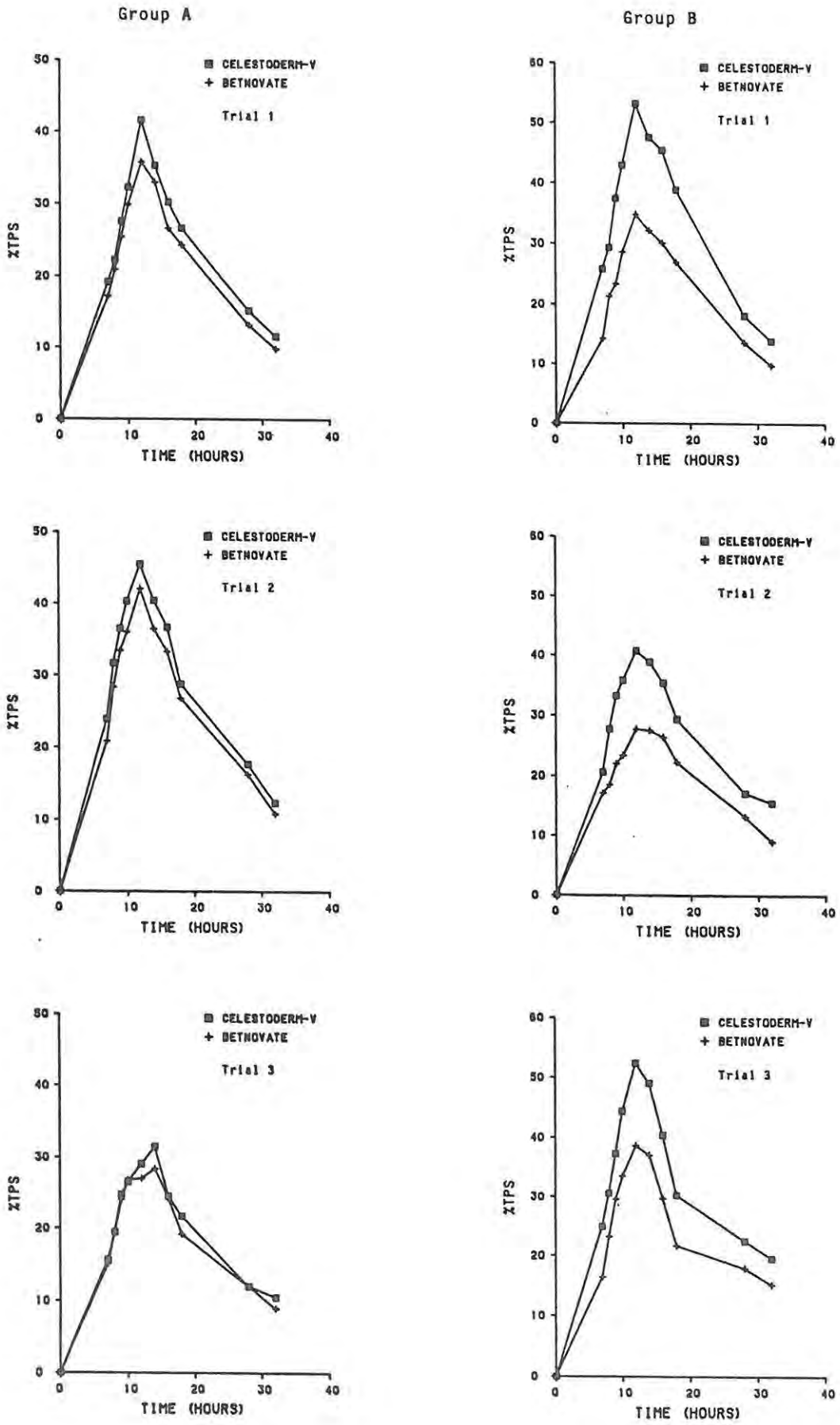


Figure 3.4.4 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations for group A (volunteers showing similar blanching between Celestoderm-V and Betnovate) or group B (volunteers showing different blanching between Celestoderm-V and Betnovate) for one trial

preparations showed similar blanching (group A), six were from group 2 and three from group 1.

The results of this re-grouping of volunteers and the re-analysing of results appear to be contradictory in places, which makes it difficult to draw conclusions and make suggestions for improved methodology of the blanching assay. It was seen from the results of group 1 and group 2 that the better blanchers were more likely to discriminate or show differences between two preparations. Better blanchers therefore appear to be more sensitive to differences in the abilities of the preparations to elicit blanching to different degrees. One would, in the light of this, be tempted to suggest that better blanchers should be used and poorer blanchers should be avoided where possible. The re-grouping for trial 3 in table 3.3 supports this suggestion in that six of the nine volunteers in the group that showed differences were from group 1. The re-grouping in trial 1 (table 3.3), however, does not support this where the groups were evenly divided between group 1 and group 2. It should also be considered that the ability to differentiate may be influenced by the potency of the corticosteroid preparations. It has been noticed in our laboratories that observers find it more difficult to make a decision in paired comparisons on very good blanchers for very potent preparations. It is therefore suggested that good blanchers be used for preparations that elicit poor blanching responses, but that for trials where potent or very potent preparations are being tested, a combination of good, average and poor blanchers should be used.

3.5 Comparison of Blanching Responses Between Males and Females (tables 3.5.1-3.5.4)

The thickness of the stratum corneum is known to be similar in males and females (161). It has been found that the rate of turnover of the stratum corneum is the same for young adult males and females, although it decreases with age, and is less rapid in older men than in older women (160). It is unclear whether different turnover rates in older persons lead to different integrity of the stratum corneum for men and women. This will, however, not influence the comparison of blanching responses between males and females in this study, as all subjects were young adults between the ages of 19 and 26 years. The ages of the male sub-

jects ranged from 19 - 26 years (average age 23 years) and of the females from 19 - 21 years (average age 20 years). A physiological factor of the stratum corneum that may influence the percutaneous penetration of corticosteroids is the size of the cells of the stratum corneum; the cells are larger in females than in males (160). It seems contradictory that the thickness of the stratum corneum in young males and females can be the same if the cell size differs, but this does not appear to have been discussed in the literature. The route of percutaneous penetration has not been fully elucidated (section 1.3.4), but the larger cell size in females may present less resistance to penetration *via* the intercellular spaces (160). This variable has not been studied.

There is a paucity of information in the literature regarding the comparative blanching responses between male and female subjects. McKenzie and Atkinson (135) and Gibson *et al.* (296) examined the blanching responses of males and females and concluded that there was no evidence of a sex related difference in skin sensitivity to corticosteroids.

The blanching responses observed in males (volunteers 1 - 9) and females (volunteers 10 - 18) in this study of three trials were analysed to ascertain whether there were differences in either preparation for males and females, and secondly to ascertain whether a difference was noted in the comparative bioavailabilities of the two preparations tested when the responses of males and females were analysed separately.

The comparison of blanching between males and females for each preparation produced interesting results. In trial 1, the profiles of blanching (figure 3.5.1) for males and females lay close together for both preparations, although the blanching elicited in female volunteers appeared to be marginally superior in the case of Betnovate. Chi-squared analysis of the graded responses however indicated significantly superior blanching at most time intervals. In the case of Celestoderm-V, significant differences were found from 9 - 14 hours after application and for Betnovate significant differences were found at all reading times except 18 hours. The maximum %TPS for Betnovate was equal for both groups as a result of more 3's and 4's being recorded in the male group than in the female group. More 3's and 4's were noted up to and including the 18 hour reading. Fewer 0's and more 1's and 2's observed in the female

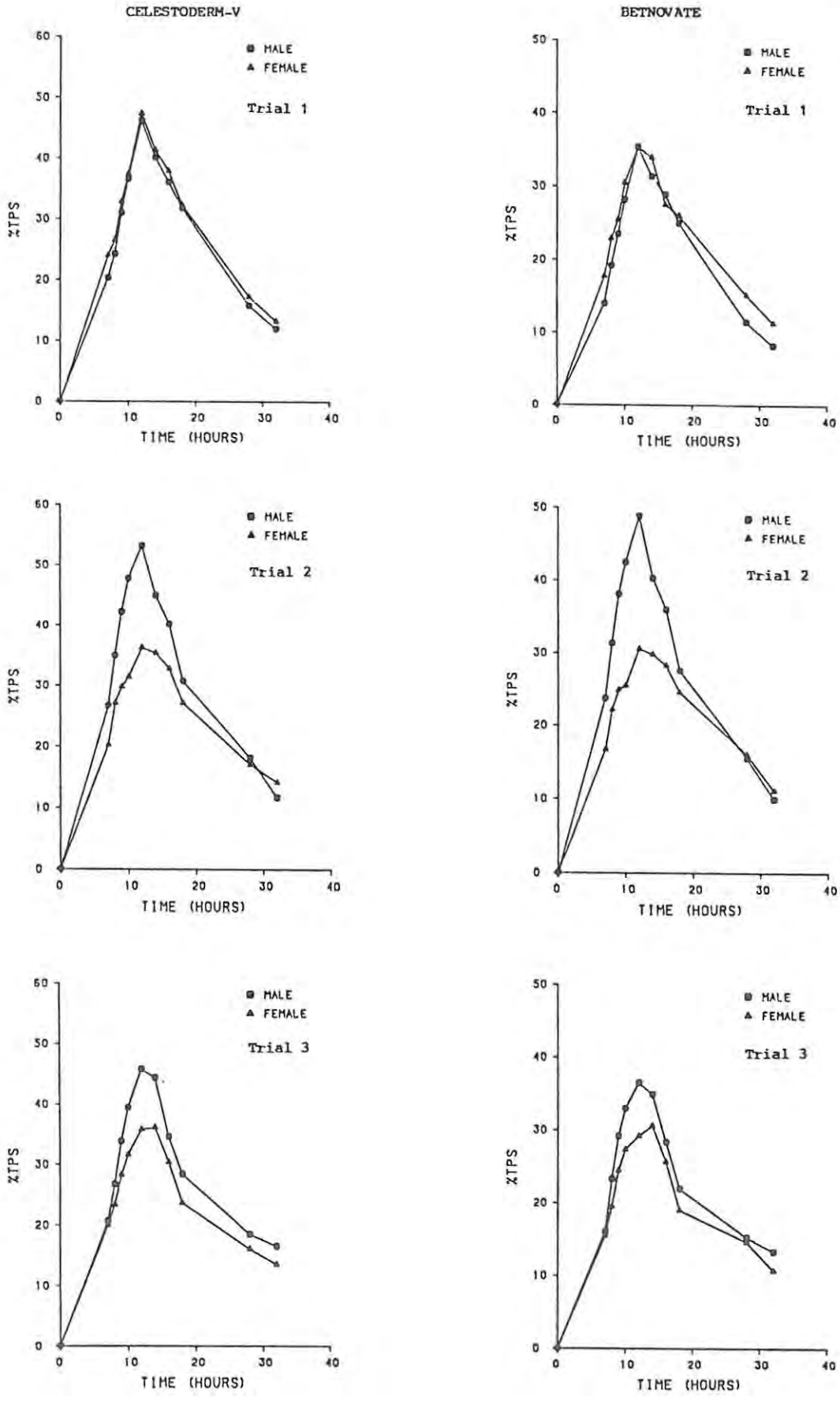


Figure 3.5.1 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents one preparation and one trial for male and female volunteers

group than in the male group accounted for the higher %TPS values and statistically significant difference of female over male.

In trial 2, the rank order of male to female was opposite to trial 1 and males showed more blanching. Statistically significant differences were noted at all readings except the 28 hour reading for Celestoderm-V. The rank order of male and female in trial 3 was as for trial 2, although fewer statistically significant differences were found. Significant differences were found only from 8 - 12 hours in the case of Betnovate and from 9 - 18 hours in the case of Celestoderm-V. It should however be noted that significant differences were found at the times where blanching was more intense, namely close to the peak blanching responses. It can therefore be seen that in trials 2 and 3, the male group of volunteers exhibited better blanching than the female group.

Caution should, however, be employed when attempting to extrapolate these results to the general population. It is possible that groups made up of different volunteers could have produced different results. It is, however, worth noting that a general observation of blanchers from the panel of the volunteers used in our laboratories has indicated that the very good blanchers are invariably males. In the volunteers used in these three trials the best blanchers were males and, when selected, were considered as being very sensitive to corticosteroid induced blanching.

It can be seen from the AUC values, %TPS values and chi-squared results that Celestoderm-V elicited a greater degree of blanching than Betnovate in both the male and the female groups, although statistically significant differences in the graded response analysis were found at only two readings in trial 2 in the male group. In the female group of volunteers, maximum blanching for both preparations was observed at 14 hours after application in trial 3, whilst all other maximum blanching responses were observed at 12 hours after application.

In the overall results reported earlier (section 3.1) the maximum blanching at 12 hours observed in trials 1 and 2 indicated definite peaks whereas in trial 3, although maximum blanching was observed at 12 hours, the differences between the 12 hour and 14 hour readings were small. In

the case of this assessment, where in the female group maximum blanching was observed at 14 hours in trial 3, the differences between the 12 and 14 hour %TPS values were small. In trials 2 and 3 the differences between the 12 and 14 hour readings in females was somewhat less than in males, but the results of trials 1 and 2 do not show a difference between time of maximum blanching between these two groups of volunteers. The overall observation therefore is that the time taken to reach maximum blanching appears to be similar in males and females.

A much more important observation from the point of view of the human skin blanching assay as a method of assessing topical corticosteroid activity is that comparative blanching responses of male and female blanchers produced the same rank order, namely Celestoderm-V being greater than Betnovate. The important conclusion is that volunteer panels can therefore be made up of male and female blanchers.

3.6 Comparison of Blanching Responses Between Arms (tables 3.6.1-3.6.7)

Very little has appeared in the literature on the comparison of the sensitivity of right and left arms to the blanching response. McKenzie and Atkinson (135) found that the left arm was more sensitive than the right arm. They do point out that the corticosteroids were applied to the right and left arms by different operators, which may account for the apparent differences in sensitivity. Gibson *et al.* (296) conversely found that the intensity of blanching was greater on the right arm, whilst Pepler *et al.* (237) found that more blanching was elicited on the right arm 8 hours after application but that the intensity of blanching was the same on both arms for the remainder of the trial. All the trials in the above experiments were performed under occlusion. It is worth noting that McKenzie and Atkinson (135) utilized a 15 hour application time, while Gibson *et al.* (296) and Pepler *et al.* (237) utilized 6 hour application periods. It is further worth noting that Gibson *et al.* observed the degree of blanching at only one reading time (7 hours after application); it would have been interesting to be able to compare results had both trials (237,296) been performed over an extended period.

No postulations have been offered as to why the right arm may be more

sensitive to corticosteroid-induced blanching than the left arm. It is possible that superficial microvasculature may be more developed and efficient in the arm in which the muscles are more active. Whilst greater oxygen requirement of and heat dissipation from these more active muscles may influence the vasculature in the muscles, it seems unlikely that the surface microvasculature should undergo any alteration. These aspects do not appear to have been discussed in the medical or physiological literature (455).

A comparison of the blanching elicited on the strong and weak arms of the volunteers was performed in this series of trials for two reasons. Firstly, to ascertain whether there was a difference between the sensitivities of the two arms to topically applied corticosteroids and secondly, to ascertain whether the blanching from either the strong arm or weak arm was responsible for the overall results of comparative bioavailability, or whether these results were an accurate combination of the two arms. Arms were, for the purpose of this section, divided into strong or dominant (writing) arms and weak arms, as opposed to right and left arms. Two of the volunteers (8 and 11) employed in this series of three trials were left-handed, whilst the remaining 16 were right-handed. Comparisons are therefore essentially between right and left arms. The profiles for each preparation are depicted in figure 3.6.1.

The AUC values for Celestoderm-V and Betnovate on the strong and weak arms were very similar within each trial. In trial 1, more blanching was noticed in the weak arms, whereas in trial 2 more blanching was found on the strong arms. In trial 3, the AUC values were almost identical with only about 1% difference between the two arms for both Celestoderm-V and Betnovate. Statistical analysis of the graded responses observed on each arm indicated very few significant differences. The results of both preparations were pooled for each arm to ascertain whether the same conclusions would be obtained compared to the results of Celestoderm-V and Betnovate analysed separately. Blanching profiles are depicted in figure 3.6.2. The same conclusions were found in terms of AUC values for all three trials, as well as for graded response analyses for trials 1 and 3. In trial 2 significant differences were found in favour of the strong arm from 9 - 16 hours after application. Contrary to the findings of Gibson *et al.* (296) and Pepler *et al.* (237), no statistically signi-

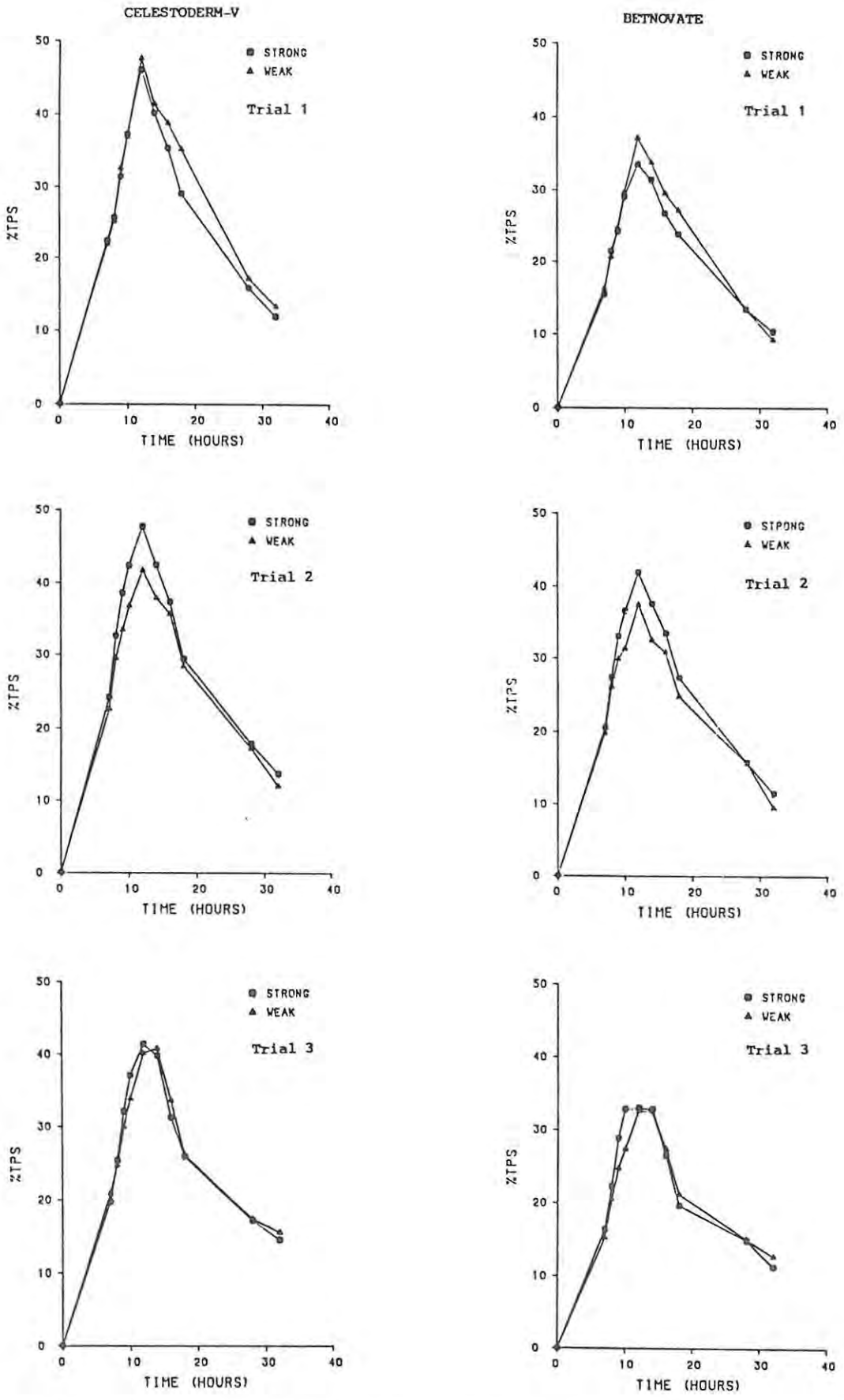


Figure 3.6.1 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents one preparation in one trial on each arm

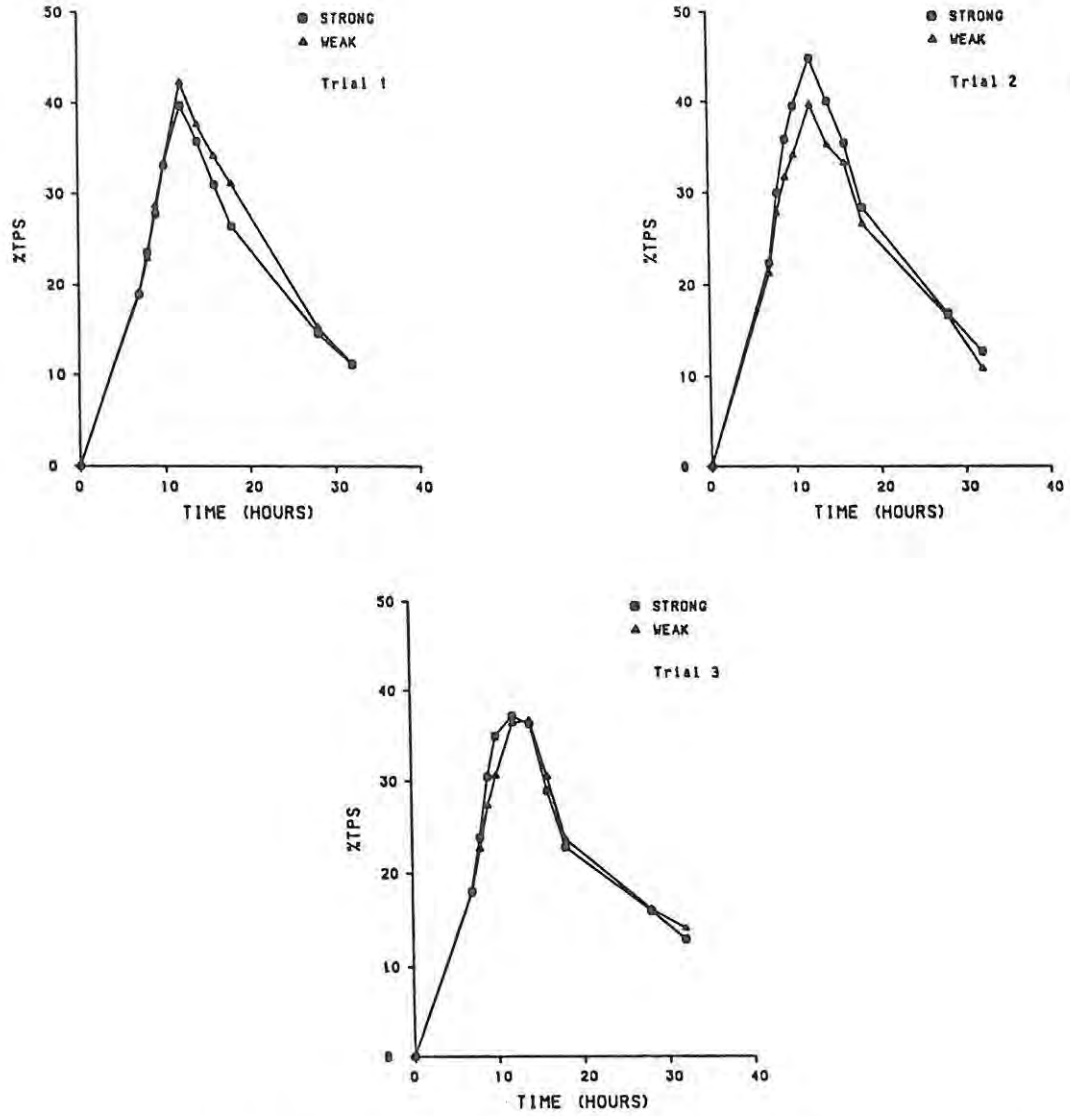


Figure 3.6.2 Blanching profiles of Celestoderm-V cream and Betnovate cream : each set of profiles represents both preparations in one trial on each arm

ficant differences were found at either the 7 hour or 8 hour readings in the series of trials reported here. The similarity in AUC values and the reversal in rank order between arms in trials 1 and 2, combined with the almost identical blanching in trial 3 and the small number of significant differences, can safely be interpreted as there being no difference between the blanching elicited on the two arms in this series of trials.

In our laboratories blanching assays are usually performed in both modes of application simultaneously, where the writing arm (usually the dominant arm) is occluded and the other arm is left unoccluded. This has been purely a practical decision based on the fact that the occlusive dressings are less cumbersome than the guards used to protect the unoc-

cluded application sites, thus allowing the volunteers to continue with their normal routine during the application phase of the trial. These results, considered with the results of Pepler *et al.* (237), indicate that this is acceptable practice, but it was decided to verify this further by assessing the comparative bioavailabilities of the two preparations on the strong and weak arms of the panel of volunteers.

The comparison of Celestoderm-V to Betnovate on the two arms compares favourably with the overall comparison of the combined results discussed in section 3.1. Maximum blanching was found at 14 hours after application on the weak arm for Celestoderm-V where the %TPS was 40,74 as opposed to 40,12 at 12 hours. The weak arm for Betnovate gave a %TPS of 32,64 at 12 hours and 32,56 at 14 hours, thereby eliciting only very slightly superior blanching at the 12 hour reading. All the other maximum responses were observed at 12 hours after application which is consistent with the findings of the pooled results. It can also be seen from the AUC values that Celestoderm-V elicited superior blanching to Betnovate for both arms in all three trials which is also consistent with the findings reported earlier.

A greater number of statistically significant differences were found in the graded response analysis in the pooled results than in the results obtained from the two arms analysed separately. In the weak arm in trial 2, statistically significant differences were only found at three reading times, which does not indicate superiority of one preparation over the other. However, when these results are studied in combination with the AUC values and the comparison of adjacent sites, Celestoderm-V is seen to elicit a superior response to Betnovate. It is therefore evident that the conclusions drawn from the pooled results were not as a result of any significant influence from any one arm, which further justifies the validity of performing the assays in two modes of application as routinely practised in these laboratories.

APPENDIX TO CHAPTER 3

The tables in this appendix contain the %TPS values and AUC values for the two preparations, as well as the chi-squared values for the comparisons. The second digit in the table numbers indicates the section in which the table was utilized. This corresponds to the digit after the point in the subsection headings in the chapter. Significant differences are based on the 95% level of significance and assuming 4 degrees of freedom (df) in the graded response analyses (unless otherwise indicated) and 1 degree of freedom in the analyses of comparisons of adjacent sites. Values greater than the following signify real differences : 1 df - 3,84; 2 df - 5,99; 3 df - 7,81; 4 df - 9,49.

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.

Table 3.1.1 FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR CELESTODERM-V CREAM all observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Trial 1										
0	188	138	93	84	38	72	76	110	248	327
1	359	374	339	275	208	242	281	291	373	316
2	88	124	159	196	233	202	198	201	27	5
3	13	12	57	79	138	118	90	46	0	0
4	0	0	0	14	31	14	3	0	0	0
%TPS	22,15	25,39	31,94	37,04	46,76	40,74	37,00	32,06	16,47	12,58
Trial 2										
0	192	93	72	64	44	56	53	106	246	358
1	328	372	333	299	254	279	313	354	354	248
2	106	123	151	160	190	204	216	170	42	40
3	22	54	70	93	115	82	63	17	6	2
4	0	6	22	32	45	27	3	1	0	0
%TPS	23,38	31,02	36,00	39,58	44,71	40,16	36,50	28,90	17,59	12,89
Trial 3										
0	204	137	92	103	87	90	106	152	251	288
1	368	385	367	289	246	264	305	342	347	331
2	70	115	136	162	174	145	180	133	50	29
3	6	11	47	70	102	107	52	19	0	0
4	0	0	6	24	39	42	5	2	0	0
%TPS	20,29	25,00	31,02	35,46	40,74	40,24	32,45	25,96	17,25	15,01

Table 3.1.2 FREQUENCIES OF BLANCHING SCORES AND %TPS VALUES FOR BETNOVATE CREAM
all observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Trial 1										
0	285	174	153	113	88	102	128	138	319	396
1	319	406	378	358	296	320	346	377	312	250
2	41	64	95	131	183	158	138	115	17	2
3	2	4	21	44	70	63	36	18	0	0
4	1	0	1	2	11	5	0	0	0	0
%TPS	15,86	21,06	24,50	29,32	35,34	32,60	28,16	25,50	13,35	9,80
Trial 2										
0	217	134	100	96	55	73	68	125	285	411
1	346	376	357	331	310	330	377	392	324	204
2	77	100	126	136	164	172	154	110	32	30
3	8	35	53	63	85	58	49	21	7	3
4	0	3	12	22	34	15	0	0	0	0
%TPS	20,22	26,74	31,48	33,95	39,70	35,03	32,10	26,04	15,78	10,53
Trial 3										
0	275	161	108	107	110	116	134	200	285	349
1	338	425	418	370	322	309	352	370	338	286
2	34	56	93	116	137	150	141	74	25	13
3	1	6	25	41	61	53	19	4	0	0
4	0	0	4	14	18	20	2	0	0	0
%TPS	15,78	21,41	26,81	30,13	32,83	32,72	26,97	20,45	14,97	12,04

Table 3.1.3 AREA UNDER THE CURVE VALUES
all observers, all volunteers

	Trial 1	Trial 2	Trial 3
Celestoderm-V	783	785	724
Betnovate	611	691	593

Table 3.1.4 COMPARISON OF ADJACENT SITES : CELESTODERM-V CREAM vs BETNOVATE CREAM
all observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Trial 1										
>	233	267	294	285	317	294	292	262	160	152
<	116	123	122	126	112	118	114	122	106	89
Trial 2										
>	220	267	254	257	265	261	267	241	183	150
<	126	137	156	154	145	151	137	156	132	119
Trial 3										
>	238	256	268	272	289	268	281	250	194	180
<	143	158	152	160	131	135	134	105	107	108

Table 3.1.5 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM
all observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Trial 1	48,44	28,62	50,50	47,03	72,97	42,35	56,85	49,89	16,60	15,57
Trial 2	13,14	14,86	12,94	17,59	14,72	16,78	22,93	17,78	5,62	9,56
Trial 3	27,83	25,74	19,79	27,85	35,31	32,93	27,99	36,25	10,61	15,22
Paired Comparison										
Trial 1	38,56	52,43	70,29	60,74	97,01	74,33	77,17	50,32	10,56	15,95
Trial 2	25,00	41,19	22,95	25,31	34,54	28,84	41,19	17,77	7,94	3,35
Trial 3	23,19	22,73	31,49	28,52	58,69	43,24	51,36	58,41	24,57	17,50

Table 3.1.6 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN TRIALS
all observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
T1 vs T2	5,43	41,50	26,26	15,53	14,06	15,24	11,37	23,17	9,77	38,82
T1 vs T3	5,39	0,55	9,87	8,68	37,26	26,86	17,45	37,90	7,83	19,76
T2 vs T3	19,17	43,36	18,54	13,68	16,15	24,87	22,60	13,37	6,82	23,24
Betnovate										
T1 vs T2	25,89	41,89	39,20	22,57	22,19	10,76	22,56	1,28	13,73	32,44
T1 vs T3	2,71	1,87	11,94	10,38	12,46	11,16	7,48	29,24	4,48	13,45
T2 vs T3	29,03	41,39	24,13	10,78	29,85	12,92	38,23	36,55	8,16	28,50

(T = Trial)

Table 3.2.1 TEMPERATURE (°C) AND RELATIVE HUMIDITY (%)

Time	Trial 1		Trial 2		Trial 3	
	Temp	RH	Temp	RH	Temp	RH
08h00 (0)*	9	75	9	80	11	80
10h00 (2)	13	70	19	40	12	70
12h00 (4)	15	55	22	32	19	57
14h00 (6)	16	50	23	30	19	65
16h00 (8)	15	55	20	35	18	67
18h00 (10)	12	80	15	75	15	80
20h00 (12)	10	90	11	87	13	86
22h00 (14)	10	90	10	87	13	86
00h01 (16)	11	87	10	87	13	85
02h00 (18)	9	90	9	87	13	87
12h00 (28)	23	25	18	45	21	60
16h00 (32)	25	17	18	37	17	80

* The figures in parentheses indicate the number of hours after application

Table 3.3.1 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM individual observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Trial 1										
Observer 1	23,50	26,50	33,45	39,70	50,58	45,02	40,86	36,46	11,46	9,03
Observer 2	19,79	26,50	35,30	40,97	52,66	44,68	39,00	30,90	18,63	12,15
Observer 3	23,15	23,15	27,08	30,44	37,04	32,52	31,13	28,82	19,33	16,55
Trial 2										
Observer 1	23,73	32,06	39,81	42,82	47,57	41,32	36,92	26,97	15,28	14,58
Observer 2	23,03	32,75	38,08	45,25	54,75	49,65	43,40	33,10	19,33	14,00
Observer 3	23,38	28,24	30,09	30,67	31,83	29,51	29,17	26,62	18,17	10,07
Trial 3										
Observer 1	24,19	28,59	36,00	41,55	45,95	45,60	36,57	25,81	15,28	12,62
Observer 2	20,25	28,13	34,26	39,70	47,22	48,26	37,04	30,21	17,13	15,05
Observer 3	16,44	18,29	22,80	25,12	29,05	26,85	23,73	21,88	19,33	17,36
Betnovate										
Trial 1										
Observer 1	18,29	22,92	25,81	29,40	36,81	33,80	29,63	29,51	8,80	7,41
Observer 2	12,96	21,53	27,89	33,45	42,25	37,96	31,48	24,65	16,44	8,91
Observer 3	16,32	18,75	19,79	25,12	26,97	26,04	23,38	22,34	14,81	13,08
Trial 2										
Observer 1	19,56	27,31	35,30	36,23	43,06	34,49	31,94	24,07	13,08	12,15
Observer 2	19,79	27,66	34,38	40,16	47,92	44,10	37,85	29,17	17,82	11,69
Observer 3	21,30	25,23	24,77	25,46	28,13	26,50	26,50	24,88	16,44	7,75
Trial 3										
Observer 1	20,02	24,54	30,09	35,76	38,66	36,23	30,56	18,98	13,19	9,49
Observer 2	14,47	23,38	31,71	34,14	38,66	41,32	30,21	23,03	13,31	11,69
Observer 3	12,85	16,32	18,63	20,49	21,18	20,60	20,14	19,33	18,40	14,93

Table 3.3.2 AREA UNDER THE CURVE VALUES
individual observers, all volunteers

	Trial 1		Trial 2		Trial 3	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Observer						
1	803	619	781	674	767	624
2	815	656	888	784	799	639
3	731	558	686	616	605	516

Table 3.3.3 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN TRIALS
individual observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Observer 1										
T1 vs T2	5,96	18,40	20,81	4,90	8,66	13,94	4,82	37,34	8,13	18,09
T1 vs T3	3,54	3,80	12,38	11,94	22,44	4,53	13,61	32,11	6,83	9,16
T2 vs T3	14,21	20,98	15,51	4,54	6,25	9,82	5,57	6,54	8,12	3,51
Observer 2										
T1 vs T2	9,09	22,00	14,74	19,42	3,45	20,64	15,60	4,41	5,16	9,60
T1 vs T3	0,93	1,89	0,75	2,81	6,60	16,64	11,44	3,64	10,59	15,92
T2 vs T3	8,49	16,79	14,44	18,36	13,15	23,91	32,01	2,95	2,86	1,29
Observer 3										
T1 vs T2	1,74	13,21	4,35	5,12	9,99	18,45	15,63	12,90	1,26	52,73
T1 vs T3	24,50	13,56	12,36	10,59	21,82	22,17	29,79	34,20	0,00	2,99
T2 vs T3	25,46	43,23	21,91	18,59	11,72	6,96	20,09	15,00	1,26	71,19
Betnovate										
Observer 1										
T1 vs T2	5,18	16,17	19,62	21,44	11,41	6,70	7,11	13,71	10,78	15,10
T1 vs T3	4,17	1,30	9,14	10,87	9,35	3,31	13,58	29,47	9,88	3,63
T2 vs T3	7,91	15,82	18,36	9,65	6,47	2,02	12,74	12,81	9,99	7,33
Observer 2										
T1 vs T2	21,31	18,00	18,66	23,06	13,70	16,56	20,73	11,52	5,12	7,77
T1 vs T3	2,75	4,48	12,13	11,61	3,34	10,91	9,41	1,43	7,10	7,17
T2 vs T3	15,38	14,15	4,31	13,07	23,31	16,86	37,12	15,10	9,34	0,09
Observer 3										
T1 vs T2	11,92	19,33	11,21	0,53	8,96	7,23	11,19	17,81	3,98	45,87
T1 vs T3	9,15	6,62	4,96	9,14	17,27	14,67	11,67	17,42	10,12	2,99
T2 vs T3	34,57	37,97	22,28	11,28	22,97	18,59	23,91	20,04	5,18	60,89

(T = Trial)

Table 3.3.4 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN TRIALS
comparison of blanching responses of individual observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Trial 1										
Observers										
1 vs 2	10,77	17,57	18,69	19,10	9,65	18,31	19,95	41,55	32,96	8,20
1 vs 3	21,30	16,74	31,54	29,01	66,95	67,76	61,77	64,77	70,17	39,49
2 vs 3	21,81	11,71	24,31	30,38	55,43	44,34	22,77	6,49	14,74	13,01
Trial 2										
1 vs 2	9,84	30,78	68,34	35,27	24,95	41,96	35,39	10,46	20,75	3,60
1 vs 3	30,52	43,26	42,81	59,67	75,08	84,82	85,13	38,14	54,55	9,56
2 vs 3	6,52	13,95	31,26	49,39	105,15	102,36	77,78	47,96	30,74	10,77
Trial 3										
1 vs 2	8,57	9,46	12,47	59,25	33,38	9,33	2,80	10,30	2,67	2,84
1 vs 3	34,41	46,26	49,81	64,19	75,02	92,31	97,90	55,99	64,32	42,44
2 vs 3	18,39	47,13	45,64	57,53	65,87	87,05	92,03	59,98	45,46	39,35
Betnovate										
Trial 1										
Observers										
1 vs 2	12,99	9,43	14,76	16,93	18,83	32,55	30,71	24,83	37,28	1,81
1 vs 3	7,68	18,35	24,04	21,04	36,10	61,70	55,38	38,54	44,74	25,63
2 vs 3	6,35	5,39	26,63	24,39	54,33	46,32	25,73	4,34	10,24	14,31
Trial 2										
1 vs 2	11,67	29,99	52,98	72,36	33,21	58,42	40,61	14,56	27,90	4,31
1 vs 3	17,48	25,10	43,61	77,55	62,50	56,94	47,64	52,07	35,31	10,73
2 vs 3	2,77	6,86	35,48	62,29	94,70	114,72	61,58	30,83	17,49	12,86
Trial 3										
1 vs 2	17,63	17,18	23,16	52,84	38,54	27,29	1,27	26,88	1,22	2,96
1 vs 3	29,39	40,64	49,39	56,56	84,37	72,96	76,19	73,98	51,34	26,84
2 vs 3	2,65	33,48	78,12	74,21	85,59	106,76	67,39	29,30	39,24	19,57

Table 3.3.5 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM individual observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Observer 1										
Trial 1	11,63	7,00	16,47	20,49	31,16	17,78	22,25	18,49	3,82	1,97
Trial 2	6,52	5,49	3,18	7,96	4,39	9,93	8,09	3,01	3,93	3,65
Trial 3	7,22	5,74	8,14	8,31	8,09	14,31	12,27	12,28	2,94	5,88
Observer 2										
Trial 1	21,48	13,98	18,19	17,08	23,33	13,60	17,65	20,35	2,61	7,52
Trial 2	8,13	9,06	7,52	7,57	13,49	11,56	12,47	10,32	0,95	2,77
Trial 3	19,55	21,09	8,45	20,47	18,58	20,75	14,75	25,79	7,67	5,89
Observer 3										
Trial 1	23,82	12,43	21,43	11,83	31,45	22,46	24,81	22,02	16,06	9,09
Trial 2	1,87	7,46	10,25	9,85	8,93	14,08	20,81	20,69	4,35	3,53
Trial 3	8,12	5,90	14,79	12,68	28,48	15,80	10,04	9,08	0,90	6,05
Paired Comparison										
Observer 1										
Trial 1	11,51	11,67	13,48	25,44	31,61	22,90	16,01	11,35	0,96	4,90
Trial 2	12,27	13,50	5,33	13,15	13,31	10,32	15,59	5,26	3,11	0,78
Trial 3	2,40	7,29	10,13	5,26	16,67	17,78	18,01	23,63	6,59	5,59
Observer 2										
Trial 1	13,67	21,82	35,17	18,13	27,05	23,35	26,41	14,86	4,73	2,87
Trial 2	4,66	12,84	7,78	5,52	8,35	9,97	11,38	5,39	1,79	1,48
Trial 3	14,26	5,65	8,44	10,75	12,23	12,82	18,67	16,46	0,44	8,64
Observer 3										
Trial 1	12,99	22,01	23,55	16,70	40,50	28,90	40,48	27,28	6,56	10,30
Trial 2	8,28	14,04	10,34	6,94	13,96	7,81	15,13	7,11	3,02	0,72
Trial 3	10,13	11,07	13,93	13,69	36,97	12,01	6,06	18,78	4,00	2,44

Table 3.3.6 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM
two observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Observers 1 + 2										
Trial 1	21,65	26,50	34,38	40,34	51,62	44,85	39,93	33,68	15,05	10,59
Trial 2	23,38	32,41	38,95	44,04	51,16	45,49	40,16	30,04	17,31	14,29
Trial 3	22,22	28,36	35,13	40,63	46,59	46,93	36,81	28,01	16,21	13,84
Observers 1 + 3										
Trial 1	23,33	24,83	30,27	35,07	43,81	38,77	36,00	32,64	15,40	12,79
Trial 2	23,56	30,15	34,95	36,75	39,70	35,42	33,05	26,80	16,73	12,33
Trial 3	20,32	23,44	29,40	33,34	37,50	36,23	30,15	23,85	17,31	14,99
Observers 2 + 3										
Trial 1	21,47	24,83	31,19	35,71	44,85	38,60	35,07	29,86	18,98	14,35
Trial 2	23,21	30,50	34,09	37,96	43,29	39,58	36,29	29,86	18,75	12,04
Trial 3	18,35	23,21	28,53	32,41	38,14	37,56	30,39	26,05	18,23	16,21
Betnovate										
Observers 1 + 2										
Trial 1	15,63	22,23	26,85	31,43	39,53	35,88	30,56	27,08	12,62	8,16
Trial 2	19,68	27,49	34,84	38,20	45,49	39,30	34,90	26,62	15,45	11,92
Trial 3	17,25	23,96	30,90	34,95	38,66	38,78	30,39	21,01	13,25	10,59
Observers 1 + 3										
Trial 1	17,31	20,84	22,80	27,26	31,89	29,92	26,51	25,93	11,81	10,25
Trial 2	20,43	26,27	30,04	30,85	35,60	30,50	29,22	24,48	14,76	9,95
Trial 3	16,44	20,43	24,36	28,13	29,92	28,42	25,35	19,16	15,80	12,21
Observers 2 + 3										
Trial 1	14,64	20,14	23,84	29,29	34,61	32,00	27,43	23,50	15,63	11,00
Trial 2	27,55	26,45	29,58	32,81	38,03	35,30	32,18	27,03	17,13	9,72
Trial 3	13,66	19,85	24,90	27,32	29,92	30,96	25,18	21,18	15,86	13,31

Table 3.3.7 AREA UNDER THE CURVE VALUES
two observers, all volunteers

	Trial 1		Trial 2		Trial 3	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Observers						
1 + 2	809	638	834	729	783	632
1 + 3	767	589	733	645	686	570
2 + 3	773	607	787	700	702	577

Table 3.3.8 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM
two observers, all volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Observers 1 + 2										
Trial 1	29,17	18,35	33,71	37,22	54,03	29,07	38,78	34,24	5,94	8,38
Trial 2	14,18	11,73	6,24	11,07	14,32	16,81	20,24	10,37	3,68	6,40
Trial 3	21,16	22,44	13,70	23,65	23,82	32,10	26,94	30,79	9,73	11,62
Observers 1 + 3										
Trial 1	30,32	15,80	33,29	30,87	53,79	32,25	40,87	30,54	15,19	9,09
Trial 2	6,79	8,55	8,59	14,58	7,83	13,90	14,45	10,69	5,26	6,92
Trial 3	13,65	8,83	16,90	12,21	20,04	21,05	15,56	16,45	3,66	9,51
Observers 2 + 3										
Trial 1	41,76	26,13	36,58	27,32	45,73	29,05	37,43	40,82	14,52	15,07
Trial 2	6,88	13,47	15,21	12,98	12,15	9,59	16,96	20,10	3,48	5,99
Trial 3	26,46	24,76	12,43	25,58	34,25	20,33	17,38	30,35	8,48	9,66
Paired comparison										
Observers 1 + 2										
Trial 1	25,66	33,67	47,35	43,77	59,21	46,98	42,78	26,76	0,08	7,84
Trial 2	16,38	26,86	13,49	18,08	21,76	20,75	27,18	11,02	5,00	2,41
Trial 3	14,10	13,28	18,99	16,05	29,19	30,96	37,34	40,03	1,06	14,69
Observers 1 + 3										
Trial 1	24,22	31,69	34,83	42,86	70,97	50,91	50,34	35,37	5,50	14,13
Trial 2	21,17	28,28	14,88	20,68	27,00	13,94	30,48	12,21	6,33	1,68
Trial 3	9,64	17,32	23,20	17,27	48,99	30,36	24,33	42,96	11,01	8,45
Observers 2 + 3										
Trial 1	26,98	43,44	59,37	34,65	64,56	50,87	63,00	39,52	10,16	10,60
Trial 2	12,65	27,12	17,58	12,20	20,65	17,99	24,96	12,20	4,52	2,42
Trial 3	24,90	15,13	20,98	24,12	41,76	24,83	24,90	34,11	0,07	11,44

Table 3.4.1 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM : TRIAL 1
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Volunteer										
1	23,61	30,56	39,58	47,92	56,25	54,17	42,36	36,11	18,75	18,75
2	31,94	38,89	52,08	66,67	70,14	58,33	55,56	40,97	12,50	10,42
3	41,67	45,83	57,64	65,28	75,69	66,67	53,47	47,22	26,39	13,89
4	10,42	17,36	22,22	25,69	37,50	27,08	35,42	36,11	18,06	11,11
5	23,61	25,69	35,42	43,06	50,69	43,06	38,89	38,19	18,06	11,81
6	19,44	23,61	27,08	24,31	35,42	28,47	29,86	28,47	15,28	6,94
7	12,50	14,58	18,75	24,31	40,97	39,58	36,11	28,47	17,36	20,83
8	10,42	13,19	15,28	16,67	25,00	22,22	14,58	11,81	4,17	4,17
9	9,03	8,33	11,11	15,97	23,61	21,53	18,75	18,75	11,11	9,72
10	30,56	45,83	49,31	51,39	60,42	63,89	64,58	50,69	16,67	11,81
11	37,50	33,33	36,11	44,44	46,53	52,08	27,78	26,39	15,97	12,50
12	18,06	20,83	25,00	36,81	48,61	36,11	38,89	34,72	17,36	11,11
13	20,14	22,22	31,94	35,42	50,69	50,00	44,44	45,14	15,28	15,28
14	22,22	23,61	29,86	36,11	47,92	50,69	47,22	34,03	17,36	14,58
15	46,53	40,28	54,86	62,50	69,44	46,53	47,22	45,14	32,64	19,44
16	20,83	28,47	35,42	38,89	45,14	41,67	35,42	28,47	18,75	14,58
17	7,64	9,72	15,97	9,03	18,75	11,11	12,50	5,56	8,33	6,94
18	12,50	14,58	17,36	22,22	38,89	20,14	22,92	20,83	12,50	12,50
Betnovate										
Volunteer										
1	22,22	27,78	39,58	46,53	57,64	53,47	46,53	40,97	22,92	22,22
2	13,89	27,77	30,56	37,50	45,83	38,19	36,11	26,39	8,33	6,25
3	36,11	36,81	50,69	54,86	67,36	58,33	45,83	35,42	15,23	6,25
4	10,42	19,44	20,83	22,22	36,11	23,61	32,64	29,86	13,19	9,03
5	9,72	15,28	16,67	21,53	31,25	22,22	22,92	24,31	13,19	6,94
6	10,42	20,14	15,28	18,75	15,97	18,75	16,67	16,67	8,33	5,56
7	8,33	10,42	11,81	19,44	27,78	30,56	27,78	20,14	11,81	9,72
8	7,64	4,86	11,81	16,67	18,75	22,22	18,06	13,89	2,78	2,78
9	6,94	10,42	14,58	15,97	17,36	14,58	13,19	17,36	7,64	5,56
10	16,67	27,78	30,56	31,94	39,58	40,97	43,06	36,11	16,67	13,89
11	32,64	35,42	34,03	43,75	44,44	54,86	22,92	28,47	15,28	13,19
12	19,44	22,22	25,00	34,72	40,97	34,03	31,94	29,86	15,97	11,81
13	11,11	18,75	20,14	24,31	30,56	34,03	27,78	29,17	11,11	6,25
14	15,97	20,14	22,92	26,39	32,64	37,50	29,17	25,69	10,42	12,50
15	27,78	29,86	38,89	49,31	54,86	34,72	37,50	37,50	29,17	17,36
16	14,58	25,00	29,86	32,64	37,50	37,50	27,08	25,69	18,06	11,81
17	6,94	9,72	6,94	6,94	9,72	9,72	8,33	4,86	5,56	6,94
18	14,58	17,36	20,83	24,31	27,78	21,53	19,44	16,67	14,58	8,33

Table 3.4.2 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM : TRIAL 2
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Volunteer										
1	53,47	64,58	78,47	86,11	84,72	75,00	63,19	56,25	46,53	40,28
2	31,25	35,42	39,58	52,08	59,03	42,36	27,78	22,22	15,97	0,69
3	29,86	41,67	43,75	50,69	58,33	28,47	27,78	18,06	15,97	17,36
4	11,11	20,83	29,86	38,89	43,75	40,28	41,67	41,67	12,50	4,86
5	25,00	33,33	37,50	38,89	50,69	48,61	43,06	29,17	17,36	17,36
6	22,92	27,78	35,42	40,28	47,22	45,14	45,14	35,42	15,28	7,64
7	50,00	54,86	67,36	67,36	72,22	69,44	53,47	38,19	18,75	9,72
8	11,11	15,97	21,53	23,61	25,00	23,61	22,92	12,50	6,25	0,00
9	4,17	20,14	26,39	31,94	37,50	31,94	36,81	22,92	14,58	6,94
10	27,08	32,64	42,36	43,75	43,06	39,58	38,19	38,19	20,14	17,36
11	39,58	54,86	51,39	54,17	50,69	50,69	40,97	27,08	20,83	16,67
12	21,53	19,44	34,03	36,81	36,11	37,50	33,33	29,17	12,50	10,42
13	13,89	20,14	20,83	22,92	23,61	31,25	30,56	22,92	15,97	9,72
14	21,53	27,78	29,17	27,78	36,11	39,58	35,42	34,03	15,97	15,28
15	22,92	34,72	34,72	36,81	53,47	43,06	40,28	25,00	29,86	31,94
16	16,67	21,53	22,22	20,14	33,33	25,69	30,56	27,78	14,58	7,64
17	9,72	17,36	20,14	25,00	28,47	28,47	25,00	20,83	13,89	11,81
18	9,03	15,28	13,19	15,28	21,53	22,22	20,83	18,75	9,72	6,25
Betnovate										
Volunteer										
1	45,14	61,11	68,06	74,31	80,56	65,28	63,19	52,78	44,44	40,97
2	25,69	32,64	40,28	50,00	57,64	45,83	31,25	20,14	18,06	3,47
3	22,92	36,11	40,28	46,53	52,08	21,53	22,92	11,11	5,56	6,25
4	14,58	20,14	24,31	31,94	39,58	38,19	35,42	34,72	8,33	4,86
5	18,06	20,83	23,61	26,39	31,25	32,64	28,47	21,53	9,03	9,72
6	18,06	25,00	31,94	34,72	43,06	42,36	40,97	34,03	18,06	11,81
7	43,06	47,22	65,28	62,50	70,14	61,81	46,53	37,50	17,36	6,94
8	12,50	17,36	20,14	23,61	27,08	27,78	22,22	14,58	9,03	2,08
9	13,19	21,53	29,17	31,94	38,19	27,08	32,64	21,53	9,72	3,47
10	22,92	23,61	30,56	30,56	32,64	28,47	29,86	30,56	16,67	9,72
11	33,33	49,31	45,14	46,53	44,44	42,36	38,89	29,17	16,67	13,19
12	18,75	20,83	29,86	29,86	32,64	29,86	29,17	26,39	18,06	8,33
13	9,72	11,11	15,28	15,28	18,75	23,61	23,61	19,44	10,42	6,94
14	12,50	19,44	24,31	25,69	31,25	36,11	30,56	30,56	13,19	11,81
15	22,22	28,47	31,94	35,42	47,92	39,58	36,81	25,69	28,47	27,78
16	11,81	18,75	16,67	16,67	27,08	23,61	25,69	24,31	15,28	10,42
17	10,42	11,11	11,81	13,19	19,44	21,53	20,83	14,58	13,89	7,64
18	9,03	16,67	18,06	15,97	20,83	22,92	18,75	20,14	11,81	4,17

Table 3.4.3 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM : TRIAL 3
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Volunteer										
1	28,47	39,58	51,39	60,42	67,36	61,11	44,44	32,64	29,86	25,69
2	22,92	31,25	36,11	47,92	50,69	40,28	38,89	18,06	25,00	20,14
3	36,81	45,14	60,42	64,58	77,08	81,25	47,22	45,14	13,19	10,42
4	25,69	27,78	40,97	49,31	57,64	59,03	49,31	43,75	16,67	19,44
5	18,75	24,31	23,61	29,86	48,61	45,83	38,89	34,72	21,53	22,22
6	11,81	18,75	22,92	20,14	25,00	27,78	25,00	23,61	15,28	14,58
7	14,58	17,36	21,53	29,17	39,58	31,94	29,86	22,22	24,31	22,92
8	12,50	15,28	23,61	28,47	25,69	23,61	17,36	11,11	5,56	4,17
9	13,89	20,83	23,61	24,31	20,14	28,47	20,14	23,61	14,58	9,03
10	27,08	38,89	46,53	52,78	56,94	51,39	45,83	45,14	31,94	22,22
11	29,17	30,56	36,11	47,92	44,44	50,69	38,19	27,08	12,50	11,11
12	9,72	15,97	23,61	21,53	23,61	22,22	19,44	15,97	11,11	11,81
13	15,28	18,06	20,14	22,22	24,31	25,00	23,61	16,67	9,72	9,72
14	21,53	22,92	29,17	31,94	39,58	42,36	40,97	27,08	20,14	18,75
15	38,89	37,50	46,53	60,42	67,36	61,81	53,47	30,56	26,39	23,61
16	21,53	22,22	27,78	29,17	36,81	46,53	32,64	31,25	18,75	15,97
17	5,56	9,72	9,03	7,64	15,97	10,42	8,33	9,03	7,64	4,86
18	11,11	13,89	15,28	10,42	12,50	14,58	10,42	9,72	6,25	3,47
Betnovate										
Volunteer										
1	19,44	29,86	38,89	47,92	50,69	43,75	32,64	27,78	26,39	21,53
2	21,53	27,08	29,86	36,81	40,28	31,25	31,25	15,28	24,31	20,14
3	24,31	33,33	47,92	53,47	61,11	65,28	36,11	27,78	5,56	7,64
4	17,36	24,31	34,03	39,58	45,14	43,06	39,58	35,42	13,89	15,97
5	15,28	20,83	22,92	25,69	34,72	33,33	28,47	23,61	14,58	11,81
6	13,19	20,83	22,22	21,53	22,92	22,92	25,00	18,75	13,19	11,81
7	7,64	13,19	18,06	21,53	27,08	28,47	27,78	20,83	20,83	20,14
8	16,67	24,31	27,78	29,17	25,69	18,75	15,97	11,81	6,94	6,25
9	9,03	15,97	20,83	20,83	20,83	27,08	18,06	15,97	11,81	4,86
10	13,89	23,61	29,17	31,25	34,72	33,33	28,47	26,39	24,31	19,44
11	24,31	27,08	36,81	43,75	42,36	48,61	44,44	30,56	16,67	15,97
12	9,03	11,11	18,06	20,14	23,61	18,75	16,67	13,89	9,72	4,17
13	9,72	11,81	20,14	14,58	15,28	20,14	10,42	8,33	6,25	4,17
14	9,72	18,06	19,44	21,53	29,86	30,56	30,56	20,14	13,89	12,50
15	26,39	31,25	39,58	48,61	54,17	47,22	41,67	25,00	25,00	18,75
16	17,36	23,61	25,69	29,86	40,28	50,00	34,72	30,56	18,75	13,89
17	13,89	11,81	13,19	15,97	9,72	11,11	10,42	4,86	9,03	4,86
18	15,28	17,36	18,06	20,14	12,50	15,28	13,19	11,11	8,33	2,78

Table 3.4.4 AREA UNDER THE CURVE VALUES
individual volunteers, all observers

	Trial 1		Trial 2		Trial 3	
	Celestoderm-V	Betnovate	Celestoderm-V.	Betnovate	Celestoderm-V	Betnovate
Volunteer						
1	927	992	1676	1549	1097	867
2	1041	640	783	774	814	706
3	1256	989	765	549	1145	804
4	685	590	752	653	996	784
5	865	508	845	552	837	604
6	644	377	825	788	552	498
7	687	480	1193	1096	693	572
8	327	298	379	432	379	415
9	417	336	577	552	536	424
10	1117	782	896	693	1120	716
11	834	814	972	867	794	829
12	772	706	692	655	438	373
13	887	570	571	423	466	287
14	843	597	753	627	773	533
15	1240	972	912	860	1111	898
16	782	671	604	534	762	756
17	260	192	523	408	239	269
18	517	489	410	432	273	339

Table 3.4.5 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN TRIALS
Celestoderm-V, individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Volunteer 1										
T1 vs T2	29,59^{2}	30,36^{2}	36,55^{3}	32,71^{3}	24,18^{2}	10,71^{3}	14,38^{2}	21,17^{2}	32,41^{2}	27,69^{1}
T1 vs T3	4,19^{2}	4,68^{1}	4,68^{2}	3,18^{2}	6,00^{3}	2,70^{3}	0,12^{2}	1,44^{1}	8,38^{2}	1,42^{1}
T2 vs T3	27,83^{2}	22,13^{2}	21,25^{3}	19,05^{2}	8,57^{2}	6,82^{3}	16,30^{2}	30,81^{2}	9,40^{1}	11,80^{1}
Volunteer 2										
T1 vs T2	0,25^{1}	2,01^{1}	7,34^{2}	6,26^{2}	4,11^{3}	6,23^{2}	23,90^{3}	10,46^{2}	1,42^{1}	15,75^{1}
T1 vs T3	15,86^{1}	4,50^{1}	14,15^{2}	11,76^{2}	10,75^{3}	11,88^{2}	12,20^{2}	20,30^{2}	7,41^{1}	9,76^{1}
T2 vs T3	12,98^{1}	0,52^{1}	0,06^{1}	1,56^{2}	1,42^{2}	1,13^{2}	6,04^{2}	4,06^{2}	2,49^{1}	42,09^{1}
Volunteer 3										
T1 vs T2	4,68^{1}	0,60^{2}	7,79^{2}	6,85^{2}	13,44^{3}	37,62^{2}	23,73^{2}	37,98^{2}	4,74^{1}	1,48^{1}
T1 vs T3	0,06^{1}	0,00^{2}	0,12^{2}	1,03^{2}	3,13^{2}	11,41^{2}	1,61^{2}	3,82^{2}	10,80^{1}	2,74^{1}
T2 vs T3	3,74^{1}	0,60^{2}	8,37^{2}	3,06^{2}	8,83^{3}	48,55^{3}	15,32^{2}	26,13^{2}	1,42^{1}	8,02^{1}
Volunteer 4										
T1 vs T2	0,06^{1}	1,79^{1}	4,80^{2}	9,00^{1}	0,50^{1}	8,67^{1}	6,77^{1}	0,23^{1}	3,74^{1}	5,17^{1}
T1 vs T3	13,33^{1}	9,30^{2}	17,71^{1}	29,41^{1}	21,65^{2}	26,84^{2}	9,40^{2}	0,23^{1}	0,57^{1}	5,71^{1}
T2 vs T3	11,80^{1}	7,45^{2}	5,63^{1}	9,12^{2}	5,58^{2}	6,36^{2}	4,23^{2}	0,00^{1}	1,42^{1}	20,20^{1}
Volunteer 5										
T1 vs T2	1,86^{1}	7,41^{1}	0,22^{1}	1,44^{1}	0,31^{2}	1,56^{2}	3,66^{1}	7,41^{1}	0,00^{1}	3,66^{1}
T1 vs T3	2,49^{1}	0,00^{1}	17,33^{1}	9,40^{1}	0,77^{2}	6,03^{2}	0,89^{1}	0,06^{1}	0,07^{1}	8,67^{1}
T2 vs T3	8,04^{1}	14,23^{1}	20,66^{1}	3,66^{1}	2,03^{2}	3,89^{2}	0,96^{1}	6,25^{1}	0,07^{1}	1,19^{1}
Volunteer 6										
T1 vs T2	0,35^{1}	0,00^{1}	4,74^{1}	10,36^{1}	6,14^{2}	12,99^{1}	9,45^{1}	0,96^{1}	0,23^{1}	0,07^{1}
T1 vs T3	7,17^{1}	1,42^{1}	0,00^{1}	0,32^{1}	5,79^{1}	0,08^{1}	8,69^{1}	8,99^{2}	0,06^{1}	5,71^{1}
T2 vs T3	10,36^{1}	0,00^{1}	10,19^{1}	22,14^{2}	20,84^{1}	11,20^{1}	30,25^{1}	10,67^{1}	0,06^{1}	4,59^{1}
Volunteer 7										
T1 vs T2	50,21^{2}	49,58^{3}	44,40^{2}	40,78^{1}	27,61^{3}	21,85^{3}	9,28^{2}	3,74^{1}	0,96^{1}	11,20^{1}
T1 vs T3	0,22^{1}	0,96^{1}	0,28^{1}	3,60^{1}	1,06^{2}	2,01^{1}	0,98^{2}	1,74^{2}	2,89^{1}	0,08^{1}
T2 vs T3	46,13^{2}	48,98^{3}	42,37^{3}	37,96^{2}	24,85^{3}	30,68^{2}	18,45^{2}	16,50^{2}	8,83^{2}	12,99^{1}
Volunteer 8										
T1 vs T2	0,06^{1}	0,91^{1}	1,60^{1}	1,68^{1}	0,23^{2}	0,98^{2}	3,13^{1}	0,06^{1}	0,76^{1}	0,00^{1}
T1 vs T3	0,06^{1}	0,06^{1}	2,36^{1}	10,94^{2}	0,09^{2}	0,98^{2}	0,23^{1}	0,06^{1}	0,35^{1}	0,00^{1}
T2 vs T3	0,00^{1}	1,42^{1}	0,08^{1}	1,72^{2}	0,09^{2}	0,00^{2}	1,68^{1}	0,22^{1}	0,08^{1}	0,00^{1}
Volunteer 9										
T1 vs T2	2,67^{1}	14,40^{1}	18,46^{1}	16,93^{2}	5,79^{1}	10,44^{2}	18,27^{2}	1,73^{2}	1,39^{1}	1,60^{1}
T1 vs T3	3,60^{1}	14,40^{1}	8,42^{1}	6,55^{1}	1,90^{2}	1,99^{2}	0,59^{2}	1,60^{2}	0,89^{1}	0,06^{1}
T2 vs T3	11,80^{1}	0,00^{1}	2,76^{2}	3,77^{1}	16,48^{2}	7,02^{2}	15,07^{2}	0,11^{2}	0,06^{1}	1,05^{1}
Volunteer 10										
T1 vs T2	0,28^{1}	14,27^{1}	9,14^{2}	5,47^{2}	13,27^{2}	25,42^{2}	31,29^{2}	10,80^{1}	0,61^{1}	1,40^{1}
T1 vs T3	2,89^{1}	3,85^{1}	2,38^{2}	1,40^{2}	1,40^{1}	8,69^{2}	15,25^{2}	2,11^{1}	22,08^{2}	8,67^{1}
T2 vs T3	1,42^{1}	3,66^{1}	2,41^{2}	3,73^{2}	9,84^{2}	6,40^{2}	5,30^{2}	3,74^{1}	6,40^{1}	3,29^{1}
Volunteer 11										
T1 vs T2	1,42^{1}	23,30^{2}	16,00^{2}	9,94^{2}	0,62^{2}	2,20^{2}	7,41^{1}	1,42^{1}	1,68^{1}	0,22^{1}
T1 vs T3	2,83^{1}	0,25^{1}	0,06^{1}	3,98^{2}	0,52^{2}	10,95^{2}	5,17^{1}	0,11^{1}	1,42^{1}	0,22^{1}
T2 vs T3	8,02^{1}	22,17^{2}	7,38^{2}	2,15^{2}	1,37^{2}	3,66^{2}	0,27^{2}	1,04^{2}	6,02^{1}	0,89^{1}
Volunteer 12										
T1 vs T2	0,07^{1}	3,50^{1}	6,24^{1}	0,22^{1}	10,70^{2}	0,06^{1}	2,03^{1}	0,25^{1}	3,66^{1}	0,23^{1}
T1 vs T3	9,45^{1}	4,43^{1}	0,00^{1}	15,13^{1}	25,50^{2}	13,88^{2}	22,89^{2}	18,77^{2}	6,77^{1}	0,00^{1}
T2 vs T3	8,02^{1}	0,06^{1}	10,19^{1}	12,09^{2}	4,83^{2}	8,64^{2}	7,45^{2}	8,86^{2}	0,51^{1}	0,23^{1}

superscipted numerals denote degrees of freedom - see introduction to this chapter

Volunteer 13										
T1 vs T2	5,17[1]	0,40[1]	12,98[1]	17,33[1]	25,36[2]	20,23[2]	18,23[1]	26,13[1]	0,00[1]	4,50[1]
T1 vs T3	3,29[1]	2,89[1]	12,89[1]	9,97[1]	20,94[2]	27,78[2]	25,46[1]	32,49[2]	3,57[1]	3,56[1]
T2 vs T3	0,23[1]	1,19[1]	0,09[1]	1,60[1]	1,64[2]	0,97[1]	0,97[1]	4,43[1]	3,57[1]	0,06[1]
Volunteer 14										
T1 vs T2	0,97[1]	0,00[1]	0,00[1]	4,96[1]	6,99[1]	6,86[2]	5,71[1]	0,24[1]	0,55[1]	0,23[1]
T1 vs T3	0,97[1]	0,00[1]	0,76[1]	0,91[1]	3,00[1]	5,68[2]	1,00[1]	3,77[1]	1,19[1]	2,17[1]
T2 vs T3	0,00[1]	0,00[1]	0,76[1]	1,68[1]	0,89[1]	1,42[1]	2,01[1]	5,79[1]	3,29[1]	1,00[1]
Volunteer 15										
T1 vs T2	29,14[1]	3,56[1]	19,21[2]	28,38[2]	7,65[2]	0,32[2]	6,67[2]	12,99[1]	1,79[1]	12,56[2]
T1 vs T3	2,93[2]	0,51[1]	5,38[2]	0,99[2]	1,43[2]	10,09[2]	2,84[2]	8,10[1]	3,77[1]	0,69[1]
T2 vs T3	17,72[1]	1,40[1]	4,53[1]	20,76[2]	5,13[2]	13,52[2]	8,00[2]	0,69[1]	0,40[1]	3,77[1]
Volunteer 16										
T1 vs T2	1,00[1]	5,28[2]	8,65[1]	16,09[1]	8,00[1]	14,96[1]	1,53[1]	0,08[2]	2,90[1]	5,63[1]
T1 vs T3	1,29[1]	0,00[1]	8,65[1]	6,25[1]	5,63[2]	5,59[2]	0,96[1]	0,00[1]	0,07[1]	0,23[1]
T2 vs T3	4,43[1]	1,60[1]	0,00[1]	5,26[1]	0,24[1]	17,00[2]	0,07[1]	0,07[1]	3,85[1]	8,02[1]
Volunteer 17										
T1 vs T2	0,55[1]	6,77[1]	1,68[1]	18,94[1]	7,72[2]	16,00[1]	9,00[1]	20,07[1]	3,60[1]	2,90[1]
T1 vs T3	0,64[1]	0,00[1]	5,56[1]	0,57[1]	0,25[1]	0,23[1]	2,06[1]	1,68[1]	0,06[1]	0,69[1]
T2 vs T3	2,36[1]	6,77[1]	12,75[1]	24,98[1]	6,24[1]	21,64[2]	18,51[1]	11,03[1]	4,59[1]	6,25[1]
Volunteer 18										
T1 vs T2	1,42[1]	0,06[1]	1,44[1]	2,36[1]	14,68[2]	11,71[2]	1,42[1]	0,28[1]	0,90[1]	4,80[1]
T1 vs T3	0,22[1]	0,06[1]	0,24[1]	9,76[1]	34,61[2]	3,13[1]	15,41[1]	9,57[1]	4,80[1]	10,80[1]
T2 vs T3	0,52[1]	0,23[1]	0,51[1]	2,72[1]	2,06[1]	0,23[1]	8,23[1]	6,77[1]	1,60[1]	1,42[1]

(T = Trial)

Table 3.4.6 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN TRIALS
Betnovate, individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Volunteer 1										
T1 vs T2	20,84[1]	32,92[2]	31,50[2]	22,00[3]	21,27[2]	4,37[2]	13,22[2]	7,73[2]	17,71[1]	24,27[1]
T1 vs T3	0,07[1]	0,08[1]	2,00[1]	2,18[2]	2,26[2]	1,64[2]	5,57[1]	11,45[1]	2,48[2]	0,40[1]
T2 vs T3	32,62[2]	26,88[2]	25,07[2]	17,12[3]	20,39[3]	10,72[2]	33,36[2]	31,37[2]	8,23[1]	21,21[1]
Volunteer 2										
T1 vs T2	8,13[1]	0,64[1]	5,84[1]	8,14[2]	3,89[2]	4,30[3]	3,35[3]	14,67[2]	6,73[1]	1,42[1]
T1 vs T3	5,17[1]	1,60[1]	0,08[1]	0,28[2]	1,39[2]	0,53[1]	0,55[1]	6,92[1]	16,37[1]	18,00[1]
T2 vs T3	0,40[1]	4,13[1]	7,17[1]	7,34[2]	7,50[2]	12,95[3]	7,59[2]	0,89[1]	2,49[1]	27,23[1]
Volunteer 3										
T1 vs T2	7,88[2]	0,00[1]	9,42[2]	2,55[2]	8,93[3]	32,29[3]	18,60[2]	28,60[2]	8,42[1]	0,00[1]
T1 vs T3	5.41[2]	0,51[1]	2,33[2]	1,33[2]	5,25[3]	13,01[3]	3,06[2]	3,00[1]	8,42[1]	0,00[1]
T2 vs T3	0,42[2]	0,51[1]	10,37[2]	0,24[2]	3,38[3]	31,04[3]	7,74[2]	13,79[1]	0,00[1]	0,00[1]
Volunteer 4										
T1 vs T2	2,00[1]	0,08[1]	2,11[1]	4,69[2]	0,22[1]	11,59[2]	0,23[1]	0,53[1]	2,78[1]	2,49[1]
T1 vs T3	5,63[1]	0,76[1]	10,98[2]	11,64[2]	0,51[1]	16,48[2]	1,42[1]	0,24[1]	0,06[1]	4,50[1]
T2 vs T3	0,96[1]	0,35[1]	7,41[1]	3,60[1]	1,47[2]	0,51[1]	0,50[1]	0,06[1]	3,60[1]	12,99[1]
Volunteer 5										
T1 vs T2	6,77[1]	1,00[1]	1,68[1]	1,60[1]	0,00[1]	8,03[2]	5,68[2]	1,42[1]	2,03[1]	1,00[1]
T1 vs T3	3,56[1]	4,43[1]	4,74[1]	0,84[1]	0,24[1]	5,91[2]	10,82[2]	3,02[2]	0,23[1]	2,90[1]
T2 vs T3	0,55[1]	1,29[1]	0,84[1]	0,00[1]	0,24[1]	0,06[1]	0,32[1]	0,11[1]	3,57[1]	0,51[1]

Volunteer 6										
T1 vs T2	5,63[1]	1,86[1]	19,09[2]	19,91[2]	29,71[2]	33,81[2]	29,84[2]	19,98[2]	6,73[1]	4,96[1]
T1 vs T3	0,50[1]	0,00[1]	5,79[1]	0,08[1]	3,13[1]	0,00[1]	3,50[1]	0,00[1]	2,78[1]	4,96[1]
T2 vs T3	2,83[1]	1,86[1]	8,69[1]	8,04[1]	19,41[2]	16,87[2]	10,36[1]	12,62[2]	0,91[1]	0,00[1]
Volunteer 7										
T1 vs T2	47,04[2]	46,62[2]	61,80[3]	54,43[2]	43,59[2]	26,47[2]	9,40[1]	21,05[2]	2,03[1]	1,00[1]
T1 vs T3	0,06[1]	0,89[1]	4,68[1]	0,35[1]	0,16[2]	0,28[2]	6,27[2]	3,62[2]	5,84[1]	9,57[1]
T2 vs T3	48,17[2]	44,13[2]	61,24[3]	41,10[2]	43,41[2]	30,26[2]	16,37[1]	18,45[2]	1,05[1]	16,07[1]
Volunteer 8										
T1 vs T2	2,83[1]	18,23[1]	5,84[1]	1,11[1]	2,11[1]	2,36[2]	0,30[1]	0,06[1]	6,24[1]	0,00[1]
T1 vs T3	5,63[1]	24,52[1]	8,67[1]	2,67[1]	0,07[1]	1,54[2]	2,17[1]	0,89[1]	3,19[1]	2,35[1]
T2 vs T3	0,50[1]	0,64[1]	4,20[2]	0,91[2]	1,42[2]	7,09[2]	4,00[1]	1,39[1]	0,57[1]	3,60[1]
Volunteer 9										
T1 vs T2	1,53[1]	9,76[1]	13,01[1]	12,04[2]	22,44[2]	9,43[1]	25,04[2]	0,64[1]	0,55[1]	0,84[1]
T1 vs T3	0,57[1]	2,72[1]	2,25[1]	4,00[1]	2,25[1]	9,47[2]	1,44[1]	0,96[1]	2,10[1]	0,08[1]
T2 vs T3	0,23[1]	2,36[1]	5,26[1]	10,21[2]	12,08[1]	2,78[2]	14,83[2]	3,13[1]	0,51[1]	0,40[1]
Volunteer 10										
T1 vs T2	1,79[1]	0,00[1]	0,30[1]	0,07[1]	5,84[1]	11,80[1]	11,20[1]	1,68[1]	0,06[1]	1,40[1]
T1 vs T3	0,94[1]	0,00[1]	0,30[1]	0,00[1]	2,03[1]	2,74[1]	9,57[1]	6,24[1]	12,77[1]	4,96[1]
T2 vs T3	5,17[1]	0,00[1]	0,00[1]	0,07[1]	1,05[1]	3,50[1]	0,08[1]	1,60[1]	14,23[1]	11,20[1]
Volunteer 11										
T1 vs T2	0,06[1]	9,76[1]	11,92[2]	0,93[2]	0,52[2]	9,67[2]	6,55[1]	0,08[1]	0,06[1]	0,22[1]
T1 vs T3	3,50[1]	4,19[1]	0,00[1]	0,65[2]	0,60[2]	6,03[2]	13,33[1]	0,00[1]	0,24[1]	0,51[1]
T2 vs T3	4,43[1]	24,52[1]	3,19[2]	0,09[2]	0,11[2]	1,33[2]	1,45[2]	0,08[1]	0,06[1]	1,40[1]
Volunteer 12										
T1 vs T2	0,28[1]	0,35[1]	4,60[1]	0,24[1]	2,72[1]	1,05[1]	0,07[1]	0,09[1]	0,57[1]	1,44[1]
T1 vs T3	10,89[1]	13,33[1]	6,82[1]	17,65[2]	15,41[1]	15,58[2]	20,12[2]	24,00[1]	6,73[1]	7,73[1]
T2 vs T3	14,27[1]	9,76[1]	10,67[2]	6,91[2]	6,21[2]	10,49[2]	11,94[2]	10,80[1]	10,92[1]	2,67[1]
Volunteer 13										
T1 vs T2	0,23[1]	6,99[1]	3,13[1]	4,19[1]	9,59[2]	10,67[1]	4,18[1]	3,60[1]	0,06[1]	0,07[1]
T1 vs T3	0,23[1]	5,84[1]	0,08[1]	4,19[1]	14,73[2]	13,71[2]	17,71[1]	26,13[1]	3,00[1]	0,76[1]
T2 vs T3	0,00[1]	0,06[1]	4,19[1]	0,00[1]	0,96[1]	0,69[1]	15,41[1]	12,59[1]	2,25[1]	1,29[1]
Volunteer 14										
T1 vs T2	2,74[1]	1,68[1]	0,00[1]	0,46[1]	0,25[1]	0,00[1]	0,28[1]	2,07[2]	0,50[1]	0,90[1]
T1 vs T3	5,56[1]	0,30[1]	0,35[1]	4,18[1]	1,05[1]	2,10[1]	0,07[1]	0,76[1]	1,39[1]	0,00[1]
T2 vs T3	0,52[1]	0,57[1]	0,35[1]	0,84[1]	0,28[1]	2,10[1]	0,07[1]	8,50[2]	0,22[1]	0,90[1]
Volunteer 15										
T1 vs T2	0,00[1]	0,30[1]	2,17[1]	11,40[2]	1,60[2]	2,06[1]	0,06[1]	4,19[1]	2,11[1]	5,67[1]
T1 vs T3	3,61[2]	0,00[1]	0,22[1]	0,58[2]	0,37[2]	5,99[2]	1,39[1]	4,19[1]	2,11[1]	0,06[1]
T2 vs T3	9,56[2]	0,30[1]	3,74[1]	8,28[2]	2,01[2]	1,83[2]	0,89[1]	0,10[2]	0,00[1]	7,53[2]
Volunteer 16										
T1 vs T2	0,50[1]	6,40[1]	14,66[2]	21,33[2]	7,73[1]	12,08[1]	0,46[1]	0,00[1]	1,53[1]	0,51[1]
T1 vs T3	0,94[1]	0,00[1]	0,32[1]	0,61[1]	0,00[1]	10,43[2]	5,45[1]	1,42[1]	0,07[1]	0,50[1]
T2 vs T3	2,78[1]	0,69[1]	3,50[1]	6,82[1]	7,73[1]	27,37[2]	8,65[1]	5,26[1]	2,25[1]	2,01[1]
Volunteer 17										
T1 vs T2	1,53[1]	0,23[1]	2,90[1]	4,68[1]	6,77[1]	8,10[1]	10,92[1]	11,45[1]	8,42[1]	0,07[1]
T1 vs T3	4,68[1]	0,51[1]	4,68[1]	9,45[1]	0,00[1]	0,23[1]	0,53[1]	0,00[1]	1,68[1]	0,69[1]
T2 vs T3	0,89[1]	0,06[1]	0,22[1]	0,91[1]	6,77[1]	5,71[1]	6,85[1]	11,45[1]	2,74[1]	1,19[1]
Volunteer 18										
T1 vs T2	3,57[1]	0,00[1]	0,30[1]	8,04[1]	0,00[1]	0,08[1]	0,07[1]	3,29[1]	0,89[1]	2,67[1]
T1 vs T3	0,06[1]	0,06[1]	0,64[1]	3,60[1]	19,43[1]	3,29[1]	2,90[1]	2,01[1]	4,53[1]	5,14[1]
T2 vs T3	4,50[1]	0,06[1]	0,07[1]	1,05[1]	8,67[1]	2,36[1]	3,85[1]	10,01[1]	1,44[1]	0,46[1]

(T = Trial)

Table 3.4.7 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM : TRIAL 1
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Volunteer										
1	0,15[2]	0,69[1]	0,50[1]	0,57[2]	2,17[2]	0,09[2]	0,36[2]	0,89[1]	1,79[1]	1,42[1]
2	20,23[2]	11,20[1]	23,07[2]	23,39[2]	16,35[3]	13,33[2]	17,19[2]	11,80[1]	2,06[1]	2,25[1]
3	0,89[1]	3,66[1]	1,39[2]	2,68[2]	1,50[2]	1,87[2]	1,66[2]	4,59[1]	8,13[1]	6,99[1]
4	0,00[1]	0,61[1]	0,30[1]	3,47[2]	0,06[1]	3,20[2]	0,25[1]	4,83[2]	2,90[1]	0,52[1]
5	12,99[1]	7,41[1]	23,74[2]	26,13[1]	15,47[2]	16,69[2]	9,73[2]	10,36[2]	2,10[1]	2,90[1]
6	9,76[1]	0,40[1]	11,57[1]	0,32[1]	18,97[2]	2,35[1]	12,13[2]	13,03[2]	3,60[1]	0,30[1]
7	2,06[1]	2,00[1]	3,66[1]	1,60[1]	9,76[1]	3,60[1]	3,14[2]	4,09[2]	3,66[1]	11,20[1]
8	0,96[1]	8,67[1]	1,40[1]	0,06[1]	0,30[1]	0,00[2]	1,53[1]	0,50[1]	0,46[1]	0,46[1]
9	0,26[1]	0,53[1]	1,39[1]	0,00[1]	4,19[1]	0,91[1]	0,23[1]	0,55[1]	1,48[1]	2,36[1]
10	16,49[2]	29,97[1]	26,97[1]	22,50[1]	22,34[2]	26,19[2]	24,40[2]	18,94[1]	0,06[1]	0,22[1]
11	1,42[1]	0,23[1]	0,23[1]	0,51[2]	0,77[2]	0,52[2]	3,61[2]	0,84[1]	0,06[1]	0,06[1]
12	0,07[1]	0,00[1]	0,00[1]	0,90[1]	3,13[1]	0,52[1]	4,68[1]	3,50[1]	0,25[1]	0,06[1]
13	10,01[1]	1,42[1]	10,00[1]	6,55[1]	14,90[2]	13,94[2]	16,17[1]	16,37[1]	1,39[1]	9,57[1]
14	6,24[1]	2,68[1]	3,60[1]	11,36[1]	11,03[1]	7,40[2]	16,07[1]	2,67[1]	5,63[1]	0,22[1]
15	16,37[1]	8,10[1]	10,08[2]	7,40[2]	9,18[3]	6,36[2]	5,17[2]	3,33[2]	0,26[1]	0,07[1]
16	2,17[1]	0,00[1]	2,25[1]	1,44[1]	2,78[1]	2,01[1]	5,45[1]	2,11[1]	0,00[1]	0,89[1]
17	0,07[1]	0,00[1]	9,45[1]	0,57[1]	6,77[1]	0,23[1]	2,06[1]	0,08[1]	1,11[1]	0,00[1]
18	0,50[1]	0,53[1]	1,11[1]	2,68[1]	13,33[1]	0,08[1]	2,11[1]	1,60[1]	0,50[1]	2,06[1]
Paired Comparison										
Volunteer										
1	0,41	0,05	0,70	0,38	0,05	1,33	1,44	2,78	4,50	1,07
2	12,00	11,17	9,63	12,12	17,63	12,90	16,69	13,79	1,39	1,78
3	0,38	0,64	0,89	0,64	1,71	0,04	1,44	2,04	6,86	5,88
4	0,06	0,41	0,00	0,05	0,38	0,41	3,12	1,71	0,56	0,90
5	9,39	6,86	20,05	15,43	14,09	10,32	7,58	11,25	0,31	0,80
6	16,96	21,33	22,32	8,04	21,81	15,75	16,00	7,26	5,06	0,36
7	4,05	4,76	7,84	7,26	7,68	3,45	8,04	5,76	4,05	16,41
8	0,17	4,90	0,84	0,00	1,71	0,00	0,00	0,07	0,17	0,00
9	0,00	0,21	0,45	0,00	1,04	2,72	2,45	0,06	0,00	1,45
10	7,58	7,68	12,19	12,19	13,47	14,45	8,45	15,43	3,27	0,00
11	0,00	0,32	0,89	0,52	0,04	4,97	0,41	0,17	0,00	0,00
12	2,12	0,94	0,06	1,39	2,45	0,21	0,00	0,21	0,44	0,00
13	13,07	13,47	15,43	14,45	14,45	13,14	14,09	12,19	0,00	1,45
14	3,70	9,48	12,89	8,26	7,03	11,17	13,79	6,76	10,32	0,94
15	12,19	7,58	4,50	3,37	5,88	3,06	1,25	0,19	0,94	0,00
16	2,23	4,00	4,35	1,57	0,70	7,84	1,71	3,37	1,39	0,00
17	0,07	0,00	1,14	0,64	5,50	1,07	3,76	0,90	1,13	0,36
18	0,84	1,71	0,21	0,24	2,45	0,00	1,25	0,05	1,23	0,94

Table 3.4.8 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM : TRIAL 2
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Volunteer										
1	4,83[2]	0,09[2]	4,41[2]	5,88[2]	1,42[2]	3,66[2]	0,23[1]	0,00[1]	1,45[2]	0,00[1]
2	1,93[1]	1,48[1]	0,13[2]	2,59[2]	0,96[3]	1,19[3]	0,30[2]	2,04[2]	0,00[1]	0,00[1]
3	3,94[2]	1,40[1]	1,91[2]	0,31[2]	2,19[3]	3,52[2]	1,77[2]	4,59[1]	12,75[1]	14,27[1]
4	1,39[1]	0,08[1]	4,13[1]	2,06[1]	1,80[2]	0,00[1]	2,72[1]	3,57[1]	2,06[1]	0,00[1]
5	5,67[1]	10,98[2]	10,55[2]	10,36[1]	16,74[2]	9,45[1]	14,27[1]	0,00[1]	8,02[1]	6,77[1]
6	1,93[1]	0,00[1]	0,57[1]	2,03[1]	0,85[2]	0,64[2]	1,38[2]	0,06[1]	0,06[1]	2,10[1]
7	4,10[2]	4,30[2]	1,46[2]	3,03[3]	0,39[2]	12,60[3]	2,52[2]	0,00[1]	0,23[1]	1,00[1]
8	0,22[1]	0,25[1]	0,00[1]	0,00[1]	0,42[2]	1,56[2]	0,00[1]	0,50[1]	1,05[1]	0,00[1]
9	5,45[1]	0,00[1]	0,40[1]	0,06[1]	0,23[1]	1,19[1]	2,17[1]	0,08[1]	2,72[1]	1,42[1]
10	2,35[2]	7,60[1]	4,68[1]	8,23[1]	8,23[1]	9,00[1]	6,02[1]	6,02[1]	1,60[1]	3,56[1]
11	4,50[1]	5,98[2]	2,11[2]	3,89[2]	1,93[2]	3,29[2]	0,22[1]	0,49[2]	1,68[1]	0,50[1]
12	0,28[1]	1,68[1]	0,57[1]	1,04[2]	0,06[1]	3,85[1]	1,05[1]	2,08[2]	4,68[1]	0,24[1]
13	2,01[1]	10,01[1]	5,45[1]	8,65[1]	1,93[1]	1,86[1]	0,00[1]	0,76[1]	2,00[1]	0,57[1]
14	10,01[1]	10,33[2]	1,42[1]	0,35[1]	1,48[1]	0,06[1]	1,48[1]	0,55[1]	0,90[1]	3,56[1]
15	0,00[1]	2,36[1]	1,53[1]	0,50[1]	1,69[2]	1,09[2]	0,89[1]	0,10[2]	0,40[1]	1,79[1]
16	1,40[1]	0,30[1]	1,68[1]	0,61[1]	2,67[1]	0,00[1]	3,19[1]	5,26[1]	0,23[1]	0,55[1]
17	0,06[1]	4,59[1]	7,17[1]	9,43[1]	6,29[2]	3,81[2]	1,49[2]	2,25[1]	0,00[1]	2,10[1]
18	0,00[1]	0,24[1]	2,90[1]	0,06[1]	1,79[1]	4,52[2]	0,00[1]	1,19[1]	0,51[1]	0,76[1]
Paired Comparison										
Volunteer										
1	18,38	21,33	11,12	13,88	12,04	12,04	12,04	11,13	7,04	0,16
2	7,04	4,65	0,04	0,03	1,75	0,04	0,04	0,00	0,04	0,80
3	11,25	6,26	3,38	6,50	4,65	7,68	10,23	5,26	10,32	11,25
4	1,45	0,84	0,00	2,23	0,70	0,76	0,70	4,00	0.24	0,10
5	3,37	1,71	2,23	1,89	5,26	8,45	9,39	10,23	6,75	11,08
6	1,14	0,64	0,16	0,00	0,15	0,15	0,64	0,59	0,76	1,14
7	2,21	4,97	0,64	0,03	0,00	0,83	0,28	0,03	1,04	0,64
8	0,75	0,84	0,05	0,17	0,06	4,35	0,06	0,94	0,17	1,33
9	2,08	0,05	0,04	0,64	0,00	1,71	2,23	0,76	0,94	0,10
10	0,50	7,58	5,50	1,89	5,26	1,25	0,76	3,37	1,07	2,72
11	2,38	4,65	4,11	4,36	2,53	4,36	2,89	0,04	0,41	0,04
12	2,77	0,94	0,06	0,06	0,24	0,24	0,06	0,06	0,10	0,00
13	1,79	0,56	0,00	1,07	3,76	3,76	0,94	0,00	0,00	0,75
14	8,65	6,76	2,21	4,00	7,50	8,83	8,26	2,21	1,25	0,17
15	1,39	0,24	0,21	0,06	0,45	0,45	2,72	0,21	0,24	0,05
16	0,84	7,04	0,84	0,50	3,68	0,19	6,26	2,23	0,00	1,56
17	0,00	1,56	3,37	8,45	4,35	4,50	2,72	1,89	2,72	0,10
18	2,40	5,26	0,84	0,00	0,21	0,76	0,50	0,00	0,00	0,00

TABLE 3.4.9 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM : TRIAL 3
individual volunteers, all observers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Volunteer										
1	6,40[1]	7,17[1]	7,15[2]	5,31[3]	5,74[3]	5,99[3]	4,50[1]	1,79[1]	0,79[1]	0,40[1]
2	1,86[1]	6,24[1]	6,02[1]	3,94[2]	4,84[2]	3,60[1]	3,66[1]	0,23[1]	0,10[1]	0,08[1]
3	8,11[2]	6,77[1]	7,78[2]	3,65[3]	6,31[3]	6,72[3]	3,05[2]	5,84[1]	6,02[1]	1,05[1]
4	1,93[1]	1,43[2]	2,72[1]	5,53[2]	7,19[2]	5,52[2]	3,45[2]	4,50[1]	0,52[1]	1,00[1]
5	0,06[1]	0,00[1]	0,00[1]	1,93[1]	9,57[1]	7,48[2]	11,45[1]	11,36[1]	1,53[1]	8,67[1]
6	0,06[1]	0,32[1]	0,00[1]	0,08[1]	0,88[2]	2,41[2]	0,00[1]	3,77[1]	0,23[1]	0,50[1]
7	4,59[1]	2,10[1]	0,07[1]	7,43[1]	8,72[2]	1,09[2]	6,88[2]	2,37[2]	1,42[1]	0,32[1]
8	1,39[1]	6,02[1]	0,85[2]	0,14[2]	0,34[2]	4,51[2]	0,52[1]	0,00[1]	0,30[1]	0,76[1]
9	2,74[1]	2,36[1]	0,08[1]	0,35[1]	0,61[1]	0,35[2]	0,00[1]	2,25[1]	0,50[1]	2,49[1]
10	14,52[1]	22,43[1]	12,51[1]	20,32[2]	25,84[2]	17,44[2]	8,23[1]	27,66[1]	11,62[1]	0,08[1]
11	2,01[2]	1,19[1]	0,06[1]	0,65[2]	0,36[2]	2,80[2]	1,42[2]	0,35[1]	2,06[1]	2,01[1]
12	0,25[1]	2,72[1]	5,14[1]	1,11[1]	0,94[2]	0,00[1]	0,06[1]	0,06[1]	0,24[1]	6,55[1]
13	3,56[1]	3,66[1]	0,00[1]	3,13[1]	3,13[1]	2,11[1]	13,33[1]	5,57[1]	1,05[1]	4,43[1]
14	14,63[1]	2,11[1]	0,00[1]	9,08[2]	6,99[1]	8,02[1]	8,10[1]	5,26[1]	5,17[1]	3,74[1]
15	4,80[1]	4,68[1]	3,50[2]	5,08[2]	6,81[2]	11,88[2]	6,46[2]	0,69[1]	0,00[1]	1,79[1]
16	2,67[1]	0,97[1]	0,97[1]	0,32[1]	0,67[2]	0,55[2]	0,96[1]	0,07[1]	0,00[1]	0,52[1]
17	7,17[1]	0,51[1]	2,03[1]	9,45[1]	4,50[1]	0,23[1]	0,53[1]	2,49[1]	0,25[1]	0,00[1]
18	2,01[1]	1,48[1]	0,55[1]	8,23[1]	0,06[1]	0,06[1]	0,89[1]	0,23[1]	0,61[1]	0,00[1]
Paired Comparison										
Volunteer										
1	11,13	13,88	14,09	21,33	21,33	14,09	20,35	12,19	5,26	1,57
2	0,04	9,63	7,50	12,89	9,63	6,50	0,83	0,76	1,25	0,04
3	11,12	7,04	5,76	7,68	8,83	6,50	12,04	10,24	5,88	0,64
4	1,04	0,05	1,88	3,38	6,50	3,05	3,12	8,52	0,06	4,50
5	0,50	1,39	0,05	6,05	5,26	4,50	7,68	10,32	3,76	6,72
6	0,04	3,12	0,96	2,04	0,15	0,15	0,89	0,89	0,24	0,76
7	2,45	10,24	4,32	6,32	7,50	1,24	7,04	1,71	0,16	2,56
8	0,00	3,05	0,05	0,05	0,70	1,14	0,19	0,27	0,17	0,00
9	1,89	2,78	0,76	0,04	2,45	1,25	1,04	4,76	4,27	0,36
10	6,86	11,25	15,43	16,41	17,39	13,14	14,45	11,25	6,05	0,56
11	1,88	0,55	0,48	0,13	0,04	0,52	3,03	1,44	0,76	2,23
12	0,10	0,56	3,37	0,00	0,21	0,94	0,24	1,23	0,31	1,13
13	6,72	0,45	0,00	5,26	1,56	4,27	5,06	4,90	0,17	2,29
14	8,04	3,45	10,32	3,45	8,83	10,32	18,58	8,83	9,48	2,78
15	0,84	1,14	4,76	6,26	3,05	10,32	5,26	3,76	6,72	2,45
16	0,05	0,64	0,05	2,23	0,84	0,04	0,00	0,45	0,05	0,00
17	4,50	0,24	2,72	7,68	0,06	1,23	0,00	0,00	1,78	0,00
18	1,25	0,76	1,39	4,35	0,06	2,23	2,72	0,56	0,00	0,57

Table 3.4.10 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM
volunteers arranged into two groups
according to individual AUC values

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Group 1										
Trial 1	30,09	34,03	42,44	49,93	58,10	52,16	45,53	39,43	19,37	14,20
Trial 2	31,48	39,89	46,30	50,93	56,09	50,47	43,75	34,80	21,92	16,28
Trial 3	27,70	33,03	41,05	49,15	56,33	55,33	43,21	34,26	21,76	18,98
Group 2										
Trial 1	14,20	16,74	21,45	24,15	35,42	29,33	28,48	24,69	13,58	10,96
Trial 2	15,28	22,14	25,69	28,24	33,33	29,86	29,24	22,99	13,28	9,49
Trial 3	12,88	16,98	20,99	21,76	25,16	25,16	21,68	17,67	12,73	11,03
Betnovate										
Group 1										
Trial 1	21,61	27,93	33,26	39,51	46,53	42,90	35,42	32,18	16,98	12,12
Trial 2	27,01	34,26	40,13	43,59	49,69	44,06	39,04	31,79	19,68	14,28
Trial 3	19,98	26,78	33,88	39,66	44,83	43,98	35,26	26,93	18,83	16,13
Group 2										
Trial 1	10,11	14,20	15,74	19,13	24,15	22,30	20,91	18,83	9,73	7,48
Trial 2	13,43	19,22	22,84	24,31	29,71	26,00	25,16	20,29	11,88	6,79
Trial 3	11,58	16,05	19,75	20,60	20,83	21,45	18,68	13,97	11,11	7,95

Table 3.4.11 AREA UNDER THE CURVE VALUES
volunteers arranged into two groups
according to individual AUC values

	Group 1		Group 2	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Trial 1	984	793	583	430
Trial 2	984	870	586	512
Trial 3	964	774	483	412

Table 3.4.12 DIFFERENCES IN AUC VALUES BETWEEN GROUP 1 AND GROUP 2

Actual differences in AUC values : Celestoderm-V - Betnovate

	Group 1	Group 2
Trial 1	191	150
Trial 2	114	74
Trial 3	190	71

Percentage differences in AUC values : $\frac{\text{Betnovate}}{\text{Celestoderm-V}} \times 100$

	Group 1	Group 2
Trial 1	80	73
Trial 2	88	87
Trial 3	80	85

TABLE 3.4.13 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM
volunteers arranged into two groups
according to individual AUC values

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Group 1										
Trial 1	41,39	28,97	39,93	38,23	41,10	31,04	40,15	33,57	4,58	4,53
Trial 2	11,73	12,37	11,63	17,49	15,00	13,23	14,72	11,62	4,82	3,20
Trial 3	39,99	31,22	22,87	27,69	38,33	33,53	27,50	33,10	8,85	7,24
Group 2										
Trial 1	18,06	6,94	26,91	28,72	57,11	23,30	25,27	21,09	15,87	13,81
Trial 2	3,46	6,30	5,82	9,58	6,04	9,38	12,57	9,90	2,92	10,01
Trial 3	2,86	3,86	5,57	16,78	13,47	9,62	8,21	10,04	2,56	9,71
Paired Comparison										
Group 1										
Trial 1	22,01	27,31	33,12	32,19	39,61	38,03	26,12	22,45	0,57	1,45
Trial 2	27,85	42,08	18,37	15,40	22,58	20,74	20,07	12,04	8,64	3,72
Trial 3	22,34	28,89	38,30	53,26	55,50	39,43	35,04	42,41	19,11	8,84
Group 2										
Trial 1	15,92	24,40	36,39	27,80	56,75	35,46	52,52	27,40	14,91	20,84
Trial 2	2,01	5,54	5,48	9,54	11,94	8,47	20,56	5,66	0,72	0,22
Trial 3	3,59	1,46	2,43	0,02	10,23	8,29	16,75	17,13	5,98	8,28

Table 3.4.14 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM volunteers arranged into groups according to comparative bioavailabilities of the two preparations in individual volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Group A										
Trial 1	19,17	22,22	27,57	32,29	41,60	35,27	30,21	26,60	15,14	11,52
Trial 2	23,93	31,67	36,52	40,32	45,51	40,42	36,72	28,80	17,68	12,36
Trial 3	15,67	19,44	24,77	26,54	29,09	31,48	24,53	21,68	12,03	10,49
Group B										
Trial 1	25,87	29,34	37,42	42,97	53,21	47,57	45,48	38,89	18,14	13,89
Trial 2	20,60	27,77	33,33	35,88	40,74	38,89	35,42	29,40	17,13	15,51
Trial 3	24,92	30,56	37,27	44,37	52,39	49,00	40,36	30,25	22,45	19,52
Betnovate										
Group A										
Trial 1	17,15	20,90	25,42	29,86	35,77	32,98	26,60	24,31	13,12	9,79
Trial 2	20,83	28,38	33,38	36,07	42,08	36,52	33,24	26,81	16,30	10,83
Trial 3	15,12	19,60	24,07	26,77	27,01	28,39	24,22	19,22	12,03	8,95
Group B										
Trial 1	14,23	21,27	23,35	28,65	34,81	32,12	30,12	27,00	13,62	9,81
Trial 2	17,13	18,52	21,99	23,38	27,77	27,54	26,39	22,22	13,19	9,02
Trial 3	16,43	23,22	29,55	33,49	38,66	37,03	29,71	21,68	17,90	15,12

Table 3.4.15 AREA UNDER THE CURVE VALUES volunteers arranged into groups according to comparative bioavailabilities of the two preparations in individual volunteers

	Group A		Group B	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Trial 1	678	608	915	616
Trial 2	791	719	755	551
Trial 3	552	521	895	665

Table 3.4.16 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM volunteers arranged into groups according to comparative bioavailabilities of the two preparations in individual volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Group A										
Trial 1	4,44	1,90	4,67	5,71	14,70	3,73	5,91	7,56	4,18	5,02
Trial 2	10,96	7,42	5,56	7,66	5,25	7,83	12,68	10,26	2,91	3,50
Trial 3	2,26	0,63	0,41	4,94	1,67	7,62	2,41	3,36	1,43	2,54
Group B										
Trial 1	74,40	42,90	80,12	69,90	86,41	73,72	72,57	57,17	15,90	14,48
Trial 2	3,80	21,06	21,59	26,57	24,55	23,61	18,61	15,31	5,60	13,37
Trial 3	46,07	38,47	28,43	37,27	53,05	40,19	49,38	43,66	14,79	15,54
Paired Comparison										
Group A										
Trial 1	0,05	0,96	2,58	3,20	10,24	8,48	9,09	2,09	0,26	3,01
Trial 2	22,17	30,49	13,33	15,51	21,19	17,63	29,98	8,02	6,85	0,36
Trial 3	0,00	0,85	0,18	3,72	1,47	0,32	0,18	6,33	0,19	1,03
Group B										
Trial 1	83,24	88,56	110,45	85,81	117,77	88,46	90,28	72,15	16,66	15,56
Trial 2	2,42	11,16	12,44	12,57	16,79	14,50	11,86	16,02	0,80	9,76
Trial 3	42,11	57,57	54,25	88,69	86,25	73,44	91,14	66,12	37,87	20,48

Table 3.4.17 CHI-SQUARED VALUES (GRADED RESPONSE) : CELESTODERM-V CREAM vs BETNOVATE CREAM TRIAL 1 : individual volunteers, all observers : adjacent cells not combined (df=4)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Volunteer										
1	0,15	1,43	4,62	2,64	7,90	3,37	2,32	1,61	1,81	2,30
2	20,23	11,74	23,96	24,35	17,85	15,28	19,46	12,12	2,06	2,25
3	5,47	5,48	4,87	6,37	3,36	3,83	4,23	7,56	9,40	6,99
4	0,00	0,61	0,31	3,47	5,81	5,95	5,02	6,50	2,90	0,52
5	15,39	10,28	23,74	27,39	16,82	20,47	12,70	11,81	2,87	2,90
6	9,76	3,35	14,96	6,57	19,20	9,00	12,16	13,03	4,80	0,30
7	2,06	2,00	5,03	4,35	10,03	4,35	3,15	4,87	3,66	12,13
8	0,96	8,67	1,40	1,13	6,22	0,00	1,53	0,50	0,46	0,46
9	1,13	0,53	1,39	0,00	4,22	3,20	5,38	6,88	1,48	2,36
10	16,49	30,19	27,99	25,00	22,34	26,49	24,40	19,00	1,13	1,14
11	3,04	1,17	0,49	0,51	1,89	0,52	3,61	0,86	0,06	0,06
12	3,38	2,07	0,35	2,14	6,79	0,55	5,17	6,25	0,25	0,06
13	10,01	2,30	13,95	9,43	16,79	14,83	17,39	16,43	2,16	9,57
14	6,24	2,70	5,08	11,38	14,68	8,42	17,84	8,25	5,63	1,14
15	19,32	8,68	10,54	8,18	9,18	6,92	5,17	3,33	2,00	2,07
16	3,40	3,20	3,85	4,53	6,82	5,17	6,24	2,53	1,02	0,89
17	0,07	0,00	9,45	0,57	7,86	0,23	2,06	0,08	1,11	0,00
18	0,50	1,42	1,32	3,83	14,03	0,40	2,14	1,65	0,50	2,06

Table 3 4.18 CHI-SQUARED VALUES (GRADED RESPONSE) : CELESTODERM-V CREAM vs BETNOVATE CREAM
TRIAL 2 : individual volunteers, all observers : adjacent cells not combined (df=4)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Volunteer										
1	4,83	1,18	4,61	5,98	1,44	3,83	2,58	3,42	1,45	0,23
2	2,84	3,20	0,13	4,67	1,90	4,74	1,99	11,25	3,21	2,91
3	3,94	2,34	1,91	1,15	4,86	4,35	1,77	5,21	12,75	14,27
4	1,39	0,08	4,13	8,03	4,46	1,31	3,05	3,65	2,06	0,00
5	6,24	10,98	11,09	11,60	16,85	14,67	15,24	9,82	8,02	6,77
6	2,65	3,21	0,84	2,23	2,85	4,27	2,71	5,43	3,13	2,10
7	5,88	5,88	5,30	3,03	0,90	12,60	3,03	1,03	3,14	1,00
8	0,22	0,25	0,75	0,00	0,42	1,56	0,13	0,50	1,05	3,13
9	7,06	1,08	1,31	3,73	0,63	2,31	2,45	2,19	2,72	1,97
10	2,35	10,69	9,59	11,45	10,03	10,33	6,94	6,41	1,64	5,54
11	6,31	7,04	2,11	7,23	3,30	5,29	0,26	1,55	1,86	0,96
12	7,59	3,93	2,26	6,65	4,53	9,82	7,25	3,44	6,17	1,13
13	2,01	10,01	6,86	8,74	2,32	6,57	8,57	1,62	3,47	1,37
14	10,38	10,33	2,16	0,42	2,22	3,06	2,15	1,08	0,92	8,44
15	0,35	4,83	2,24	1,07	1,75	1,68	1,76	0,10	0,40	1,85
16	3,00	1,22	3,08	2,45	5,18	1,17	3,24	6,48	0,70	1,36
17	0,06	4,59	7,75	12,63	6,29	3,81	1,49	4,70	0,00	2,10
18	0,00	0,24	2,90	0,06	8,50	4,52	3,18	3,58	0,51	0,76

Table 3.4.19 CHI-SQUARED VALUES (GRADED RESPONSE) : CELESTODERM-V CREAM vs BETNOVATE CREAM
TRIAL 3 : individual volunteers, all observers : adjacent cells not combined (df=4)

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Volunteer										
1	8,29	7,72	7,90	5,70	6,11	7,47	8,28	2,42	0,79	2,41
2	4,20	9,44	8,67	4,17	8,80	4,45	5,61	1,14	0,10	0,47
3	8,79	7,94	10,19	3,65	6,91	7,32	4,75	12,67	6,41	1,21
4	7,49	1,43	3,20	5,76	7,85	9,78	4,82	5,13	1,41	1,20
5	4,26	3,14	0,98	1,93	10,95	7,85	11,46	11,43	6,00	10,42
6	1,03	1,27	0,35	0,26	0,88	2,41	8,87	3,88	1,15	1,37
7	5,11	2,10	4,24	7,87	8,73	1,81	6,88	2,37	1,46	0,92
8	1,41	8,18	1,27	4,09	4,61	5,62	0,93	1,03	0,30	0,76
9	2,74	2,50	1,94	1,59	1,51	5,24	1,51	3,89	1,37	2,49
10	17,54	23,80	16,46	20,83	25,84	17,76	15,11	28,37	12,33	3,14
11	2,01	1,88	0,97	0,78	0,93	2,82	3,85	1,71	2,06	2,73
12	0,85	2,80	5,23	2,36	1,00	2,37	1,26	1,03	0,27	6,86
13	3,56	4,33	0,00	6,33	6,65	2,99	15,28	6,63	1,75	4,43
14	14,64	3,83	14,07	9,08	7,26	8,59	9,53	6,04	5,17	4,43
15	8,69	5,08	4,99	5,08	6,81	12,02	6,47	3,39	0,57	2,02
16	2,69	6,96	8,29	0,66	0,68	0,57	1,58	0,07	0,00	0,52
17	7,54	0,51	2,03	11,46	4,50	1,41	0,53	2,49	0,25	0,00
18	2,01	1,48	1,47	9,30	1,14	0,06	0,89	0,23	0,61	0,13

Table 3.5.1 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM
male and female volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Male										
Trial 1	20,29	24,23	31,02	36,65	46,14	40,12	36,11	31,79	15,74	11,96
Trial 2	26,54	34,95	42,21	47,76	53,16	44,98	40,20	30,71	18,13	11,65
Trial 3	20,60	26,70	33,80	39,35	45,76	44,37	34,57	28,32	18,44	16,51
Female										
Trial 1	24,00	26,54	32,87	37,42	47,38	41,36	37,89	32,33	17,21	13,19
Trial 2	20,22	27,08	29,78	31,40	36,27	35,34	32,79	27,08	17,05	14,12
Trial 3	19,98	23,30	28,24	31,56	35,73	36,11	30,32	23,61	16,05	13,50
Betnovate										
Male										
Trial 1	13,97	19,21	23,53	28,16	35,34	31,33	28,86	25,00	11,50	8,26
Trial 2	23,69	31,33	38,12	42,44	48,84	40,28	35,96	27,55	15,51	9,95
Trial 3	16,05	23,30	29,17	32,95	36,50	34,88	28,32	21,91	15,28	13,35
Female										
Trial 1	17,75	22,92	25,46	30,48	35,34	33,87	27,47	26,00	15,20	11,34
Trial 2	16,74	22,15	24,85	25,46	30,56	29,78	28,24	24,54	16,05	11,11
Trial 3	15,51	19,52	24,46	27,31	29,17	30,56	25,62	18,98	14,66	10,73

Table 3.5.2 AREA UNDER THE CURVE VALUES
male and female volunteers

	Male		Female	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Trial 1	761	579	806	644
Trial 2	866	772	704	611
Trial 3	783	631	664	556

Table 3.5.3 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN SEXES
male vs female

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Trial 1	9,09	9,12	14,98	24,13	10,44	19,09	9,23	3,54	2,12	1,51
Trial 2	25,46	24,85	46,82	70,21	73,59	32,65	35,80	13,40	8,80	9,99
Trial 3	0,49	6,87	14,29	21,89	26,07	24,83	13,75	10,82	5,10	7,25
Betnovate										
Trial 1	18,97	24,61	23,72	21,59	29,11	32,12	18,44	6,40	11,90	10,44
Trial 2	30,96	36,23	59,57	82,32	89,05	54,73	40,02	31,07	23,67	10,07
Trial 3	3,04	11,10	15,35	11,75	16,51	8,98	9,81	5,80	5,45	6,35

Table 3.5.4 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM male and female volunteers

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Male										
Trial 1	25,65	14,79	22,54	22,38	33,45	24,94	17,96	24,30	15,25	14,28
Trial 2	7,12	4,83	4,70	6,91	4,33	6,25	11,64	10,99	7,26	3,77
Trial 3	16,03	13,47	14,63	15,12	23,21	21,70	18,56	23,83	7,21	8,23
Female										
Trial 1	24,93	16,44	30,08	27,37	52,08	28,35	47,25	26,00	3,53	3,74
Trial 2	10,46	13,47	11,77	17,19	14,99	18,29	13,79	10,26	1,96	6,63
Trial 3	12,69	13,15	8,33	14,94	13,85	13,21	10,86	13,32	5,06	7,17
Paired Comparison										
Male										
Trial 1	24,85	31,84	31,31	23,89	50,21	25,80	37,67	24,38	10,24	19,85
Trial 2	21,14	27,45	11,63	8,04	13,82	14,00	22,46	11,13	12,35	5,98
Trial 3	16,90	0,06	20,92	34,09	52,38	35,70	44,44	46,52	18,48	16,59
Female										
Trial 1	13,97	20,46	38,62	37,24	45,85	50,52	38,72	25,27	1,57	1,84
Trial 2	5,65	13,84	10,85	18,59	20,58	14,33	18,13	6,45	0,11	0,03
Trial 3	6,73	5,15	10,63	2,52	11,20	10,14	10,66	14,31	6,87	2,59

Table 3.6.1 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM strong arm and weak arm

Time (Hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Strong										
Trial 1	22,38	25,62	31,33	37,19	45,99	40,13	35,26	29,01	15,82	11,88
Trial 2	24,15	32,56	38,58	42,36	47,69	42,44	37,35	29,40	17,90	13,73
Trial 3	19,68	25,31	32,10	37,04	41,36	39,74	31,25	25,85	17,13	14,51
Weak										
Trial 1	21,91	25,15	32,56	36,88	47,53	41,36	38,73	35,11	17,13	13,27
Trial 2	22,61	29,48	33,41	36,81	41,74	37,89	35,65	28,40	17,28	12,04
Trial 3	20,91	24,69	29,94	33,87	40,12	40,74	33,64	26,08	17,36	15,51
Betnovate										
Strong										
Trial 1	15,43	21,45	24,23	29,01	33,56	31,40	26,77	23,84	13,35	10,34
Trial 2	20,60	27,39	33,02	36,57	41,90	37,58	33,41	27,31	15,82	11,50
Trial 3	16,36	22,30	28,86	32,87	33,02	32,87	26,54	19,68	14,89	11,34
Weak										
Trial 1	16,28	20,68	24,77	29,63	37,11	33,80	29,55	27,16	13,35	9,26
Trial 2	19,83	26,08	29,94	31,33	37,50	32,48	30,79	24,77	15,74	9,57
Trial 3	15,20	20,52	24,77	27,39	32,64	32,56	27,39	21,22	15,05	12,73

Table 3.6.2 AREA UNDER THE CURVE VALUES
strong arm and weak arm

	Strong		Weak	
	Celestoderm-V	Betnovate	Celestoderm-V	Betnovate
Trial 1	753	592	813	631
Trial 2	815	721	754	662
Trial 3	720	596	727	590

Table 3.6.3 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN ARMS
strong arm vs weak arm

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Celestoderm-V										
Trial 1	4,22	1,44	1,26	1,54	11,69	2,51	8,37	14,65	4,83	2,57
Trial 2	1,42	3,76	9,18	10,93	18,07	13,15	8,34	2,82	3,85	4,74
Trial 3	2,31	0,90	2,21	4,49	6,21	2,99	5,63	13,48	0,40	6,34
Betnovate										
Trial 1	6,87	3,95	1,67	3,67	5,32	3,05	11,37	6,63	0,07	1,28
Trial 2	1,30	2,09	4,95	8,96	5,20	13,45	11,88	5,46	2,03	9,59
Trial 3	2,24	2,66	10,18	10,88	3,78	2,96	7,04	5,22	4,17	1,84

Table 3.6.4 CHI-SQUARED VALUES : CELESTODERM-V CREAM vs BETNOVATE CREAM
strong arm and weak arm

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Strong										
Trial 1	30,95	10,61	22,83	27,57	48,22	23,03	28,91	19,94	5,40	2,80
Trial 2	9,60	10,67	9,55	10,15	12,30	7,86	10,56	7,34	4,42	4,78
Trial 3	7,45	9,48	6,93	10,04	18,37	14,84	9,23	21,44	8,00	9,09
Weak										
Trial 1	21,95	21,72	27,83	22,42	32,66	19,70	29,63	30,89	14,00	16,32
Trial 2	4,33	5,35	6,88	8,44	8,88	9,16	15,17	11,91	3,12	13,57
Trial 3	23,58	16,95	15,93	20,39	22,62	22,90	24,87	25,41	4,04	7,85
Paired Comparison										
Strong										
Trial 1	20,39	21,11	27,90	25,79	53,00	34,10	36,56	21,24	2,91	1,77
Trial 2	14,45	20,38	19,08	23,67	21,67	10,03	19,98	8,69	7,56	0,74
Trial 3	7,13	7,88	10,52	6,98	21,11	16,74	12,08	33,61	12,51	9,78
Weak										
Trial 1	17,66	30,90	42,28	34,40	43,27	39,43	39,74	28,45	7,67	16,99
Trial 2	10,20	20,18	5,39	4,71	12,94	19,12	20,58	8,66	1,39	2,69
Trial 3	16,59	14,86	21,38	23,56	38,53	26,38	43,39	24,28	11,56	7,36

Table 3.6.5 %TPS VALUES FOR CELESTODERM-V CREAM AND BETNOVATE CREAM
pooled results of both preparations

Time (hrs)	7.	8	9	10	12	14	16	18	28	32
Strong										
Trial 1	18,90	23,53	27,78	33,10	39,78	35,76	31,02	26,43	14,59	11,11
Trial 2	22,38	29,98	35,80	39,47	44,80	40,01	35,38	28,36	16,86	12,62
Trial 3	18,02	23,81	30,48	34,96	37,19	36,31	28,90	22,77	16,01	12,93
Weak										
Trial 1	19,10	22,92	28,67	33,26	42,32	37,58	34,14	31,14	15,24	11,27
Trial 2	21,22	27,78	31,68	34,07	39,62	35,19	33,22	26,59	16,51	10,81
Trial 3	18,06	22,61	27,36	30,63	36,38	36,65	30,52	23,65	16,21	14,12

Table 3.6.6 AREA UNDER THE CURVE VALUES
pooled results of both preparations

	Trial 1	Trial 2	Trial 3
Strong	673	768	658
Weak	722	708	659

Table 3.6.7 CHI-SQUARED VALUES (GRADED RESPONSE) : COMPARISON BETWEEN ARMS
strong arm vs weak arm; pooled results of both preparations

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Trial 1	4,43	1,45	2,75	0,54	7,87	5,15	18,01	20,33	2,05	0,24
Trial 2	1,78	4,60	10,65	18,89	17,40	26,46	17,54	6,82	4,01	5,53
Trial 3	0,60	2,78	9,74	12,76	4,10	0,65	5,65	7,67	3,02	6,45

CHAPTER 4

THE INFLUENCE OF INTERNATIONAL TRANSPORTATION ON THE BIOAVAILABILITY OF BETAMETHASONE 17-VALERATE FROM BETNOVATE FORMULATIONS

It has been reported (section 1.1) that skin disorders are the second most common minor illness and, considering the increase in international travel, it is likely that topical corticosteroid preparations are frequently transported in the luggage of patients in the holds of aircraft or ships. An even more important consideration is the transportation of topical formulations by manufacturers for sale in different countries. The influence of temperature on chemical reactions is a well known scientific phenomenon, routinely utilized by pharmacists in accelerated stability studies and warnings to store medicines in a cool place. Elevated temperatures have been employed in analytical studies on the degradation of corticosteroids in diluted preparations (section 1.6.4), but the influence of very high or very low temperatures on the bioavailabilities of corticosteroids from formulated products does not appear to have been investigated.

Claims for damages to various cosmetic products (mainly lipsticks and eyebrow pencils) have been made against retail outlets, these damages allegedly being due to excessive heat during transportation (456). Coleman (437) found that Topilar cream, imported from the United Kingdom to South Africa, elicited considerably less blanching than expected. Postulated reasons for this were that the cream was formulated for use in a temperate climate and the base may not have been a good releasing medium in a subtropical climate, or that the wide temperature ranges that may have been encountered during importation may in some way have altered the release characteristics of the vehicle. The cream was imported into South Africa by the pharmaceutical distributor and the mode of transportation is not known, although it was probably transported by sea. Shipping agents, harbour authorities, airlines and research organizations were contacted during the planning of the experiment reported in this chapter to obtain information pertaining to temperature conditions in the holds of aircraft and ships. These conditions have, however, not been extensively investigated. Temperatures in the cargo hold of a ship have been found to vary from 1°C to 32°C (456,457) during the cool

season (June/July from South Africa to the United Kingdom and December/January from the United Kingdom to Hong Kong). It is likely that these temperatures may be considerably higher on the north/south route during the hotter equatorial summers. Information about temperatures in the hold of an aircraft has been more difficult to obtain, but cargo may be exposed to temperatures of below 0°C, depending on the cruising altitude of the aircraft and the duration of the flight (458). The ideal vehicle should be able to withstand storage over a temperature range of 5°C to 30°C (190), limits which were exceeded according to the above communications.

In view of the above, it was decided to investigate whether the method of transportation influences the bioavailability of commercially available Betnovate cream, ointment and lotion, each containing 0,1% betamethasone (as the 17-valerate). The influence of temperature extremes on the blanching response could have been investigated after storage of the different preparations in refrigerators or ovens for varying lengths of time under controlled conditions. It was however decided that this experiment would be designed to emulate practical conditions. None of the parcels was sent through a shipping agent; no requests were therefore made for special storage conditions during transportation and holding, and it was therefore not possible to trace the conditions to which the preparations were exposed. Whilst this was identified as a potential disadvantage, it was decided that for the purposes of this experiment the emulation of practical conditions outweighed the usefulness of knowing the conditions to which the products had been exposed. Containers of the same batch of each preparation were purchased from a pharmacy in the United Kingdom. The batch numbers and expiry dates of the preparations are as follows: creams - 2LP 493, October 1985; ointments - 2FP 314, June 1985 and lotions - 2CP 600, March 1985. The trials were performed well within the above expiry dates. One parcel containing each of the formulations was transported to South Africa in the hand luggage of one of the investigators in our laboratories. This parcel was therefore maintained at a reasonably constant temperature range from the time of purchase until the time of testing. A second such parcel was posted to South Africa by surface mail and, as this occurred during the equatorial and southern hemisphere summer, this parcel may have been exposed to relatively high temperatures for up to 8 weeks. A third set of con-

tainers was transported to South Africa in a suitcase in the hold of an aircraft and may therefore have been exposed to very low temperatures for 10 - 15 hours.

The bioavailability of each preparation was assessed by means of the human skin blanching assay within six weeks after the arrival of all the preparations in our laboratories. Three trials were mounted, one for each of the formulation types. The general methodology employed is described in detail in section 2.3. The preparations were applied to a total of 12 sites on each arm of 12 subjects for 6 hours. The same subjects were not necessarily employed in each trial, but were selected from a panel of volunteers known to be sensitive to corticosteroid-induced blanching. The writing arm was occluded on all subjects and the other arm remained unoccluded. Betnovate cream (manufactured and purchased in South Africa) was included in each trial as a standard preparation. All preparations were therefore applied to three sites on each arm. The degree of pallor was assessed independently by three experienced observers on a 0 - 4 scale at several predetermined times from 7 - 32 hours after application. This allowed the construction of the blanching profiles depicted in figures 4.1 - 4.3. The raw data and statistical analyses utilized in this chapter are presented in the appendix at the end of the chapter.

4.1 Assessment of creams (tables 4.1,4.4,4.5)

It can be seen from figure 4.1 that the blanching elicited by Betnovate cream in the occluded mode was similar irrespective of the method of transportation. The AUC value of the cream transported in the cabin of the aircraft was moderately higher than that transported in the hold of the aircraft and that transported by ship, the latter two being almost identical. The method of transportation was further found not to influence the rate of onset or the duration of action of the creams. Maximum blanching was recorded 14 hours after application in all cases with the exception of the cream transported in the hold of the aircraft. In this cream the difference between the 14 and 16 hour readings was, however, only 0,46 %TPS.

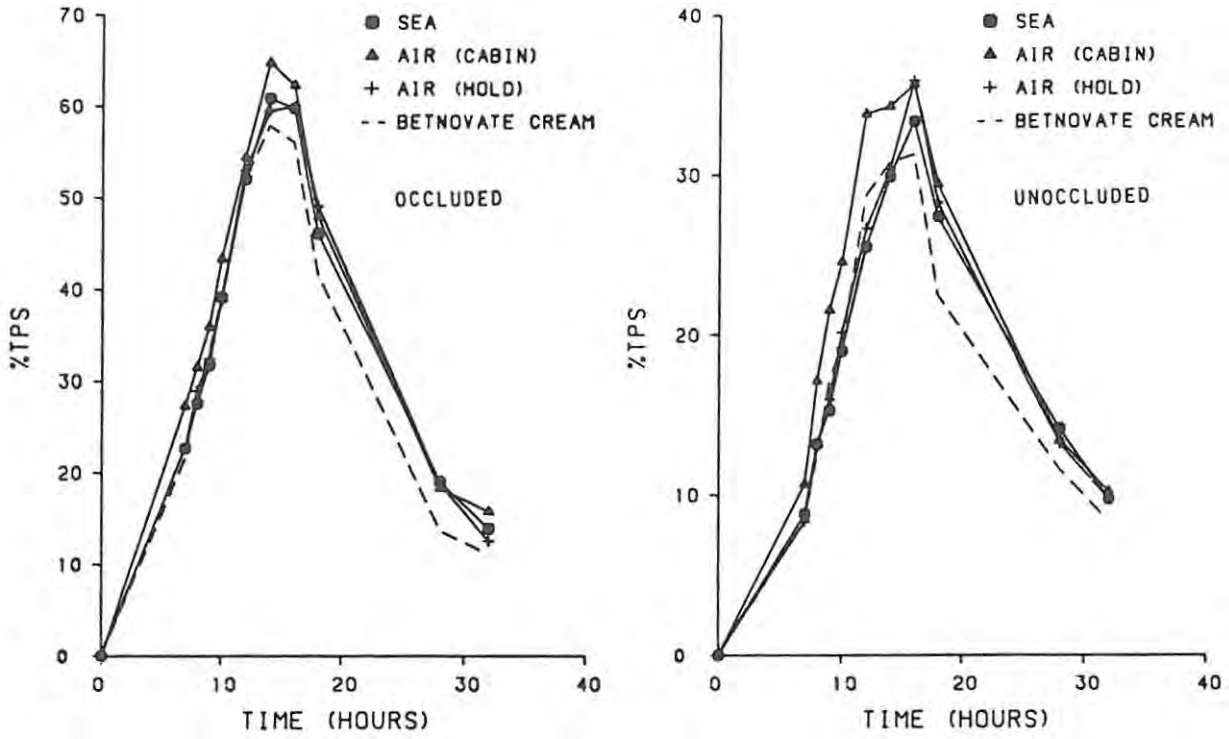


Figure 4.1 Blanching profiles of Betnovate creams

Statistical analysis of the graded responses showed significant differences at only one reading time, namely the 7 hour reading of the comparison between aircraft hold and cabin. Statistical analysis of the comparison of adjacent sites produced a greater number of significant differences, which were found in the comparison of the creams transported in the cabin of the aircraft compared with both the other forms of transportation. Only four differences occurred in each of these comparisons and, when considered with the graded response analyses, the AUC values and the blanching profiles, they do not influence the final conclusion that the method of transportation had little effect on the blanching abilities of the creams applied under occlusion.

Maximum blanching in the unoccluded creams occurred at 16 hours after application, but the cream transported in the cabin of the aircraft had a more rapid onset of action. Statistical analyses also indicated a greater number of significant differences in the unoccluded creams. While only two significant differences were found in the graded response analysis, several were found in the comparison of adjacent sites. The majority of these were found in the comparison of transportation in the cabin of the aircraft with the other two transport modes, and all were

found at or before the peak blanching response. The more rapid onset of action of the creams transported in the cabin of the aircraft was therefore statistically significant in terms of the analysis of adjacent sites.

It can thus be seen from the above that the method of transportation did not influence the release of the active ingredient from the creams applied under occlusive dressings, but appeared to adversely affect the release from the creams transported by sea and in the hold of the aircraft, and applied without occlusion. The rank order of the preparations was, however, the same in both modes of application.

4.2 Assessment of ointments (tables 4.2,4.4,4.6)

The blanching elicited by ointments applied in the occluded mode of application appeared, as in the case of creams, to be only slightly affected by the method of transportation of the preparations. The range of AUC values obtained for the occluded ointments was greater than for the occluded creams. Whilst this may be a function of the difference in release characteristics of creams and ointments, it is possible that the transportation method had a moderately greater influence on ointments than on creams. The AUC values for the occluded ointments transported in the cabin of the aircraft were higher than for the two other modes of transportation, which produced very similar AUC values. Maximum blanching occurred in all three cases at 14 hours after application, and the rate of onset and duration of action were similar in all three ointments. It is interesting to note that the Betnovate cream included as a standard preparation elicited a greater degree of blanching than the Betnovate ointments. Betnovate cream has also been reported by Smith (271) to elicit more blanching than Betnovate ointment, when applied under occlusion.

In the analysis of paired comparisons of adjacent sites of the ointments applied under occlusion, a statistically significant difference was obtained at only one observation time in the comparisons of the ointment transported by sea with those transported in the aircraft hold or aircraft cabin, and no statistically significant differences were found in the graded response analysis. Significant differences were found at

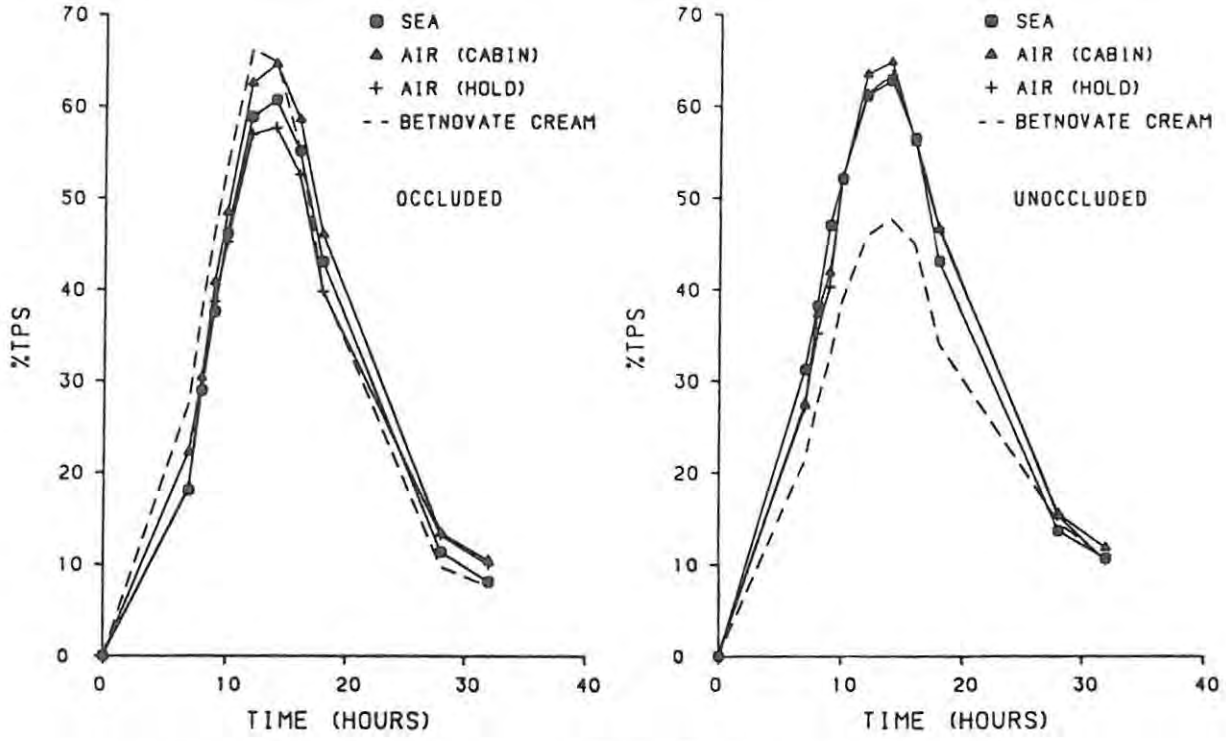


Figure 4.2 Blanching profiles of Betnovate ointments

more intervals in the comparison of aircraft hold with the cabin. The range of reading times at which these differences occurred and the relatively small number of differences do not, however, allow the conclusion to be drawn that the ointment transported in the cabin of the aircraft elicited superior blanching to that transported in the hold of the aircraft, when applied under occlusion.

A similar situation occurred in the ointments applied without occlusion. The highest AUC value was noted for the ointment transported in the cabin of the aircraft, but the three ointments applied in the unoccluded mode produced very similar AUC values, and the blanching profiles are almost superimposed. As was the case with the occluded ointments, the unoccluded preparations produced profiles indicating similar rates of onset and duration of action. It is interesting to note that slightly more intense blanching responses were obtained without occlusion. This phenomenon has been noted in our laboratories on previous occasions with topical corticosteroid preparations that elicit a strong blanching response. It is postulated that this is due to a well formulated vehicle which facilitates release of the corticosteroid without occlusion. It has further been found that, due to the inherent occlusivity of ointment

bases, the differences between blanching responses elicited by ointments in the occluded and open application modes are frequently smaller than the differences in blanching elicited by creams in the two modes.

The few statistically significant differences found in both forms of chi-squared analysis are distributed throughout the trial and the comparisons of the methods of transportation. The blanching elicited by the three unoccluded ointments was therefore similar and only influenced to a small extent by the method of transportation. It was further noted that whilst the differences in blanching responses of the ointments transported in different ways were not statistically significant, the rank order was not consistent between the two application modes. In terms of AUC values, the ointments transported in the cabin of the aircraft elicited the greatest degree of blanching in both modes of application. However, in the occluded mode the ointment transported in the hold of the aircraft elicited the lowest degree of blanching, while in the unoccluded mode the ointment transported by sea elicited the lowest degree of blanching.

4.3 Assessment of lotions (tables 4.3,4.4,4.7)

The profiles and AUC values obtained for the lotions applied under occlusion indicate greater differences than for the creams or ointments. The rate of onset was similar for all three lotions and maximum blanching was observed at 12 hours after application, which is two hours earlier than for the Betnovate cream (standard). Lotions could be expected to exhibit a more rapid onset of action than creams and ointments due to the lower viscosity of lotions and the less complex interaction between the corticosteroid and the lotion base.

Statistically significant differences were noted in a few cases in the graded response analyses and in most cases in the analysis of the comparison of adjacent sites. In the comparison of the lotions transported by sea and in the hold of the aircraft and applied in the occluded mode, significant differences occurred at three reading times in the graded response analysis and at all except the 28 hour and 32 hour readings in the comparisons of adjacent sites. A similar situation pertained to the comparison of the lotions transported by sea and in the cabin of the

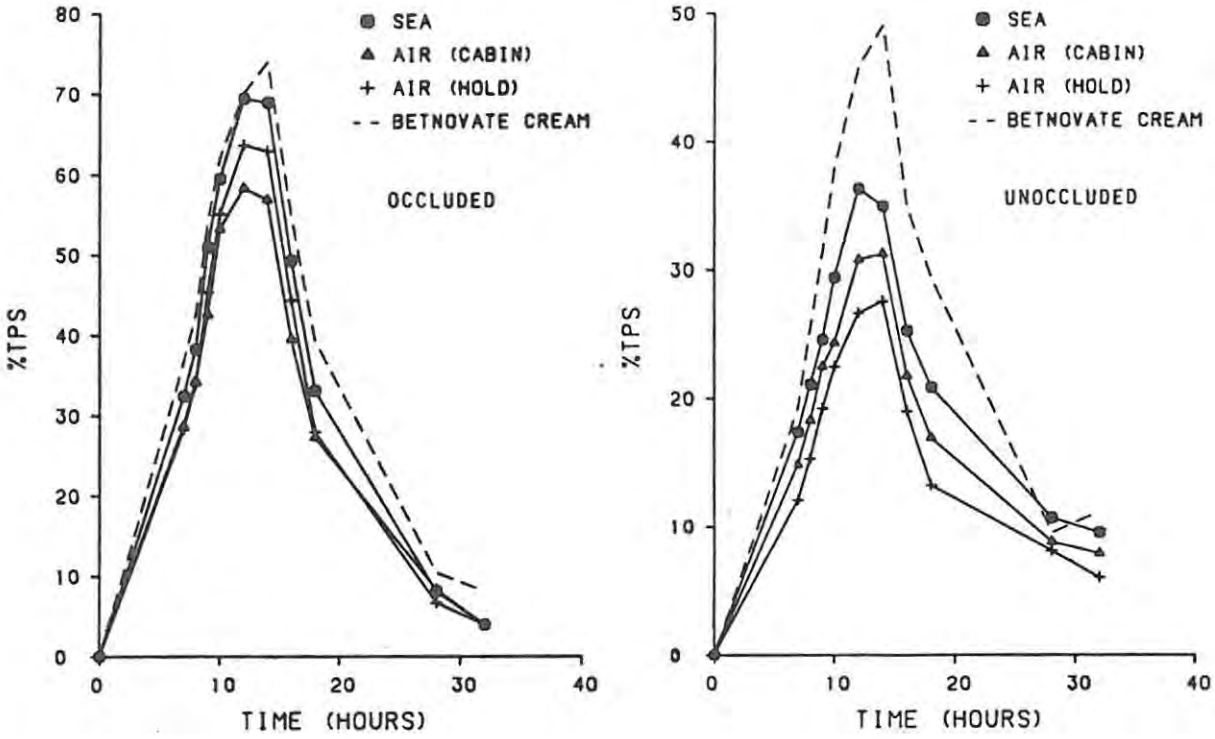


Figure 4.3 Blanching profiles of Betnovate lotions

aircraft, with an additional significant difference in the graded response analysis. No statistically significant differences were noted in the graded response analysis of the comparison of lotions transported in the hold and cabin of the aircraft, but significant differences were found in all comparisons of adjacent sites up to and including 16 hours after application.

The general observations for the lotions applied without occlusive dressings were similar to those applied under occlusion. The lotion transported by sea elicited the greatest degree of blanching in both modes of application. In the occluded mode, the lotion transported in the hold of the ship elicited the second greatest degree of blanching and the lotion transported in the cabin of the aircraft elicited the lowest degree of blanching. In the unoccluded mode, the lotion transported in the cabin of the aircraft was ranked second and the lotion transported in the hold of the aircraft elicited the lowest degree of blanching. The differences in blanching intensities observed in the unoccluded mode also appeared to be greater in the lotions than in the creams and ointments. The lotion transported by sea and applied in the unoccluded mode produced

maximum blanching at the 12 hour reading, which is two hours earlier than the other two lotions.

The statistical analysis of the graded responses produced only four significant differences, all of which occurred in the comparison of the lotions transported by sea and in the hold of the aircraft. In the comparisons of adjacent sites, significant differences were, however, noted at all times except the 7, 28 and 32 hour readings in the comparison of the unoccluded lotions transported by sea and in the cabin of the aircraft. Statistically significant differences were found at all readings in the comparison of the lotions transported by sea and in the hold of the aircraft, whilst no significant differences were found in the comparisons of the lotions transported in the hold and cabin of the aircraft.

Statistical analysis of the lotions did not give unequivocal results in both forms of analysis, in that few differences were noted in the graded responses. The comparisons of adjacent sites, however, indicate significant differences between the lotions transported in the three different ways. This, read with the AUC values and the blanching profiles, allows the conclusion that the lotions transported by sea and air elicited different degrees of blanching.

4.4 Discussion

Some overall observations and conclusions noted in this series of three trials are worthy of comment. The shapes of the blanching profiles obtained for the Betnovate cream manufactured in South Africa and incorporated into all three of these trials as a standard preparation were as expected, thus giving credence to the results of the trials. Higher AUC values were obtained by the standard preparation in the occluded over the unoccluded mode of application in all three trials. In the lotions and creams trials, the rank order of Betnovate cream to the other preparations was unaffected by the mode of application. It is however interesting to note that in the ointment trial, Betnovate cream elicited a greater blanching response than the ointments in the occluded mode, and a lower response than the ointments in the unoccluded mode. This response in the occluded mode is almost certainly due to the fact that

ointments, because of their inherent occlusive properties, are less affected by an occlusive dressing than are creams and lotions.

The conditions under which the preparations were transported only slightly influenced the blanching abilities of the ointments and creams applied under occlusion, but appeared to affect the lotions and the creams applied without occlusion. This may well be an example of occlusion masking the release characteristics of the vehicle (section 2.2.2). It should be remembered that topical corticosteroids are normally applied in the clinical situation without occlusion. It is worth noting that the cream and ointment transported in the cabin of the aircraft, and therefore maintained at essentially normal ambient temperatures, elicited higher degrees of blanching in both modes of application than those transported by sea or in the hold of the aircraft. This suggests that even though differences were not always statistically significant, extremes in temperature appeared to have some effect on the bioavailabilities of the corticosteroid from these formulations.

If percutaneous absorption is adversely influenced by temperature conditions during transportation, the intensity of blanching would be expected to be lower for the preparations stored in the hold of the aircraft and those transported by sea, compared with those transported in the cabin of the aircraft. This was the case with creams and ointments, but not with the lotions. The lotions transported by ship elicited superior blanching when compared to the other two lotions in both modes of application. This is difficult to explain, but may be due to the lotions having been exposed to high or low temperatures which in some way affected the release of corticosteroid from the vehicle. It would, however, be presumed that if this was the case, the creams and ointments transported in the same parcel as the lotions would have been adversely affected. An alteration in the release characteristics also appears to have caused a decreased blanching response in the creams applied without occlusion. The mechanism of this alteration however remains to be elucidated. Exposure of the semisolid emulsion to high or low temperatures, which may have occurred during transportation, possibly led to an alteration in the viscosities of the creams when compared to the cream transported in the cabin of the aircraft. A further possibility is that the physico-chemical properties of the vehicle were influenced by possible

exposure to high or low temperatures. The results of this experiment indicate that further studies, including assessment of blanching, HPLC verification of corticosteroid content, viscosity studies and electron microscopic examination of the formulations, would be useful to attempt to elucidate the cause of the different blanching responses noted after exposure of corticosteroid formulations to different temperatures. There also does not appear to be a clear explanation for the reversal in rank order of the lotions transported in the hold and the cabin of the aircraft when applied in the two modes of application.

All the preparations studied in this chapter were assayed for betamethasone 17-valerate content by means of a high performance liquid chromatographic technique described by Smith *et al.* (459). It can be seen from the table below that the corticosteroid content was essentially the same within each formulation type, irrespective of the method of transportation. Differences in blanching responses were therefore not due to variations in corticosteroid content, and must have been a result of alteration of the release characteristics of the base during transportation.

HPLC ASSAY VALUES OF BETNOVATE PRODUCTS (PERCENTAGE PURITY)

	Sea	Air cabin	Air hold
Cream	100,06	100,92	98,96
Ointment	101,78	101,51	99,36
Lotion	94,63	92,51	94,69
Betnovate cream (standard)	94,95		

This series of three trials was performed on three types of formulations containing the same corticosteroid, and it would therefore be unwise to extrapolate these results to all topically applied medicaments. The practical consideration is however worth noting, namely that the method of transportation may influence the efficacy of topically applied drugs and other medicaments. A further consideration is that elevated temperatures during transportation may hasten the degradation of the drug and render the expiry date of the product inaccurate. Pharmacists and medical practitioners should therefore recommend that travellers transport medicines in their hand luggage, which is kept in the cabin of the

aircraft or in the cabin of the ship on which they are travelling. Exporters of preparations for topical use, as well as manufacturers transporting goods across country by rail, should make use of special transportation and storage conditions. These precautions will aid in preventing the possible adverse effects of extremes in temperature on topical formulations.

APPENDIX TO CHAPTER 4

The tables in this appendix contain the %TPS values and AUC values for the various preparations, as well as the chi-squared values for the comparisons. Significant differences are based on the 95% level of significance and assuming 4 degrees of freedom in the graded response analyses and 1 degree of freedom in the analyses of the comparisons of adjacent sites. Values greater than 9,49 and 3,84, respectively, signify real differences.

The following abbreviations are used in the tables: "Sea" = those preparations transported in the hold of the ship, "cabin" = those preparations transported in the cabin of the aircraft, and "hold" = those preparations transported in the hold of the aircraft. "Standard" = the Betnovate cream (manufactured and purchased in South Africa) included in all the trials as a standard preparation. In the tables of chi-squared values "Cabin/Hold" is the comparisons of the preparations transported in the cabin of the aircraft with the preparations transported in the hold of the aircraft, etc.

Table 4.1 %TPS VALUES FOR THE TRIAL ON BETNOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
Sea	22,69	27,55	31,71	39,12	52,03	60,88	59,72	46,06	18,98	13,89
Cabin	27,31	31,48	35,88	43,29	54,40	64,81	62,29	47,92	18,29	15,74
Hold	22,69	28,94	32,44	38,66	53,01	59,49	59,95	49,07	18,75	12,50
Standard	21,53	28,24	33,10	39,12	52,08	57,87	56,02	41,67	13,66	11,11
Unoccluded										
Sea	8,80	13,19	15,28	18,98	25,46	29,86	33,33	27,31	14,12	9,72
Cabin	10,65	17,13	21,53	24,54	33,80	34,26	35,65	29,40	13,43	10,19
Hold	8,33	12,96	15,97	20,14	26,62	30,32	35,88	28,24	13,19	9,72
Standard	8,33	12,50	17,13	19,44	28,70	30,79	31,25	22,45	11,57	8,33

Table 4.2 %TPS VALUES FOR THE TRIAL ON BETNOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
Sea	18,06	28,94	37,50	46,06	58,80	60,65	55,09	43,06	11,34	8,10
Cabin	22,22	30,32	40,74	48,38	62,50	64,58	58,56	46,06	13,43	10,42
Hold	18,29	29,17	38,66	45,14	56,94	57,64	52,55	39,81	13,19	9,95
Standard	27,55	37,73	46,53	53,47	66,20	64,81	55,32	40,05	9,72	7,64
Unoccluded										
Sea	31,25	38,19	46,99	52,08	61,11	62,73	56,48	43,06	13,66	10,65
Cabin	27,55	37,27	41,90	51,85	63,43	64,81	56,02	46,53	15,51	11,81
Hold	27,08	35,19	40,28	52,31	61,34	63,43	56,25	46,76	15,28	10,42
Standard	21,30	27,55	32,41	38,66	45,83	47,69	44,91	34,03	14,58	10,65

Table 4.3 %TPS VALUES FOR THE TRIAL ON BETNOVATE LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
Sea	32,41	38,19	50,93	59,49	69,44	68,98	49,31	33,10	8,10	3,94
Cabin	28,70	34,26	42,59	53,24	58,33	56,94	39,58	27,31	8,33	3,94
Hold	28,24	33,80	45,37	55,09	63,66	62,96	44,44	28,01	6,71	3,94
Standard	36,57	42,36	53,94	62,04	70,14	74,07	54,86	39,35	10,42	8,10
Unoccluded										
Sea	17,36	21,06	24,54	29,40	36,34	34,95	25,23	20,83	10,65	9,49
Cabin	14,81	18,29	22,45	24,31	30,79	31,25	21,76	16,90	8,80	7,87
Hold	12,04	15,28	19,21	22,45	26,62	27,55	18,98	13,19	8,10	6,02
Standard	19,21	25,46	31,25	37,96	45,83	49,07	34,95	29,40	9,49	11,11

Table 4.4 AREA UNDER THE CURVE VALUES OF BETNOVATE FORMULATIONS

	Creams		Ointments		Lotions	
	Occluded	Unoccluded	Occluded	Unoccluded	Occluded	Unoccluded
Sea	992	552	911	1025	946	571
Cabin	1051	616	995	1047	811	483
Hold	1006	559	894	1033	840	411
Standard	906	506	971	808	1059	716

Table 4.5 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
Sea/Hold	4,23	1,33	1,05	3,19	3,47	3,22	1,31	5,93	1,02	0,69
Sea/Cabin	4,21	4,61	5,52	4,80	1,37	1,98	0,80	2,24	0,19	0,93
Hold/Cabin	10,51	6,86	5,78	7,36	1,99	5,74	1,71	1,41	0,54	2,79
Unoccluded										
Sea/Hold	0,09	1,40	1,40	1,76	1,47	2,03	10,67	2,28	0,18	1,87
Sea/Cabin	1,28	4,08	10,90	8,75	8,86	3,31	2,13	2,51	1,20	0,08
Hold/Cabin	2,02	5,26	6,75	3,44	7,15	4,01	4,71	2,45	1,27	2,36
Paired Comparison										
Occluded										
Sea/Hold	3,13	0,31	1,48	0,11	0,00	1,04	0,11	0,33	0,06	0,02
Sea/Cabin	1,51	4,94	2,28	6,78	3,08	4,40	2,56	4,90	0,22	1,75
Hold/Cabin	9,45	6,15	3,72	11,92	6,01	1,34	0,53	0,00	1,78	0,42
Unoccluded										
Sea/Hold	0,60	0,54	7,89	4,75	0,48	0,31	0,00	0,00	0,07	0,33
Sea/Cabin	3,02	8,22	8,56	7,29	11,44	6,62	1,48	1,87	0,00	0,20
Hold/Cabin	7,35	10,74	7,11	10,01	14,90	4,82	7,11	2,28	0,25	0,07

Table 4.6 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
Sea/Hold	0,19	3,78	2,95	0,09	2,45	3,77	4,02	6,30	1,26	2,47
Sea/Cabin	2,97	2,68	5,96	1,06	4,59	1,69	8,81	6,66	1,22	2,94
Hold/Cabin	2,21	0,55	1,52	1,19	12,66	8,32	13,80	12,62	0,74	0,80
Unoccluded										
Sea/Hold	2,50	4,53	10,07	6,59	3,90	5,86	1,01	6,26	0,68	4,75
Sea/Cabin	4,96	5,98	8,70	6,95	9,34	11,34	6,51	8,79	1,97	1,02
Hold/Cabin	1,97	0,78	0,22	3,63	10,80	13,73	5,79	7,27	0,55	2,75
Paired Comparison										
Occluded										
Sea/Hold	0,16	0,14	1,37	0,11	0,58	0,21	0,06	0,33	1,26	3,69
Sea/Cabin	4,13	1,41	0,21	0,20	0,01	0,47	0,21	0,81	0,42	0,77
Hold/Cabin	5,63	4,88	4,75	2,01	0,75	2,56	3,90	5,69	1,05	0,94
Unoccluded										
Sea/Hold	7,11	2,89	0,00	0,01	1,00	2,42	0,77	0,48	0,46	0,02
Sea/Cabin	3,61	6,13	5,25	5,56	0,00	5,33	0,65	0,11	0,91	0,09
Hold/Cabin	5,51	2,48	1,48	5,80	1,73	0,00	1,01	1,92	0,00	0,00

Table 4.7 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
Sea/Hold	5,27	9,69	11,31	5,14	9,79	7,01	4,03	6,76	2,35	0,00
Sea/Cabin	2,96	7,57	16,88	6,89	19,79	18,10	12,05	5,96	0,02	0,00
Hold/Cabin	2,09	7,73	3,09	2,25	6,62	5,34	4,49	0,14	2,71	0,00
Unoccluded										
Sea/Hold	9,12	11,46	3,98	13,00	17,25	9,19	5,89	12,63	3,23	4,87
Sea/Cabin	6,18	2,78	3,14	7,52	7,69	2,68	2,04	3,10	2,47	1,00
Hold/Cabin	6,13	5,68	2,55	2,94	2,49	2,88	1,78	3,66	0,19	1,48
Paired Comparison										
Occluded										
Sea/Hold	7,11	8,48	4,69	10,34	10,22	4,90	7,27	10,84	1,69	0,03
Sea/Cabin	5,20	21,50	13,92	18,11	14,90	16,53	16,23	10,51	0,09	0,00
Hold/Cabin	5,01	8,10	5,75	10,78	17,51	9,34	10,22	1,97	0,34	0,00
Unoccluded										
Sea/Hold	13,02	6,49	10,41	16,12	23,52	9,26	15,56	16,79	4,00	7,85
Sea/Cabin	0,57	4,70	3,91	6,78	13,28	6,29	6,82	5,97	3,44	0,54
Hold/Cabin	2,53	2,29	0,06	0,23	0,00	0,06	0,25	0,88	0,18	1,63

CHAPTER 5

COMPARATIVE BIOAVAILABILITY STUDIES ON PROPRIETARY CORTICOSTEROID-CONTAINING PREPARATIONS FROM THREE COUNTRIES

One of the factors that requires consideration during the development of topical formulations is the climatic conditions under which the medicament is to be utilized. Preparations formulated for use in temperate climates may not be suitable for use in tropical or sub-tropical climates and *vice versa*. The influence of the vehicle on the release of corticosteroids has been thoroughly studied and is discussed in section 1.6. It was decided to investigate the blanching activities of proprietary corticosteroid formulations purchased in three countries, namely South Africa (SA), Australia and the United Kingdom (UK), the first two countries representing sub-tropical climatic conditions and the UK representing temperate conditions. The products used in this study were Betnovate cream, ointment and lotion (0,1% betamethasone as the 17-valerate), Synalar cream, ointment and lotion (0,025% fluocinolone 16,17-acetonide), Eumovate cream and ointment (0,05% clobetasone 17-butyrate) and Locoid cream and ointment (0,1% hydrocortisone 17-butyrate). The preparations used, together with the manufacturing company and place of manufacture are summarized in table 5.1. Manufacturers were consulted to ascertain whether there were differences between the formulations available in the three countries; this information is presented in section 5.5.

The preparations were purchased from pharmacies in Farnham (UK), Sydney (Australia) and Grahamstown (SA) shortly before the commencement of the series of trials. The UK and Australian preparations were transported to South Africa in the hand luggage of the purchasers to avoid possible adverse effects of temperature extremes during transportation (see Chapter 4). Ten trials were mounted for this study. Each of the different products from the three countries was assayed in the same trial; the Betnovate creams from the three countries were assayed in one trial, the Betnovate ointments in another, *etc*. The general methodology employed in the trials is described in detail in section 2.3. The preparations were applied to a total of 12 sites on each arm of 12 subjects for 6 hours. The same subjects were not necessarily employed in each trial but were

TABLE 5.1 PROPRIETARY PREPARATIONS USED IN THIS STUDY

Preparation	Purchased	Manufactured
Betnovate cream	South Africa	South Africa
	Australia	Australia
	United Kingdom	United Kingdom
Betnovate ointment	South Africa	South Africa
	Australia	Australia
	United Kingdom	United Kingdom
Betnovate lotion	South Africa	South Africa
	Australia	Australia
	United Kingdom	United Kingdom
Synalar cream	South Africa	South Africa
	Australia	United Kingdom
	United Kingdom	United Kingdom
Synalar ointment	South Africa	South Africa
	Australia	United Kingdom
	United Kingdom	United Kingdom
Synalar lotion	South Africa	South Africa
	Australia	United Kingdom
	United Kingdom	United Kingdom
Locoid cream	South Africa	South Africa*
	Australia	Netherlands
	United Kingdom	Netherlands
Locoid ointment	South Africa	South Africa*
	Australia	Netherlands
	United Kingdom	Netherlands
Eumovate cream	South Africa	South Africa
	Australia	Australia
	United Kingdom	United Kingdom
Eumovate ointment	South Africa	South Africa
	Australia	Australia
	United Kingdom	United Kingdom

Preparation	Manufacturer
Betnovate	Glaxo Pharmaceuticals
Eumovate	Glaxo Pharmaceuticals
Locoid*	Gist-Brocades nv
Synalar	Imperial Chemical Industries (I.C.I.)

* manufactured under licence in South Africa

selected from a panel of volunteers known to be sensitive to corticosteroid-induced blanching. The writing arm was occluded on all subjects and the other arm remained unoccluded. Betnovate cream (manufactured and purchased in South Africa) was included in each trial as a standard preparation. Each preparation was therefore applied to three sites on each arm. The degree of pallor was assessed independently by three experienced observers on a 0 - 4 scale at several predetermined times from 7 - 32 hours after application. This allowed the construction of the blanching profiles depicted in figures 5.1.1 - 5.4.2. The blanching profiles designated SA (Standard) in figure 5.1.1 and Betnovate cream in the remaining figures represent the Betnovate cream included in all trials as the standard preparation. The raw data and statistical analyses for this study are presented in the appendix at the end of the chapter.

The preparations utilized in this study have documented clinical efficacies (cited in table 5.2) and blanching activities (cited in table 5.3). Whilst the different proprietary preparations were not compared to each other in this study, the summary in table 5.3 gives a general overview of the relative blanching abilities. There are several other reports in the literature on comparative blanching abilities of the constituent corticosteroids in various vehicles, but only those studies in which the tradename products used in this study were reported are cited in table 5.3. Numerous authors have stated that commercially available products were used, but have not reported the tradenames. In several cases where

TABLE 5.2 REPORTS ON CLINICAL EFFICACIES OF THE PREPARATIONS USED IN THIS STUDY

Preparation	References
Betnovate cream	268,460-462
Betnovate ointment	54,461,463-465
Betnovate lotion	55
Synalar cream	172,460,466
Synalar ointment	54,118,467
Locoid cream	29,468-470
Locoid ointment	92,471
Eumovate cream	472*
Eumovate ointment	92,472*

* reported as Molivate, the previous tradename for Eumovate in the United Kingdom.

TABLE 5.3 REPORTS ON COMPARATIVE BLANCHING RESPONSES OF THE PREPARATIONS USED IN THIS STUDY

COMPARATIVE BLANCHING	REFERENCES
Betnovate > Synalar	
creams (occluded)	68*,380*
creams (unoccluded)	380*
ointments (occluded)	204*,296,297,304*,380*
ointments (unoccluded)	380*
Synalar > Betnovate	
creams (occluded)	113*
creams (unoccluded)	215*
ointments (unoccluded)	96*
Betnovate > Locoid	
creams (unoccluded)	215*
ointments (unoccluded)	96
Locoid > Betnovate	
creams (occluded)	101*
Betnovate > Eumovate	
creams (occluded)	447
creams (unoccluded)	447
ointments (occluded)	96,295*,304,447
Eumovate > Betnovate	
ointments (occluded)	296*,297*
ointments (unoccluded)	447
Synalar > Eumovate	
ointments (occluded)	296,304*
ointments (unoccluded)	96
Eumovate > Synalar	
ointments (occluded)	297
Synalar > Locoid	
creams (unoccluded)	215*
ointments (unoccluded)	96
Eumovate > Locoid	
ointments (unoccluded)	96*

* not statistically significantly different

proprietary products were used, the name or names of only the principal products are presented. A similar situation was noted when reviewing the literature on clinical trials. It is important to bear in mind that, for example, commercially available betamethasone 17-valerate cream is not synonymous with Betnovate cream, and inter-laboratory comparisons are unreliable unless tradenames or formulae of vehicles are reported. Authors should therefore, where possible, report the tradenames or formulae of the products assayed.

5.1 Results and discussion

5.1.1 Comparisons of Betnovate creams, ointments and lotions

5.1.1.1 Comparison of creams (tables 5.1.1,5.1.4-5.1.6)

The profiles obtained for the Betnovate creams from the three countries are depicted in figure 5.1.1. It can be seen from these and the AUC values (see appendix) that a substantially greater intensity of blanching was seen in the occluded mode of application compared to the unoccluded mode. Maximum blanching occurred between 12 and 14 hours after application in all cases, with the peaks being relatively broad.

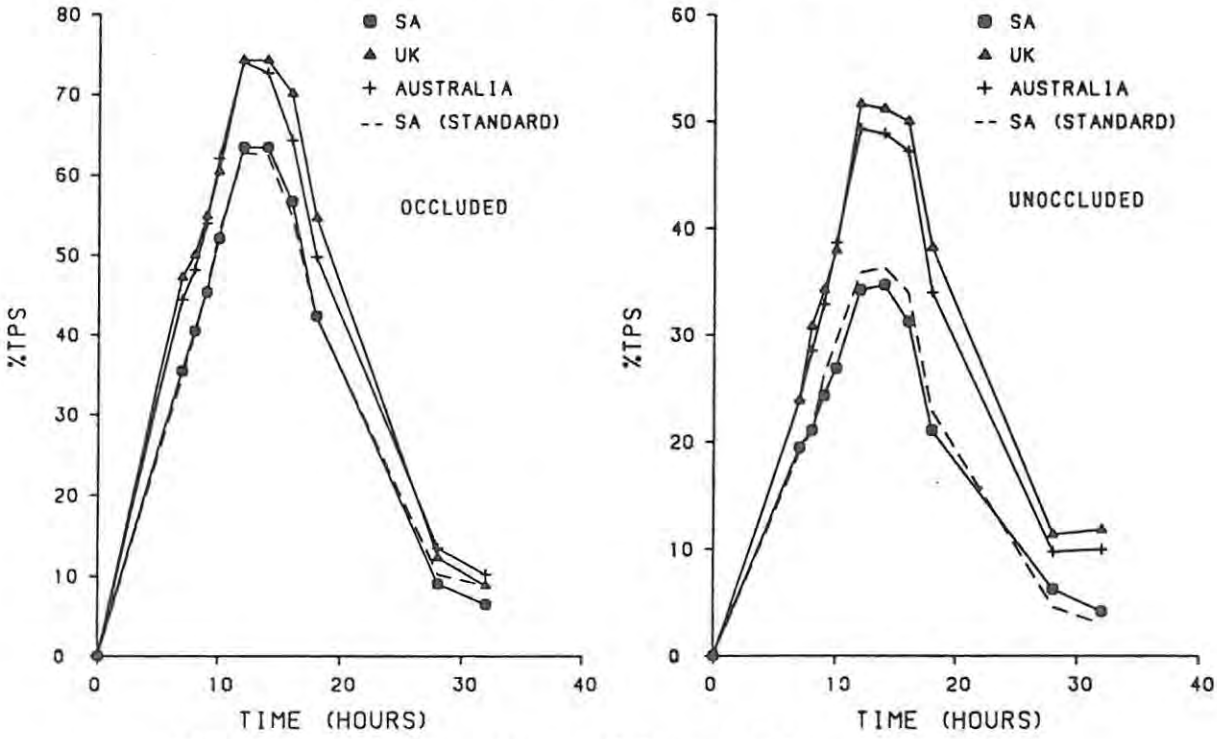


Figure 5.1.1 Blanching profiles of Betnovate creams

Statistical analysis of the comparison of the UK cream with the SA cream indicated superiority of the former in both modes of application. In the graded response analysis, significant differences were found at all but three observations in each application mode, and in the paired comparisons of adjacent sites significant differences were found in favour of the UK formulation at all observation times. In the comparison of the Australian cream to the SA cream applied under occlusion, statistically significant differences were found in the graded response analysis from 7 - 12 hours after application and at all readings in the comparison of adjacent sites. In the unoccluded application mode statistically sig-

nificant differences were found at all but two readings in the graded response analysis and at all reading times in the comparison of adjacent sites. The Australian cream therefore also elicited superior blanching to the SA cream in both application modes.

In the comparison of the UK cream to the Australian cream, a small number of statistically significant differences were found in both forms of analysis in the occluded mode, but these were not sufficient to indicate a statistically significant difference overall. In the unoccluded mode no significant differences were found in the graded response analysis, while differences were found at all but three of the readings in the comparison of adjacent sites. A study of the profiles, AUC values and results of statistical analysis therefore indicates that the blanching abilities of the UK cream and Australian cream are similar to each other in both application modes.

As was previously mentioned, Betnovate cream (SA) was incorporated into each of the trials in this study as a standard preparation. The application pattern for this trial on Betnovate creams from the three countries was not altered to avoid the inclusion of two SA Betnovate creams, that is to say Betnovate cream SA was incorporated under two different codes. It is particularly interesting and encouraging to note that the observed blanching responses of the two SA Betnovate creams were almost identical in the occluded application mode and very similar in the unoccluded mode. In the occluded mode the AUC values varied by 1 unit, whereas in the unoccluded mode the difference was slightly larger; the AUC values were 542 and 556. This marginally greater difference was probably due to the increased difficulty of assessing weaker blanching responses. The trial design did not include the standard preparation in the comparisons of adjacent sites. The graded responses were however analysed, and it was found that no statistically significant differences occurred in either application mode. As explained in the section on methodology, the trial design does not allow observers to identify preparations until the code is broken after the conclusion of the trial. The observers therefore were not aware during the trial which sites contained the differently coded SA Betnovate creams. Whilst it cannot be denied that the visual method of observation utilized in the blanching assay is subjective, the reliability of visual assessment performed by experienced

observers is undoubtedly demonstrated by the similarity in blanching responses reported here.

5.1.1.2 Comparison of ointments (tables 5.1.2,5.1.4,5.1.7)

Figure 5.1.2 depicts the profiles obtained for the Betnovate ointments applied with and without occlusion. Maximum blanching occurred at 14 hours after application for all the ointment preparations and the shapes of the blanching profiles were similar in both modes of application. The intensity of blanching was similar in both application modes, which is often the case in corticosteroid preparations that elicit a good response, and especially when incorporated into an ointment base (see also section 4.2).

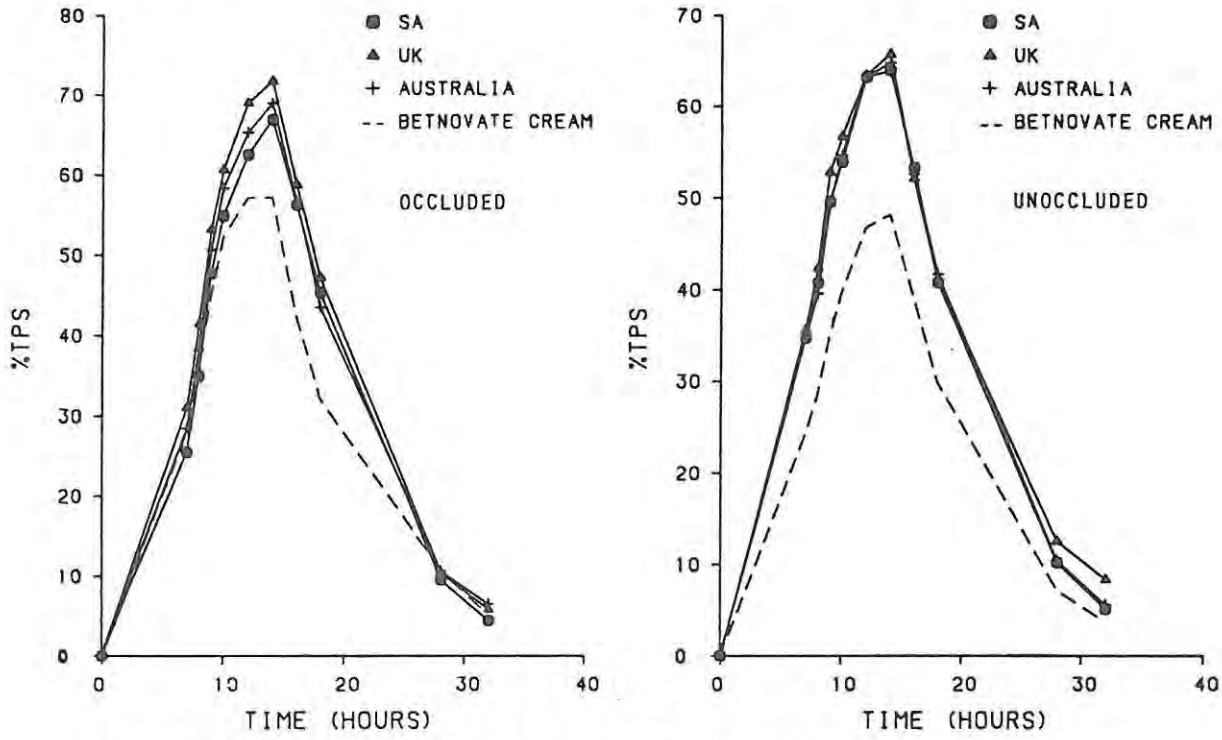


Figure 5.1.2 Blanching profiles of Betnovate ointments

Only one statistically significant difference was found in the graded response analyses of the ointments in both modes of application. Several significant differences were, however, found in the comparisons of adjacent sites. Significant differences were found from 7 - 18 hours after application in the comparison of the UK ointment to the SA ointment in the occluded mode, and at five readings in the unoccluded mode. It is significant that the chi-squared values obtained in this analysis of

adjacent sites were larger in the occluded than the unoccluded mode due to the substantial number of site pairs in which the UK ointment elicited greater blanching than the SA ointment in the occluded mode. Although there was one significant difference in the graded response analysis, and the chi-squared values in the comparison of adjacent sites were fairly high, when these are studied in combination with the AUC values and blanching profiles it is concluded that the ointments purchased in the UK and SA did not elicit significantly different blanching responses, when applied under occlusion. In all the other ointment comparisons the differences were smaller and, as there were no differences in the graded response analyses, it can be concluded that the blanching abilities were similar.

5.1.1.3 Comparison of lotions (tables 5.1.3,5.1.4,5.1.8)

The profiles obtained for the Betnovate lotions are reproduced in figure 5.1.3. It can be seen from the profiles and AUC values that occlusion of the application sites gave rise to a considerably enhanced degree of blanching. The maximum blanching responses in the occluded mode occurred two hours prior to those in the unoccluded mode, namely at 12 hours and 14 hours after application. This could be expected due to the lack of occlusivity of lotions; hydration of the stratum corneum by the occlu-

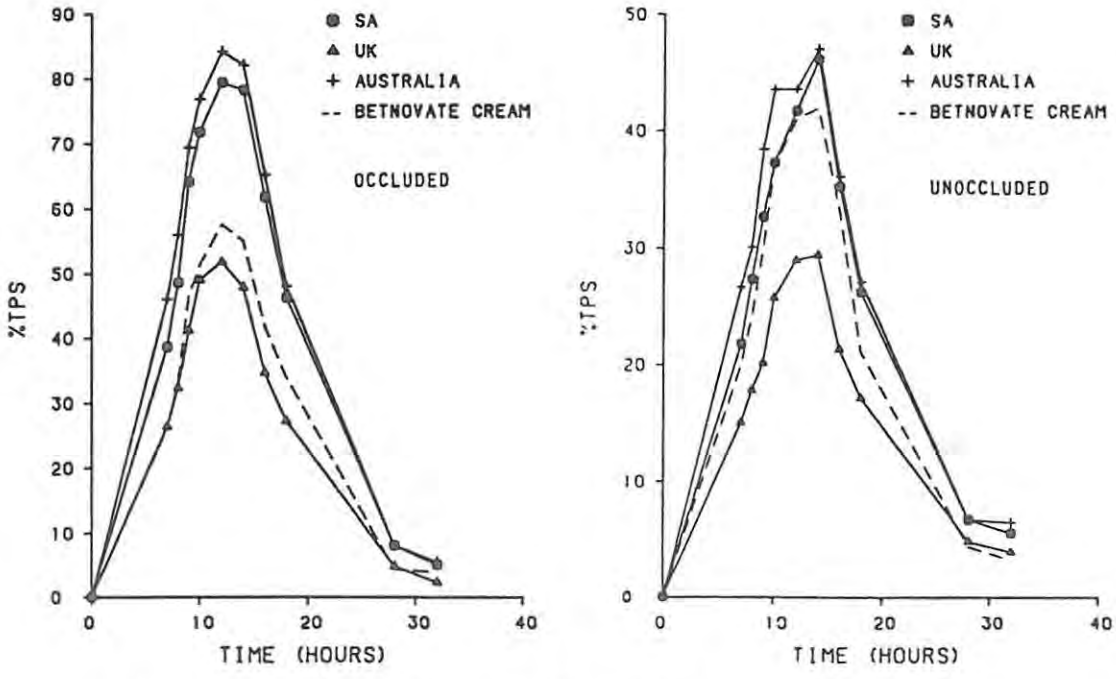


Figure 5.1.3 Blanching profiles of Betnovate lotions

sive dressing would therefore have a greater influence on the rate and extent of the percutaneous penetration of corticosteroids from lotions than from creams and ointments. It is interesting to note from the profiles of the unoccluded lotions that the Australian lotion, besides eliciting more blanching than the other two lotions, demonstrated a more rapid onset of action. The shoulder noted just before the peak blanching response has been found on previous occasions in our laboratories. It is usual practice in our laboratories to join points on the blanching profiles with straight lines and not employ computer assisted smoothing which would hide this shoulder.

In the statistical analyses of the comparison of the UK lotion with the Australian and SA lotions, significant differences were found at all readings except 28 and 32 hours after application in both application modes in the graded response analysis, as well as the paired comparisons of adjacent sites in the unoccluded mode. In the comparison of adjacent sites in the occluded mode, significant differences were found at all readings except 32 hours after application. The UK lotion therefore elicited considerably less blanching than the SA and Australian lotions in both modes of application.

In the comparison of the SA lotion with the Australian lotion, no statistically significant differences were found in the graded response analyses in either application mode and only two were found in the comparison of adjacent sites in the unoccluded mode. In the comparison of adjacent sites in the occluded mode, statistically significant differences were found at all but three readings. Considering the absence of significant differences in the graded response analysis and the relatively small difference in AUC values, it seems reasonable to conclude that the Australian and SA lotions elicited similar blanching responses to each other in both application modes.

5.1.2 Comparisons of Synalar creams, ointments and lotions

5.1.2.1 Comparison of creams (tables 5.2.1,5.2.4,5.2.5)

The blanching profiles of the Synalar creams applied in both modes of application are reproduced in figure 5.2.1. Comparison of the blanching profiles of the Synalar creams to Betnovate cream demonstrated that in

the occluded application mode Betnovate elicited a more rapid onset of action than two of the Synalar preparations (SA and UK), but had a shorter duration of action than all three Synalar creams. In the unoccluded mode the four preparations exhibited similar onset of action, but the Synalar creams had a longer duration of action. The relatively longer duration of action of Synalar preparations has been reported before (68, 96,204,367). It can further be seen from the profiles and AUC values that the rank order of the Synalar creams (Australia > SA > UK) was similar in both modes of application. The difference in the blanching intensities between the two modes of application was small, a phenomenon that is frequently noted for corticosteroid preparations that elicit a good blanching response (see also sections 4.2 and 5.1.1.2).

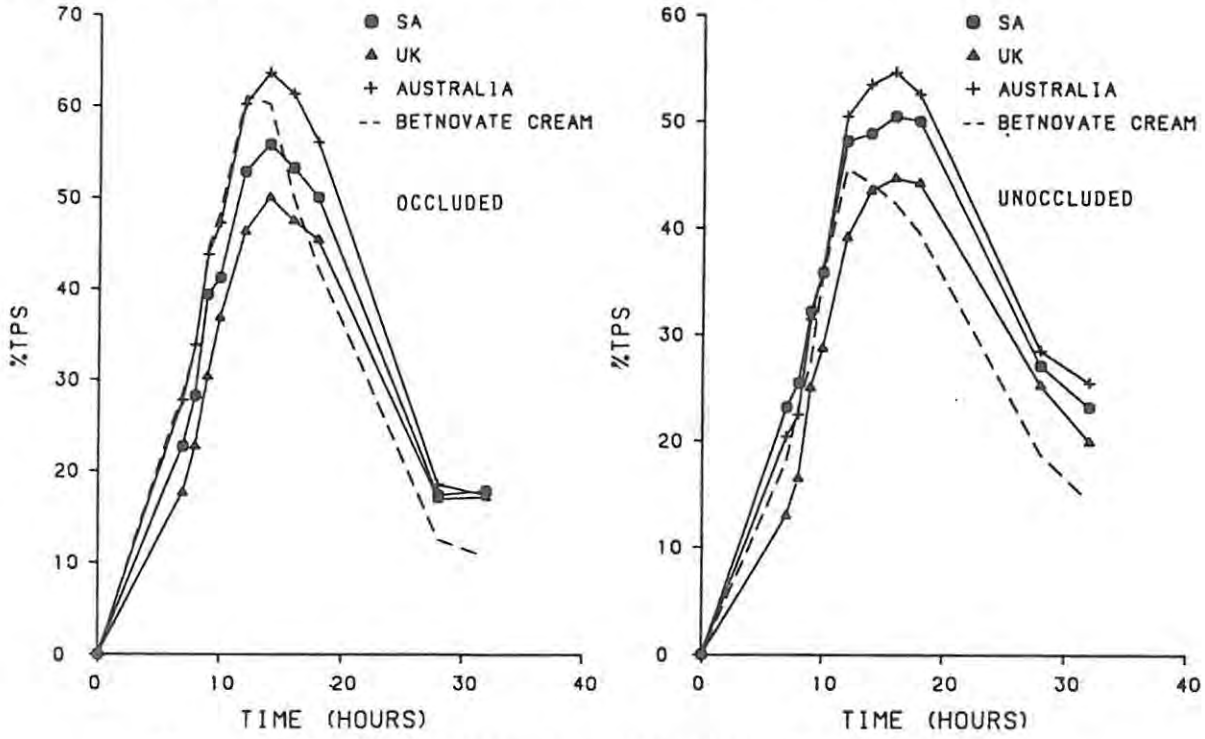


Figure 5.2.1 Blanching profiles of Synalar creams

No significant differences were found in the graded response analysis of the comparison of the SA cream with the UK cream in the occluded mode of application, while significant differences were found at all but the last two readings in the paired comparisons of adjacent sites. The chi-squared values obtained in the paired comparison analysis were large, and when these were studied with the AUC values and blanching profiles it was concluded that the SA cream elicited a greater intensity of blanching than the UK cream in the occluded mode, even though no significant

differences were found in the graded response analysis. In the unoccluded mode, significant differences were found at the 7 and 8 hour readings in the graded response analysis and at all except the 18 and 28 hour readings in the analysis of the paired comparisons. However, if the same principles as above are applied in this case, it can be concluded that the SA cream also elicited a greater degree of blanching than the UK cream in the unoccluded mode of application.

In the comparison of the SA cream with the Australian cream in the occluded application mode, significant differences were found in the graded response analysis at 10, 12 and 16 hours after application, and at all except the 28 and 32 hour readings in the comparisons of adjacent sites. In the unoccluded application mode, no statistically significant differences were found in the graded response analysis, whilst significant differences were found at the 7, 14, 16 and 32 hour readings in the comparisons of adjacent sites. A study of the statistical analyses, AUC values and blanching profiles therefore indicates that the Australian Synalar cream elicited more blanching than the SA Synalar cream in the occluded mode. The two preparations elicited similar blanching responses to each other in the unoccluded application mode, although the Australian cream elicited somewhat more blanching from 12 - 18 hours after application.

Statistical analyses of the comparison of the Australian cream to the UK cream were found to allow more definite conclusions. Statistically significant differences were found in both application modes at most of the readings in the graded response analyses and at all except the 28 and 32 hour readings in the analysis of paired comparisons of adjacent sites. A study of the statistical analyses, AUC values and blanching profiles therefore indicates clear-cut superiority of the blanching response of the Australian cream compared with the UK cream in both modes of application.

5.1.2.2 Comparison of ointments (tables 5.2.2,5.2.4,5.2.6)

Figure 5.2.2 depicts the blanching profiles of the Synalar ointments. It can be seen from the profiles and %TPS values that the time of maximum blanching for each preparation, except the SA preparation applied

under occlusion, was 14 hours after application. Maximum blanching for the SA ointment applied under occlusion was recorded at 16 hours, but the difference between the 14 and 16 hour readings was only 0,47 %TPS. It can further be seen that the blanching responses elicited by the three preparations were similar between the two application modes (see also sections 4.2, 5.1.1.2 and 5.1.2.1).

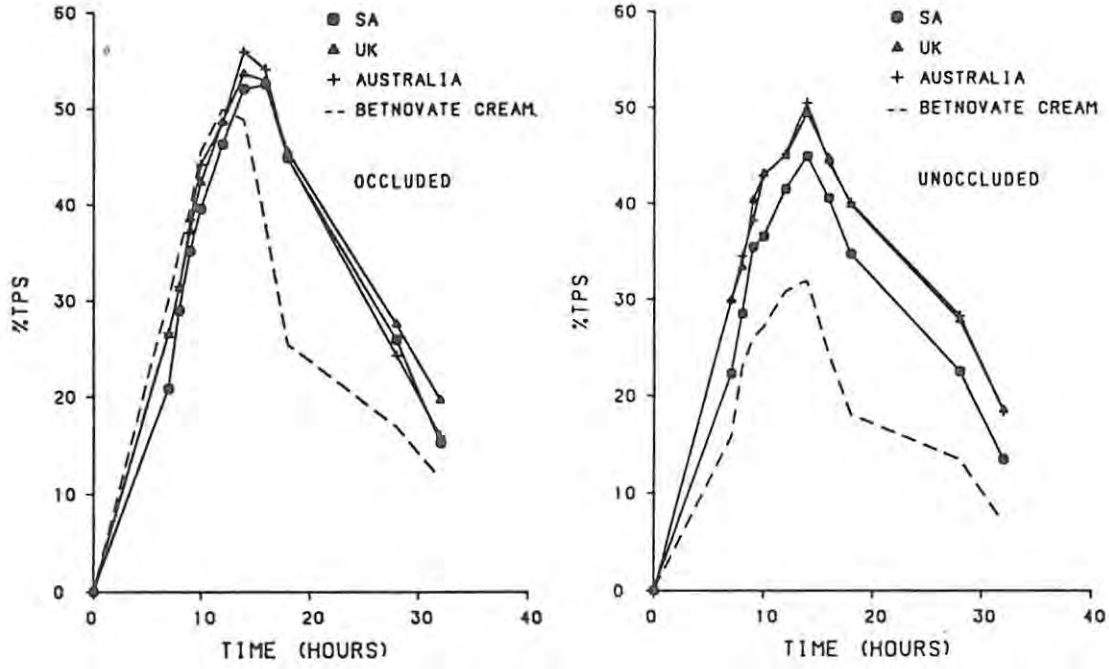


Figure 5.2.2 Blanching profiles of Synalar ointments

In the comparison of the Australian to the UK ointment no statistically significant differences were found in either mode of application. The AUC values between the two preparations were similar in both modes of application, and in the unoccluded mode the blanching profiles were virtually superimposed for the duration of the trial. These two ointments therefore elicited similar blanching responses in both modes of application.

In the statistical analyses of the comparison of the UK ointment with the SA ointment, no significant differences were found in the graded response analysis in the occluded mode of application and differences were found only at the 28 and 32 hour readings in the unoccluded mode. All the comparisons of adjacent sites between these two ointments were, however, significantly statistically different. In the occluded mode the

difference as indicated by the profiles and AUC values was not very large, whereas in the unoccluded mode the difference was greater. A study of the statistical analyses, AUC values and blanching profiles indicates that these preparations elicited similar blanching responses in the occluded application mode, whilst the UK ointment elicited superior blanching to the SA ointment in the unoccluded mode.

No statistically significant differences were found in the graded response analysis of the comparison of the ointments from SA and Australia. In the comparison of adjacent sites, differences were found in the occluded mode at all except one reading time until 14 hours after application, and in the unoccluded mode at all reading times. A study of the statistical analyses, together with the AUC values and blanching profiles therefore indicates that these preparations elicited similar blanching intensities in the occluded application mode, whilst the Australian preparation elicited more blanching than the SA preparation in the unoccluded mode.

5.1.2.3 Comparison of lotions (tables 5.2.3,5.2.4,5.2.7)

The blanching profiles obtained for Synalar lotions are depicted in figure 5.2.3. The UK lotion elicited a greater intensity of blanching than the Australian and SA lotions in both modes of application. In the occluded mode the Australian and SA lotions elicited very similar blanching responses to each other, whilst in the unoccluded mode the SA lotion elicited somewhat greater blanching than the Australian lotion. The AUC values obtained for the SA and Australian lotions applied under occlusion were identical. Maximum blanching in the occluded mode was noted at 14 hours after application and in the unoccluded mode at 16 hours after application (see also section 5.1.1.3). It is interesting to note that all three Synalar lotions elicited a more intense blanching response when applied without occlusion. In the Australian preparation the increase in blanching in the unoccluded mode was small, in the SA preparation it was slightly larger, whilst in the UK preparation the blanching elicited without occlusion was considerably greater than that elicited with occlusion. The Betnovate cream included as a standard preparation however elicited a more intense blanching response in the occluded mode. A similar situation to this occurred in the

assessment of Eumovate ointments (section 5.1.4.2).

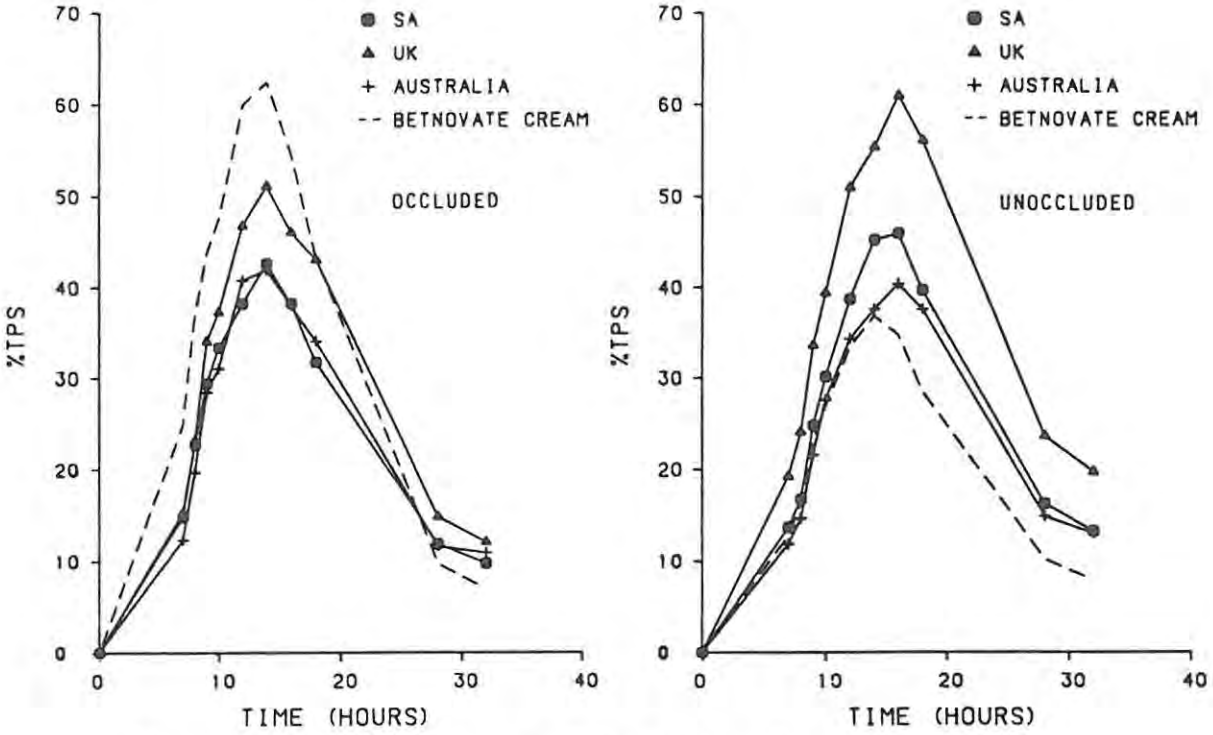


Figure 5.2.3 Blanching profiles of Synalar lotions

In the occluded application mode, no significant differences were found between the Australian lotion and SA lotion in either form of statistical analysis and, as the AUC values were identical and the blanching profiles were virtually superimposed, it is concluded that these two lotions elicited the same degree of blanching in the occluded mode. In the unoccluded mode, the SA lotion can be seen to have elicited a slightly greater degree of blanching than the Australian lotion in terms of the blanching profiles and AUC values. However, as only one statistically significant difference was observed (12 hours after application in the paired comparison of adjacent sites), it is concluded that the difference between these two preparations is not significant.

In the comparison of the UK lotion with the SA lotion applied under occlusion, a significant difference was found at 18 hours after application in the graded response analysis and from 10 - 18 hours after application in the analysis of the paired comparisons. In the unoccluded mode significant differences occurred at 9, 16 and 18 hours after application in the graded response analysis and at all reading times in the analysis of paired comparisons. Considering the statistical analyses with the

blanching profiles and AUC values, it can be seen that although not always statistically significant, the UK lotion elicited a more intense blanching response than the SA lotion in both modes of application.

Statistical analyses of the graded responses of the comparison of the UK lotion with the Australian lotion applied under occlusion showed significant differences at 10 and 14 hours after application. In the analysis of paired comparisons, statistically significant differences were found at 7 hours and from 10 - 18 hours after application. It can however be seen from the blanching profiles and AUC values that although the differences were not statistically significant, the UK lotion elicited a greater degree of blanching than the Australian lotion in the occluded application mode. In the unoccluded mode, the only statistically non-significant difference that occurred was at the 7 hour reading in the graded response analysis. This, together with the AUC values and blanching profiles indicates that the UK lotion also elicited superior blanching to the Australian lotion in the unoccluded mode of application.

5.1.3 Comparisons of Locoid creams and ointments

5.1.3.1 Comparison of creams (tables 5.3.1,5.3.3,5.3.4)

The blanching profiles of the Locoid creams are depicted in figure 5.3.1. It can be seen from the profiles that maximum blanching occurred at 12 hours after application and that a greater intensity of blanching was observed in the occluded than in the unoccluded application mode.

In the comparison of the UK cream to the Australian cream, no significant differences were found in the graded response analyses in either application mode and few significant differences were found in the analyses of the paired comparisons of adjacent sites in both modes of application. It can therefore be concluded that the slightly superior blanching response of the UK cream to the Australian cream was not statistically significant.

In the comparison of the UK cream to the SA cream in the occluded mode of application, one significant difference was found in the graded response analysis, whilst differences were found at all readings except

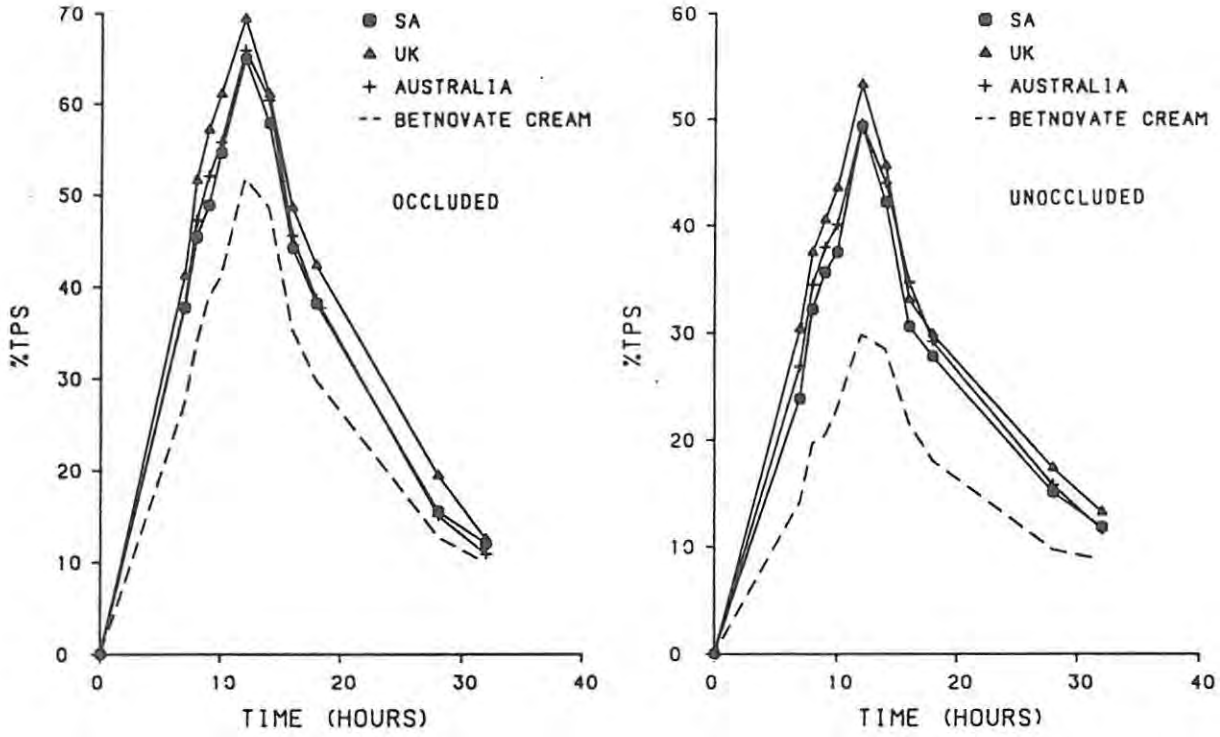


Figure 5.3.1 Blanching profiles of Locoid creams

32 hours after application in the comparisons of adjacent sites. In the unoccluded mode no statistically significant differences were found in the graded response analysis, while significant differences were found from 7 - 9 hours after application in the comparisons of adjacent sites. Considering the AUC values, blanching profiles and statistical analyses, it can be concluded that whilst the UK cream elicited a greater degree of blanching than the SA cream, the superior blanching response was not statistically significant.

Only one significant difference was found in the comparison of the SA cream to the Australian cream; this was at the 14 hour reading in the comparison of adjacent sites in the unoccluded mode of application. The AUC values in the occluded mode were very similar and whilst the difference in the AUC values in the unoccluded mode was slightly greater than in the occluded mode, when these are studied with the blanching profiles and statistical analyses, it can be concluded that the SA cream and the Australian cream elicited similar degrees of blanching in both modes of application.

5.1.3.2 Comparison of ointments (tables 5.3.2,5.3.3,5.3.5)

The blanching profiles of the ointments are depicted in figure 5.3.2. In the occluded mode the SA ointment exhibited a slightly more rapid onset and shorter duration of action than the Australian and UK ointments, whilst the UK ointment exhibited a longer duration of action than the other two ointments in the unoccluded mode. The trough in the curves at the 8 hour reading may be due to a sudden change in ambient temperature which may have influenced the superficial vasculature of the forearm. In the unoccluded mode, a trough was only noted for the SA ointment whereas the other two Locoid ointments exhibited shoulders at this point, whilst the blanching elicited by Betnovate cream seemed to be unaffected. The absence of any irregularity in the profile of Betnovate cream may be due to the weak blanching response at this reading. It has been reported (135,184,203,325) that the hydration of the stratum corneum after occlusion is still evident at the 7 hour reading; this may in some way render the surface vasculature more sensitive to sudden changes in ambient temperature. Transient erythema has been observed around the borders of occluded sites for up to one hour after removal of occlusive dressings (325). The contrast between erythematous skin and the sites of application may have led to observers recording more blanching than would

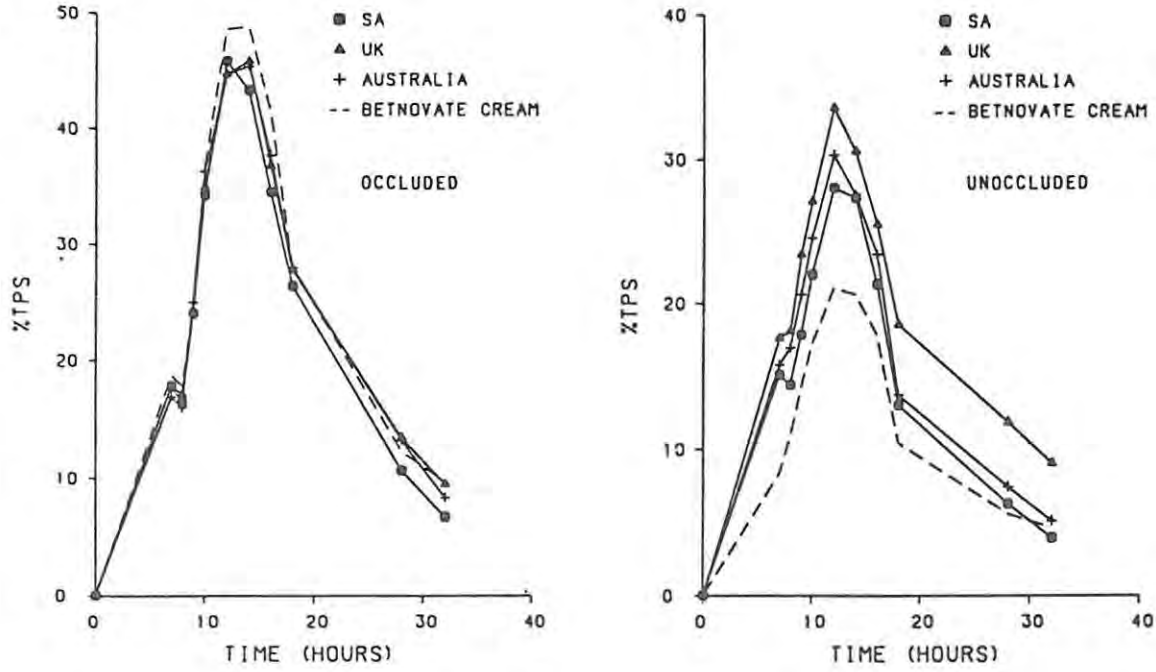


Figure 5.3.2 Blanching profiles of Locoid ointments

have been recorded had blanching sites been surrounded by normal skin at the 7 hour reading. The trough at the 8 hour reading therefore may not be due to decreased blanching at this reading, but to an artefactual peak at the 7 hour reading.

The blanching elicited by Betnovate cream in this trial is also worth noting. Whereas in the occluded mode Betnovate cream elicited a slightly higher degree of blanching than the Locoid ointments, in the unoccluded mode the blanching elicited by Betnovate cream was substantially lower than the Locoid ointments. This is almost certainly due to the inherent occlusivity of ointment bases which makes the release of corticosteroids from ointments less susceptible to the effects of occlusive dressings (see also section 4.2).

The only statistically significant difference found in the occluded mode was the comparison of adjacent sites between the SA and Australian ointments 28 hours after application. In the unoccluded mode no significant differences were found in the comparison of the UK ointment with the Australian ointment, and the SA ointment with the Australian ointment in the graded response analysis, whilst a small number of differences were found in each of these ointment pairs in the comparisons of adjacent sites. In the comparison of the SA ointment with the UK ointment, significant differences were found at the 28 hour and 32 hour readings in the graded response analysis, and at all readings in the paired comparisons of adjacent sites.

It can therefore be concluded that in the occluded mode the three ointments elicited similar blanching responses to each other. In the unoccluded mode the UK ointment elicited the greatest degree of blanching, and the difference between the UK ointment and the SA ointment was statistically significant in terms of the comparisons of adjacent sites.

5.1.4 Comparisons of Eumovate creams and ointments

5.1.4.1 Comparison of creams (tables 5.4.1,5.4.3,5.4.4)

The blanching profiles of the Eumovate creams are depicted in figure 5.4.1. The AUC values are similar for the three preparations in each mode of application, with the occluded creams having exhibited consider-

ably greater blanching responses than the unoccluded creams. Maximum blanching was recorded for the occluded UK cream 10 hours after application, which was two hours before the other creams. The difference between the 10 and 12 hour readings was small, and maximum blanching may have occurred at some time between these observations. The unoccluded Eumovate creams elicited very poor blanching responses. The irregular shapes of the curves is most probably attributable to the difficulty in assessment of such weak responses. The shapes of the Betnovate cream profiles and the weaker blanching of Betnovate cream in the unoccluded mode indicates that the Eumovate results are a function of the corticosteroid and/or formulation and not of the volunteers, observers or trial design. This illustrates the usefulness of incorporating a standard preparation into blanching trials.

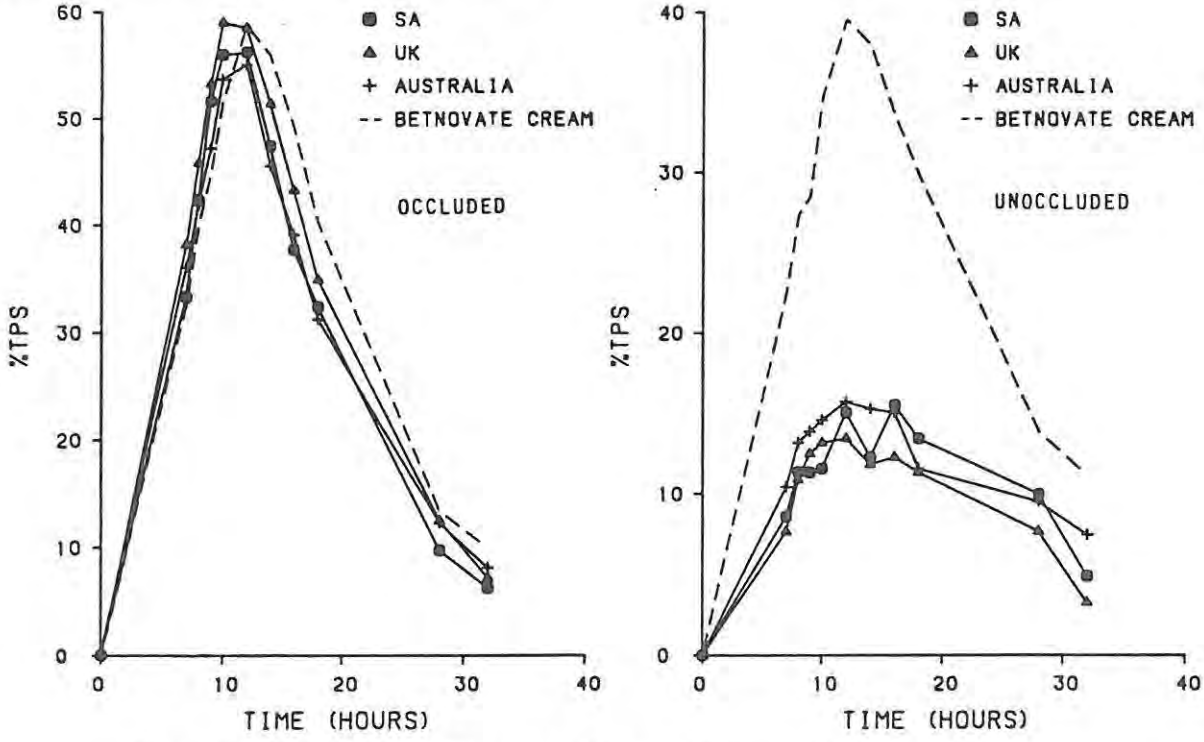


Figure 5.4.1 Blanching profiles of Eumovate creams

No statistically significant differences were found in the graded response analyses in either mode of application. In the comparison of adjacent sites, significant differences were found at most readings in the comparison of the UK cream with the Australian cream in the occluded mode, and at the 7, 16 and 32 hour readings in the unoccluded mode. In the comparison of the UK cream with the SA cream, significant differences were found in the paired comparisons at four reading times in the

occluded mode and at none in the unoccluded mode, whilst in the comparison of the Australian cream with the SA cream no differences were found in the occluded mode and one (9 hours after application) was found in the unoccluded mode. A study of the blanching profiles, AUC values and statistical analyses therefore indicates that the creams from Australia and SA elicited similar blanching responses to each other in both modes of application.

5.1.4.2 Comparison of ointments (tables 5.4.2,5.4.3,5.4.5)

The profiles of the Eumovate ointments are depicted in figure 5.4.2. The superior blanching response of Eumovate ointment in the unoccluded mode of application as compared to the occluded mode of application has been noted on previous occasions in our laboratories (244,447,473). Possible explanations for this anomalous behaviour have been suggested elsewhere (244,447). In previous experiments where this phenomenon was observed, the Eumovate ointments had been purchased in SA, but in this experiment the ointments were from three different countries and they all exhibited this behaviour. The Betnovate cream incorporated into this experiment as a standard preparation exhibited the expected superior blanching in the occluded application mode and it can be seen in section 5.1.4.1 that

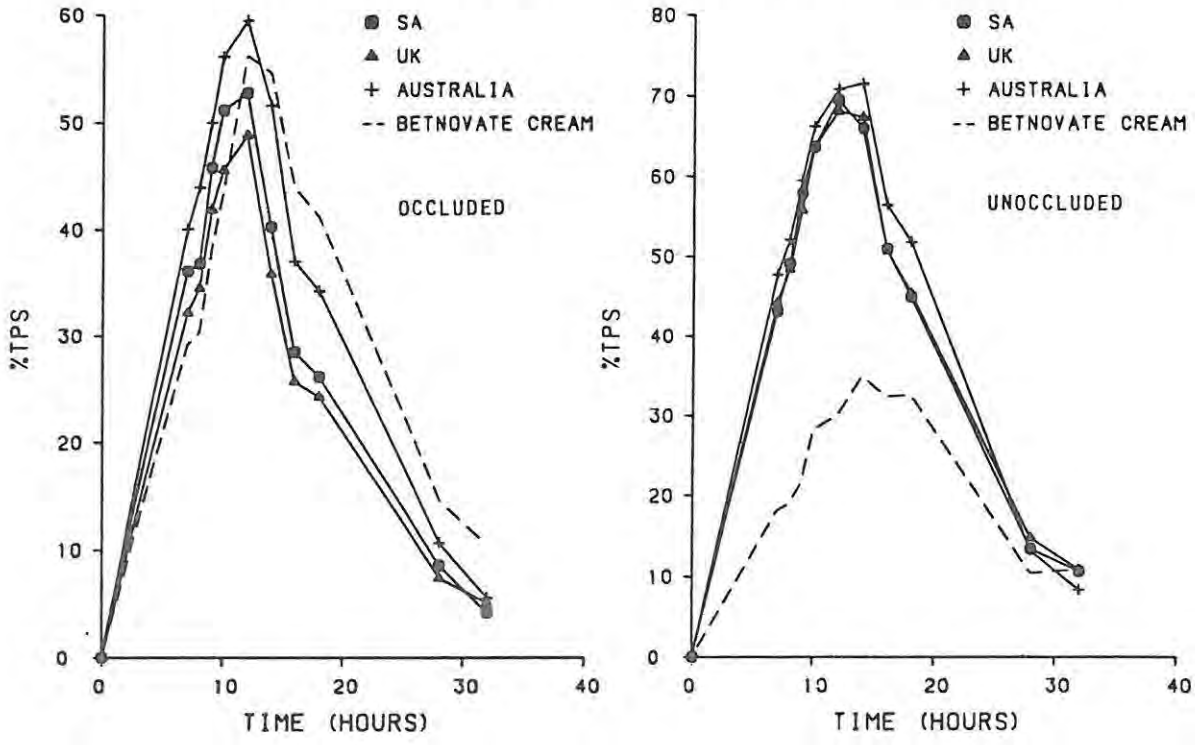


Figure 5.4.2 Blanching profiles of Eumovate ointments

Eumovate cream elicited more blanching in the occluded than in the unoccluded mode of application. Further research is required to ascertain whether this phenomenon exhibited by Eumovate ointment is a characteristic of clobetasone 17-butyrate in all ointment bases, or whether the particular formulation of Eumovate ointment base will alter the release characteristics of other corticosteroids in the same way.

In the graded response analysis of the comparison between the Australian ointment and the SA ointment applied under occlusion, statistically significant differences were found at 14, 16 and 18 hours after application. Significant differences were noted at all except the last two readings in the comparison of adjacent sites. The Australian ointment therefore exhibited statistically significantly superior blanching to the SA ointment from 14 - 18 hours after application. In the comparison of the Australian ointment with the UK ointment, statistically significant differences were noted from 7 - 18 hours after application in favour of the Australian ointment in both the graded response and comparison of adjacent sites analyses. No significant differences were found in the graded response analysis of the comparison between the SA ointment and the UK ointment, while significant differences were found at only three reading times in the comparison of adjacent sites.

The graded response analysis for the unoccluded ointments showed no significant differences for any of the ointment comparisons at any of the reading times. In the analysis of the comparisons of adjacent sites between the SA ointment and Australian ointment, differences were noted at all but three reading times, whilst no significant differences were found in the comparisons of the UK with the SA and Australian ointments. The UK and SA ointments elicited almost identical blanching responses, and whilst these were lower than the Australian ointment the differences were not statistically significant.

5.2 Summary and conclusions

A summary of the results of the comparisons and overall conclusions is presented in table 5.4. It can be seen from the overall conclusions that there was no definite trend of products from one country consistently exhibiting superior blanching to products from the other two

Table 5.4 SUMMARY OF COMPARISONS OF THE PREPARATIONS STUDIED

Comparison	AUC	Graded Response	Paired Comparison	Conclusion
Betnovate creams				
Occluded	Australia > SA	Australia > SA	Australia > SA	Australia > SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA < UK	SA < UK	SA < UK	SA < UK
	SA = SA	SA ≡ SA		SA = SA
Unoccluded	Australia > SA	Australia > SA	Australia > SA	Australia > SA
	Australia ≡ UK	Australia ≡ UK	Australia < UK	Australia ≡ UK
	SA < UK	SA < UK	SA < UK	SA < UK
	SA ≡ SA	SA ≡ SA		SA ≡ SA
Betnovate ointments				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia < UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA < UK	SA ≡ UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA < UK	SA ≡ UK
Betnovate lotions				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia > SA	Australia ≡ SA
	Australia > UK	Australia > UK	Australia > UK	Australia > UK
	SA > UK	SA > UK	SA > UK	SA > UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia > UK	Australia > UK	Australia > UK	Australia > UK
	SA > UK	SA > UK	SA > UK	SA > UK
Synalar creams				
Occluded	Australia > SA	Australia ≡ SA	Australia > SA	Australia > SA
	Australia > UK	Australia > UK	Australia > UK	Australia > UK
	SA > UK	SA ≡ UK	SA > UK	SA > UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia > UK	Australia > UK	Australia > UK	Australia > UK
	SA > UK	SA ≡ UK	SA > UK	SA > UK
Synalar ointments				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA < UK	SA ≡ UK
Unoccluded	Australia > SA	Australia ≡ SA	Australia > SA	Australia > SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA < UK	SA ≡ UK	SA < UK	SA < UK
Synalar lotions				
Occluded	Australia = SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia < UK	Australia ≡ UK	Australia < UK	Australia < UK
	SA < UK	SA ≡ UK	SA < UK	SA < UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia < UK	Australia < UK	Australia < UK	Australia < UK
	SA < UK	SA ≡ UK	SA < UK	SA < UK

continued on next page

Locoid creams				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia < UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA < UK	SA ≡ UK	SA < UK	SA ≡ UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia < UK	Australia ≡ UK
	SA < UK	SA ≡ UK	SA ≡ UK	SA ≡ UK
Locoid ointments				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA ≡ UK	SA ≡ UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia < UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA < UK	SA ≡ UK	SA < UK	SA < UK
Eumovate creams				
Occluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia < UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA ≡ UK	SA ≡ UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA	Australia ≡ SA
	Australia > UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA > UK	SA ≡ UK	SA ≡ UK	SA ≡ UK
Eumovate ointments				
Occluded	Australia > SA	Australia ≡ SA	Australia > SA	Australia > SA
	Australia > UK	Australia > UK	Australia > UK	Australia > UK
	SA ≡ UK	SA ≡ UK	SA ≡ UK	SA ≡ UK
Unoccluded	Australia ≡ SA	Australia ≡ SA	Australia > SA	Australia ≡ SA
	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK	Australia ≡ UK
	SA ≡ UK	SA ≡ UK	SA ≡ UK	SA ≡ UK

countries, or products from one country consistently exhibiting the lowest degree of blanching.

An important factor that emerged from this study is the necessity of drawing overall conclusions from the statistical analyses in combination with a study of the blanching profiles and AUC values (see also section 2.4.4). There were several instances in this series of trials where the statistical analyses did not concur, or where statistical analyses indicated differences between two preparations that appeared to produce similar blanching responses in terms of AUC values and the blanching profiles. An example is the comparison between the Australian and SA Synalar creams applied under occlusion. The AUC values are 1130 and 1001, respectively, and the blanching profiles indicate that the Australian cream exhibited a higher degree of blanching that the SA cream. The graded response analyses, however, only indicated significant dif-

ferences at three reading times (10, 12 and 16 hours after application), whilst analysis of comparisons of adjacent sites indicated significant differences at all but the last two readings. A study of the graded response analysis in isolation would therefore have led to the conclusion that these preparations elicited similar degrees of blanching, whilst examination of the above parameters in combination allow the conclusion to be drawn that the Australian cream elicited a greater degree of blanching than the SA cream. A similar situation occurred in the comparison of the UK Synalar cream with the SA Synalar cream in both modes of application, as well as in the comparisons of the Synalar lotions from SA and Australia with the UK lotion in the occluded mode, and the UK with the SA lotion in the unoccluded mode. Whilst this also appears to have occurred in the comparison of the Australian Synalar cream with the SA Synalar cream in the unoccluded application mode, closer study shows that the AUC values are similar (1072 and 1035), the %TPS values from 12 - 18 hours after application are similar and both forms of statistical analysis did not indicate significant differences. This also occurred in the comparison of Locoid ointments from the UK and Australia applied in the unoccluded mode.

Another aspect of the interpretation of results that was discussed in section 2.4.4 was the occurrence of statistical differences in the analysis of paired comparisons in trials where AUC values, blanching profiles and analysis of graded responses indicated similar blanching responses. It was argued in section 2.4.3.2 that this may be due to a theoretically unlimited number of subdivisions of the scoring system, depending on how many subdivisions the observers believe they are able to distinguish when comparing blanching responses at adjacent sites. An example of this can be seen in the comparison of unoccluded Betnovate ointments from SA and the UK. The AUC values were 997 and 1036, the profiles were similar and no significant differences were found in the graded response analysis, but differences occurred at five reading times in the paired comparison analysis. A further example of this is the comparison of the occluded Synalar ointments from the UK and SA, where no significant differences were found in the graded response analysis, while differences were found at all readings in the comparisons of adjacent sites. An interesting example of this occurred in the comparison of the Eumovate ointments applied without occlusion. The difference

between the AUC values of the UK and SA ointments was very small (14 units), whilst the difference between the Australian ointment and the other two ointments was larger. However, whilst the paired comparisons indicated no significant differences between the Australian and UK ointments, significant differences were found at most readings between the Australian and SA ointments.

A possible means of avoiding the inconsistencies discussed above is the substitution of the statistical methods with a single method that indicates statistical significance, or the lack thereof, of preparations over the entire duration of the trial, as opposed to at each reading time. A form of statistical analysis that achieves this is utilized by Woodford and co-workers (14,68,101,113,138,194,195,204-208,215,223,272, 309,370). Analysis of blanching responses at each reading time however allows the assessment of whether differences (where they occur) in rates of onset or duration of action are statistically significant.

Whilst the importance of statistical analyses is generally recognized, the relevance of statistically significant differences in blanching assays performed to predict clinical efficacy requires consideration. Statistical methods that are too sensitive may indicate significant differences between preparations in blanching trials that are of little relevance in the clinical situation. However, it seems difficult to ascertain the magnitude of the difference required in blanching assays to reliably predict differences in clinical efficacy, especially when considering that results of comparative clinical efficacies of topical corticosteroids may depend on the prevailing disease being treated, as well as the duration of treatment (section 2.2.8). The absence of a correlation between clinical efficacy and blanching responses in some of the comparisons cited in section 2.2.8 may have been a result of the choice of statistical methods.

Another general observation that emerged from this series of trials is the usefulness of incorporating a standard preparation in all trials. Woodford and co-workers (68,101,113,138,204-208,309,370) have routinely included a standard preparation into blanching trials which, with the necessary manipulation, allows comparisons between trials. Whilst the objective of this study did not require comparisons between trials, the

blanching profiles of Betnovate cream give credence to the results of the trials. This was particularly useful in the trial on Eumovate creams (section 5.1.4.1) where the blanching responses of the unoccluded creams were very weak. The blanching responses of Betnovate creams for the trials in this thesis are discussed in Chapter 7.

It was further found that, with the exceptions of the Synalar lotions and Eumovate ointments, the blanching responses were, as expected, greater when applied under occlusion than when left unoccluded. The blanching profiles are slightly narrower for the preparations applied under occlusion than for the preparations assayed without the use of occlusive dressings. This is most likely due to enhanced penetration through hydrated skin leading to a more rapid onset of action and shorter duration of action of the preparations applied under occlusion. The times at which maximum blanching responses were recorded ranged over the ten trials from 10 - 16 hours after application in the occluded mode and from 12 - 16 hours after application in the unoccluded mode. The average time after application at which maximum blanching was recorded was 12,85 hours in the occluded mode and 13,45 hours in the unoccluded mode. It can be seen from table 5.5 that the influence of occlusion on the time to peak was, as expected, greatest in the case of lotions, followed by creams and was smallest in case of ointments. Occlusion could be expected to influence the release of corticosteroids from lotions more than from creams due to the relatively simple formulation of lotions compared to creams. On the other hand, the release of corticosteroids could be expected not to be influenced to as great an extent from ointments than from creams, due to the inherent occlusivity of ointment bases.

Table 5.5 AVERAGE TIME OF MAXIMUM BLANCHING (HOURS AFTER APPLICATION) OF THE PREPARATIONS STUDIED IN THIS CHAPTER

	Occluded	Unoccluded
Creams	12,38	13,00
Ointments	13,31	13,25
Lotions	13,00	14,75

As was mentioned in the introduction to this chapter, manufacturers were asked whether the formulations of their products varied between the three countries (474-476). The manufacturers understandably were not

prepared to divulge the specific formulae or methods of manufacture, but it was ascertained that the Betnovate ointment base is variable depending on circumstances, while the bases used for the other products are manufactured to the same specifications for sale in the three countries. No information was forthcoming on the Betnovate lotion base. The superior blanching responses of the Betnovate ointment purchased in the UK compared with that purchased in SA, applied under occlusion, may therefore be due to different formulations, although it should be noted that the manufacturer stated only that the bases differ "according to local circumstances", but did not specify whether these two bases were different.

Differences in the blanching responses that occurred in some of the other preparations can therefore not be attributed to differences in vehicles, as the manufacturers stated that the vehicles were the same. There is, however, a possibility that the universal formulations are not ideally suited to the climatic conditions of all countries. Whilst it appears from our correspondence with the manufacturers that the universal bases are manufactured from raw materials of the same specifications, it is unlikely that these are purchased from the same manufacturer or the same manufacturing plants. It should be borne in mind that specifications of raw materials are usually quoted with upper and lower limits, and whilst all the raw materials may have met the specifications, differences in purities may have contributed to the different blanching responses. The quantity of the preparations used in the different countries, and the relative market shares will dictate the scale of manufacture, which may influence the characteristics of the bases. Release characteristics of the Synalar and Locoid preparations purchased in Australia may also have been influenced by the transportation from Europe (see table 5.1 and Chapter 4), although the blanching responses elicited by the Locoid preparations purchased in the three countries were generally similar.

Manufacturers should take cognizance of the results of this study and consider the reformulation of certain products with the objective of obtaining maximum release of the corticosteroids from their vehicles under the specific climatic conditions under which they are to be utilized. It may further be necessary in a country such as Australia,

to design different vehicles for use in the hot Northern Territory and the cooler conditions of Tasmania. Manufacturers and clinicians should also bear in mind that results of comparative clinical trials performed in temperate climates may differ were the trials to be performed under tropical or sub-tropical conditions.

APPENDIX TO CHAPTER 5

The tables in this appendix contain the %TPS values and AUC values for the various preparations, as well as the chi-squared values for the comparisons. Significant differences are based on the 95% level of significance and assuming 4 degrees of freedom in the graded response analyses and 1 degree of freedom in the analyses of the comparisons of adjacent sites.

The following abbreviations are used in the tables: "SA" refers to those preparations purchased in South Africa, "UK" refers to those preparations purchased in the United Kingdom and "Aust" refers to those preparations purchased in Australia. "Betnovate" refers to the Betnovate cream (manufactured and purchased in South Africa) included in all the trials as a standard preparation. In the tables of chi-squared values "SA/UK" is the comparisons of the preparations purchased in South Africa and the United Kingdom, etc.

Table 5.1.1 %TPS VALUES FOR BETNOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	35,42	40,47	45,37	52,08	63,43	63,43	56,71	42,36	9,03	6,48
UK	47,22	50,00	54,86	60,42	74,31	74,31	70,14	54,63	12,27	8,80
Australia	44,44	48,15	53,94	62,04	74,07	72,69	64,35	49,77	13,43	10,19
Betnovate	34,49	40,05	45,60	52,78	62,73	62,50	54,86	42,13	10,19	8,80
Unoccluded										
SA	19,44	21,06	24,31	26,85	34,26	34,72	31,25	21,06	6,25	4,17
UK	23,84	30,79	34,26	37,96	51,62	51,16	50,00	38,19	11,34	11,81
Australia	23,84	28,47	32,87	38,66	49,31	48,84	47,22	34,03	9,72	9,95
Betnovate	18,98	21,06	26,16	29,40	35,88	36,34	34,03	22,92	4,63	3,01

Table 5.1.2 %TPS VALUES FOR BETNOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	25,46	34,95	47,69	54,86	62,50	66,90	56,25	45,37	9,49	4,40
UK	31,02	41,44	53,24	60,65	68,98	71,76	58,80	47,22	10,42	5,79
Australia	28,47	38,19	50,69	58,33	65,28	68,98	56,71	43,52	10,65	6,48
Betnovate	28,01	36,34	45,14	52,31	57,18	57,18	42,36	32,18	10,65	5,09
Unoccluded										
SA	34,72	40,74	49,54	53,94	63,19	63,89	53,24	40,74	10,19	5,09
UK	35,65	42,36	52,78	56,71	63,43	65,74	52,31	40,97	12,50	8,33
Australia	34,72	39,58	49,31	54,63	63,19	64,81	52,55	41,67	10,42	5,56
Betnovate	24,31	28,47	34,95	39,81	46,76	48,15	39,12	29,86	7,18	3,70

Table 5.1.3 %TPS VALUES FOR BETNOVATE LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	38,66	48,61	64,12	71,76	79,40	78,24	61,81	46,30	8,10	5,09
UK	26,39	32,41	41,20	49,07	51,85	47,92	34,72	27,31	4,86	2,31
Australia	46,06	56,02	69,44	76,85	84,26	82,18	65,28	48,15	8,10	5,56
Betnovate	26,16	33,33	46,53	51,39	57,64	55,09	41,90	34,03	4,40	3,94
Unoccluded										
SA	21,76	27,31	32,64	37,27	41,67	46,06	35,19	26,16	6,71	5,56
UK	15,05	17,82	20,14	25,69	28,94	29,40	21,30	17,13	4,86	3,94
Australia	26,62	30,09	38,43	43,52	43,52	46,99	36,11	27,08	6,71	6,48
Betnovate	19,91	24,07	29,86	37,04	40,97	41,90	33,10	21,06	4,40	3,24

Table 5.1.4 AREA UNDER THE CURVE VALUES : BETNOVATE PREPARATIONS

	Creams		Ointments		Lotions	
	Occluded	Unoccluded	Occluded	Unoccluded	Occluded	Unoccluded
SA	1003	542	986	997	1159	664
UK	1253	855	1077	1036	724	441
Australia	1208	798	1021	1055	1245	716
Betnovate	1004	556	863	725	814	585

Table 5.1.5 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	14,63	9,96	10,92	6,70	15,19	16,16	22,13	17,87	3,95	2,18
SA/Aust	14,28	9,96	9,61	11,41	12,31	8,78	8,82	7,26	6,96	5,33
UK/Aust	10,33	3,59	3,74	3,52	11,39	4,11	11,51	3,11	1,09	0,71
Unoccluded										
SA/UK	3,01	10,48	8,81	11,42	20,21	20,34	30,47	37,06	9,13	21,39
SA/Aust	4,09	10,59	11,32	16,79	20,63	22,11	21,01	21,12	7,04	14,28
UK/Aust	0,98	2,45	4,00	3,25	6,94	5,52	2,44	3,56	2,30	2,47
Paired Comparison										
Occluded										
SA/UK	32,47	30,12	32,66	27,28	33,52	43,27	47,76	44,95	11,46	5,11
SA/Aust	17,40	18,39	25,69	18,11	25,10	33,44	25,69	25,31	7,93	4,65
UK/Aust	2,06	5,25	0,57	0,99	1,23	1,33	3,71	6,45	0,06	2,24
Unoccluded										
SA/UK	8,48	21,02	33,47	46,51	58,88	53,15	50,07	47,46	10,78	25,35
SA/Aust	4,20	10,41	25,81	38,72	40,48	45,57	52,48	40,88	10,30	14,38
UK/Aust	4,34	4,56	2,96	6,13	4,00	9,35	4,40	6,78	2,73	2,84

Table 5.1.6 CHI-SQUARED VALUES (GRADED RESPONSE) : BETNOVATE CREAM : SA vs SA

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded	5,25	2,77	1,45	0,80	0,56	0,43	4,49	1,77	0,49	2,18
Unoccluded	0,49	0,11	0,48	2,00	1,47	4,71	1,90	1,11	1,77	0,94

Table 5.1.7 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	5,31	5,30	5,39	3,29	3,74	6,60	11,65	2,23	8,45	1,53
SA/Aust	2,27	6,90	4,19	1,79	0,93	5,92	6,06	1,64	6,66	2,47
UK/Aust	3,02	3,08	1,39	0,74	2,10	1,43	1,30	1,62	0,24	0,24
Unoccluded										
SA/UK	5,11	2,50	4,10	3,65	0,90	1,19	2,71	1,34	5,75	9,06
SA/Aust	0,49	3,35	3,00	0,54	2,06	2,29	4,20	1,41	0,09	2,04
UK/Aust	6,76	3,00	5,75	3,18	2,55	1,83	2,59	2,00	6,79	3,48
Paired Comparison										
Occluded										
SA/UK	18,50	22,50	18,28	29,92	30,78	16,30	20,78	9,26	0,68	0,48
SA/Aust	0,88	0,21	0,05	0,66	0,01	0,33	1,19	0,55	0,08	3,18
UK/Aust	5,80	11,68	4,88	1,45	3,31	9,01	9,19	2,84	0,57	0,12
Unoccluded										
SA/UK	0,77	7,29	6,78	2,81	5,96	11,05	6,30	1,73	2,62	3,35
SA/Aust	0,68	2,28	0,36	0,83	0,01	0,01	0,06	1,64	0,09	0,00
UK/Aust	0,10	2,25	1,78	2,17	1,49	0,01	0,51	0,23	2,82	0,80

Table 5.1.8 CHI-SQUARED VALUES : COMPARISON OF BETNOVATE LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	14,80	21,50	39,22	38,58	57,62	68,62	53,73	33,34	5,02	5,28
SA/Aust	6,96	9,37	4,43	3,44	3,11	2,81	5,39	0,44	0,00	1,01
UK/Aust	36,90	43,63	51,57	53,44	77,77	85,43	65,05	38,51	5,02	6,42
Unoccluded										
SA/UK	11,96	19,09	26,04	16,27	12,69	28,93	22,87	14,60	1,56	1,60
SA/Aust	4,43	1,85	4,50	8,42	2,39	5,57	7,84	6,02	1,90	0,47
UK/Aust	23,29	24,31	35,43	25,42	18,46	27,42	25,21	14,00	4,40	2,98
Paired Comparison										
Occluded										
SA/UK	19,38	27,28	44,04	51,58	46,76	69,86	77,66	55,51	8,89	2,56
SA/Aust	17,40	16,28	11,36	6,49	6,49	9,80	2,93	6,13	0,03	3,23
UK/Aust	70,78	78,22	89,25	94,01	90,09	102,01	84,79	79,84	5,36	1,94
Unoccluded										
SA/UK	22,22	30,67	43,69	42,45	49,61	47,46	36,01	9,19	0,66	0,52
SA/Aust	2,55	5,80	1,37	6,49	2,56	2,22	0,05	2,35	1,02	0,03
UK/Aust	56,68	49,61	61,48	51,25	55,36	69,34	51,25	44,85	3,03	1,73

Table 5.2.1 %TPS VALUES FOR SYNALAR CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	22,69	28,24	39,35	41,20	52,78	55,79	53,24	50,00	17,36	17,82
UK	17,59	22,69	30,32	36,81	46,30	50,00	47,45	45,37	16,90	17,13
Australia	27,78	33,80	43,75	47,22	60,19	63,66	61,34	56,02	18,52	17,36
Betnovate	28,70	33,56	44,44	48,61	61,11	60,19	50,00	42,36	12,50	10,65
Unoccluded										
SA	23,15	25,46	32,18	35,88	48,15	48,84	50,46	50,00	27,08	23,15
UK	12,96	16,44	25,00	28,70	39,12	43,52	44,68	44,21	25,23	19,91
Australia	20,37	22,45	31,48	36,11	50,46	53,47	54,63	52,55	28,47	25,46
Betnovate	18,06	23,15	27,31	35,42	45,60	44,21	42,36	39,58	18,75	14,35

Table 5.2.2 %TPS VALUES FOR SYNALAR OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	20,83	28,94	35,19	39,58	46,30	52,08	52,55	44,91	25,93	15,27
UK	26,39	31,25	37,27	42,36	48,61	53,70	53,01	45,60	27,55	19,67
Australia	26,62	31,48	38,43	44,21	48,61	56,02	54,17	44,91	24,31	15,97
Betnovate	30,09	35,19	39,81	45,60	50,00	48,84	37,73	25,46	16,90	11,57
Unoccluded										
SA	22,22	28,47	35,42	36,57	41,44	44,91	40,51	34,72	22,45	13,43
UK	29,86	33,33	40,28	43,06	44,91	49,31	44,68	39,81	27,78	18,52
Australia	29,86	34,49	38,19	42,82	45,14	50,46	44,21	40,05	28,24	18,29
Betnovate	15,74	22,92	25,93	27,08	30,79	31,94	24,31	18,06	13,43	6,94

Table 5.2.3 %TPS VALUES FOR SYNALAR LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	14,81	22,69	29,40	33,33	38,19	42,59	38,19	31,17	11,81	9,72
UK	15,28	23,15	34,03	37,27	46,76	51,16	46,06	43,06	14,81	12,04
Australia	12,27	19,68	28,47	31,02	40,74	41,90	37,96	34,03	11,57	10,88
Betnovate	24,77	36,81	43,75	47,45	59,95	62,50	54,63	43,29	9,72	6,94
Unoccluded										
SA	13,66	16,67	24,77	30,09	38,66	45,14	45,83	39,58	16,20	13,19
UK	19,21	24,07	33,56	39,35	50,93	55,32	60,88	56,02	23,61	19,68
Australia	11,81	14,58	21,53	27,55	34,26	37,50	40,28	37,50	14,81	12,96
Betnovate	12,73	16,20	23,15	26,85	33,33	36,81	34,72	28,47	10,19	7,87

Table 5.2.4 AREA UNDER THE CURVE VALUES : SYNALAR PREPARATIONS

	Creams		Ointments		Lotions	
	Occluded	Unoccluded	Occluded	Unoccluded	Occluded	Unoccluded
SA	1001	1035	990	854	692	778
UK	891	872	1054	1006	848	1069
Australia	1130	1072	1032	1011	692	703
Betnovate	971	838	831	543	947	598

Table 5.2.5 CHI-SQUARED VALUES : COMPARISON OF SYNALAR CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	5,34	9,42	9,00	3,51	6,46	3,37	3,64	1,98	4,49	0,47
SA/Aust	4,12	5,10	3,52	10,35	10,23	7,97	12,06	8,32	4,36	0,08
UK/Aust	17,15	20,07	21,72	24,81	17,60	17,32	21,62	14,64	0,52	0,57
Unoccluded										
SA/UK	17,58	14,94	6,88	6,77	8,34	2,91	2,83	7,46	0,88	1,93
SA/Aust	7,88	8,61	1,91	2,95	6,74	5,61	2,72	5,45	1,80	1,75
UK/Aust	11,28	15,76	6,25	10,68	21,58	12,11	9,20	5,42	2,06	4,93
Paired Comparison										
Occluded										
SA/UK	8,49	22,22	23,52	21,33	24,16	9,78	23,90	11,07	1,56	1,84
SA/Aust	5,33	10,45	13,93	10,51	20,50	25,89	29,41	21,50	0,02	0,00
UK/Aust	42,26	46,51	46,02	49,35	53,63	36,92	40,48	32,04	0,29	0,08
Unoccluded										
SA/UK	21,39	32,47	13,30	15,61	13,93	11,77	7,95	3,52	2,09	5,97
SA/Aust	4,13	3,04	0,12	0,61	0,20	6,13	9,78	1,51	0,98	7,11
UK/Aust	14,42	9,11	16,60	14,58	31,21	26,48	30,24	22,55	0,25	1,02

Table 5.2.6 CHI-SQUARED VALUES : COMPARISON OF SYNALAR OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	4,84	0,75	4,07	7,66	1,07	1,55	0,78	0,99	2,91	5,20
SA/Aust	3,76	0,88	1,83	5,30	1,54	1,27	2,07	4,21	2,73	0,18
UK/Aust	2,50	0,41	3,98	1,56	1,37	1,95	0,78	1,83	2,72	3,77
Unoccluded										
SA/UK	6,66	2,93	2,48	6,98	2,13	1,70	1,37	5,70	10,63	11,01
SA/Aust	7,24	6,83	3,53	5,28	2,42	6,27	1,21	4,29	6,46	5,73
UK/Aust	1,58	3,86	1,43	0,85	5,25	2,66	0,29	0,79	3,20	6,38
Paired Comparison										
Occluded										
SA/UK	8,82	9,47	9,85	6,30	7,01	12,33	4,70	9,26	9,47	6,78
SA/Aust	11,04	5,48	5,80	8,14	3,71	9,68	1,73	1,27	0,23	1,11
UK/Aust	0,00	2,84	0,05	0,05	0,51	0,06	0,05	0,01	0,88	2,09
Unoccluded										
SA/UK	26,64	26,64	20,50	31,65	36,01	42,35	16,00	24,51	15,13	22,37
SA/Aust	16,01	14,72	8,56	10,78	8,89	10,84	9,92	6,78	13,65	12,74
UK/Aust	1,97	0,34	0,66	0,33	2,76	0,05	2,28	0,00	0,52	0,96

Table 5.2.7 CHI-SQUARED VALUES : COMPARISON OF SYNALAR LOTIONS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	0,06	1,24	4,41	5,53	7,02	4,74	8,39	14,79	3,54	2,29
SA/Aust	3,60	1,93	0,28	5,57	3,08	2,65	0,13	5,75	1,28	1,15
UK/Aust	4,43	2,86	5,64	10,70	6,48	10,94	7,62	8,90	2,06	1,10
Unoccluded										
SA/UK	6,14	8,65	9,99	7,56	9,31	7,87	16,39	18,46	8,92	8,00
SA/Aust	2,71	1,53	1,67	0,91	1,61	7,93	8,57	4,98	1,29	4,51
UK/Aust	8,45	13,25	18,59	11,92	16,02	15,82	20,09	17,88	12,96	10,63
Paired Comparison										
Occluded										
SA/UK	0,02	0,06	2,84	7,15	7,62	25,96	19,01	17,75	0,84	1,42
SA/Aust	2,53	1,95	0,00	0,00	0,00	1,23	1,41	1,23	0,45	0,20
UK/Aust	5,82	3,51	3,44	7,11	10,51	10,26	23,52	20,78	0,17	0,00
Unoccluded										
SA/UK	7,93	8,93	18,55	16,00	19,28	26,64	29,41	40,61	18,50	12,15
SA/Aust	3,70	1,59	0,68	3,61	9,11	2,55	1,30	2,36	1,31	0,46
UK/Aust	15,75	29,28	23,50	31,35	56,68	51,96	56,68	39,62	13,04	8,27

Table 5.3.1 %TPS VALUES FOR LOCOID CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	37,73	45,37	48,84	54,63	65,05	57,87	44,21	38,19	15,51	12,04
UK	41,20	51,62	57,18	61,11	69,44	61,11	48,61	42,36	19,44	12,50
Australia	37,73	47,22	52,08	55,79	65,97	60,42	45,60	38,66	15,05	10,88
Betnovate	27,31	33,80	38,89	41,20	51,85	48,61	35,42	29,86	12,73	9,95
Unoccluded										
SA	23,84	32,18	35,65	37,50	49,31	42,13	30,56	27,78	15,05	11,81
UK	30,32	37,50	40.51	43,52	53,24	45,60	33,10	29,86	17,36	13,19
Australia	26,85	34,49	37,96	40,05	49,54	43,98	34,72	29,17	15,74	11,57
Betnovate	14,12	19,68	20,37	22,92	29,86	28,47	21,53	18,06	9,72	8,80

Table 5.3.2 %TPS VALUES FOR LOCOID OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	17,82	16,44	24,07	34,26	45,83	43,29	34,49	26,39	10,65	6,71
UK	17,82	17,13	24,31	34,95	44,68	45,83	36,81	27,78	13,43	9,49
Australia	16,90	15,97	25,00	36,34	44,68	45,37	37,73	28,01	13,19	8,33
Betnovate	18,75	17,82	24,77	36,81	48,61	48,84	41,44	28,01	12,27	9,72
Unoccluded										
SA	15,05	14,35	17,82	21,99	28,01	27,31	21,30	12,96	6,25	3,94
UK	17,59	18,06	23,38	27,08	33,56	30,56	25,46	18,52	11,81	9,03
Australia	15,74	16,90	20,60	24,54	30,32	27,55	23,38	13,66	7,41	5,09
Betnovate	8,33	10,88	14,12	17,13	21,06	20,60	17,82	10,42	5,56	4,63

Table 5.3.3 AREA UNDER THE CURVE VALUES : LOCOID PREPARATIONS

	Creams		Ointments	
	Occluded	Unoccluded	Occluded	Unoccluded
SA	1023	759	657	408
UK	1139	856	699	543
Australia	1037	805	696	444
Betnovate	804	485	724	314

Table 5.3.4 CHI-SQUARED VALUES : COMPARISON OF LOCOID CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	2,29	8,45	10,45	4,92	3,17	2,93	3,69	3,61	5,09	1,04
SA/Aust	3,53	3,06	1,35	1,48	1,92	6,56	1,10	2,52	0,08	0,58
UK/Aust	8,03	8,82	5,04	3,42	3,93	3,59	3,68	3,35	6,43	0,96
Unoccluded										
SA/UK	5,50	3,58	2,69	3,56	2,51	4,22	2,27	2,88	2,78	1,88
SA/Aust	1,64	3,12	1,04	1,01	2,34	5,59	3,30	3,07	2,42	1,34
UK/Aust	2,67	1,79	0,76	2,41	6,40	5,74	0,74	0,13	0,68	0,72
Paired Comparison										
Occluded										
SA/UK	9,85	15,61	24,70	21,78	20,01	15,01	17,01	7,89	4,20	0,02
SA/Aust	1,07	1,07	0,20	0,94	0,01	2,55	1,73	0,11	0,02	0,02
UK/Aust	8,78	8,01	5,98	2,36	5,19	0,22	0,60	0,01	0,16	0,09
Unoccluded										
SA/UK	11,36	5,96	6,13	2,96	0,29	1,30	1,33	0,70	0,07	2,09
SA/Aust	0,06	3,41	0,12	0,61	1,55	7,22	1,78	1,54	0,02	0,30
UK/Aust	7,89	12,68	6,63	4,32	2,48	0,54	0,06	4,84	0,61	0,50

Table 5.3.5 CHI-SQUARED VALUES : COMPARISON OF LOCOID OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	1,83	1,35	2,76	2,91	2,72	3,31	2,17	3,92	2,37	4,11
SA/Aust	6,71	3,35	2,79	7,93	3,00	1,70	1,73	9,40	3,08	1,63
UK/Aust	2,61	6,71	7,62	3,52	1,92	1,00	1,46	8,62	0,69	1,08
Unoccluded										
SA/UK	1,93	3,10	5,94	6,53	4,60	1,68	2,61	7,63	11,53	11,67
SA/Aust	2,58	3,28	2,35	5,26	4,33	2,74	2,47	0,18	0,58	0,78
UK/Aust	1,33	2,04	2,76	4,20	3,69	5,65	1,77	5,69	7,06	6,60
Paired Comparison										
Occluded										
SA/UK	2,61	0,14	0,06	0,21	0,01	1,89	0,98	0,23	0,94	0,60
SA/Aust	0,01	0,15	0,16	0,00	0,01	0,21	0,85	2,84	4,49	3,52
UK/Aust	0,22	0,06	0,00	1,45	0,23	0,00	0,90	0,00	0,00	0,18
Unoccluded										
SA/UK	5,16	4,66	7,62	16,70	10,18	11,69	15,56	9,80	13,50	14,38
SA/Aust	0,02	0,07	0,37	0,06	0,33	4,56	3,94	0,29	0,03	0,15
UK/Aust	1,68	0,06	1,51	5,33	3,04	0,64	0,00	1,54	9,13	9,30

Table 5.4.1 %TPS VALUES FOR EUMOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	33,33	42,36	51,62	56,02	56,25	47,45	37,73	32,41	9,72	6,25
UK	38,19	45,83	53,24	59,03	58,56	51,39	43,29	34,95	12,50	7,18
Australia	36,11	41,90	47,22	53,70	55,09	45,60	39,12	31,25	12,27	8,10
Betnovate	32,41	39,81	44,68	51,39	58,80	56,02	49,31	40,28	13,43	9,95
Unoccluded										
SA	8,56	11,34	11,34	11,57	15,05	12,27	15,51	13,43	9,95	4,86
UK	7,64	10,88	12,50	13,19	13,43	11,81	12,27	11,34	7,64	3,24
Australia	10,42	13,19	13,89	14,58	15,74	15,28	15,05	11,57	9,49	7,41
Betnovate	22,45	27,31	28,47	34,72	39,58	37,96	33,56	29,86	13,89	11,11

Table 5.4.2 %TPS VALUES FOR EUMOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
SA	36,11	36,81	45,83	51,16	52,78	40,28	28,47	26,16	8,56	4,17
UK	32,18	34,49	41,90	45,60	48,84	35,88	25,69	24,31	7,41	5,09
Australia	40,05	43,98	50,00	56,12	59,49	51,62	37,04	34,26	10,65	5,56
Betnovate	29,17	30,32	38,19	43,52	56,25	54,63	43,98	41,20	14,58	10,42
Unoccluded										
SA	43,06	49,07	57,57	63,66	69,44	65,97	50,93	44,91	13,43	10,65
UK	44,21	48,38	55,79	63,66	68,06	67,36	50,69	45,37	14,81	10,88
Australia	47,69	52,08	59,49	66,20	70,83	71,53	56,48	51,85	13,19	8,33
Betnovate	18,06	18,98	21,53	28,24	30,09	34,95	32,41	32,64	10,42	10,88

Table 5.4.3 AREA UNDER THE CURVE VALUES : EUMOVATE PREPARATIONS

	Creams		Ointments	
	Occluded	Unoccluded	Occluded	Unoccluded
SA	869	320	772	1132
UK	958	274	702	1146
Australia	883	333	926	1219
Betnovate	975	718	930	641

Table 5.4.4 CHI-SQUARED VALUES : COMPARISON OF EUMOVATE CREAMS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	2,01	3,04	6,80	4,36	5,29	4,03	4,45	4,86	2,37	1,80
SA/Aust	3,70	4,88	7,39	9,03	3,50	6,36	0,90	0,21	4,66	3,19
UK/Aust	2,74	1,91	4,04	7,85	3,25	4,54	1,86	6,35	1,31	0,35
Unoccluded										
SA/UK	0,46	0,21	0,35	2,56	4,68	0,08	2,36	4,46	3,75	1,67
SA/Aust	2,24	3,35	4,48	6,98	4,24	2,44	6,85	2,05	1,25	3,03
UK/Aust	2,97	2,85	3,47	2,06	1,87	3,28	4,35	1,37	7,29	8,95
Paired Comparison										
Occluded										
SA/UK	8,93	8,22	3,41	1,55	0,85	0,51	11,70	4,44	1,40	0,00
SA/Aust	0,21	0,05	0,11	0,01	1,07	2,03	0,24	0,96	1,50	0,60
UK/Aust	4,98	11,07	13,35	13,65	14,63	13,96	9,19	5,48	3,84	0,60
Unoccluded										
SA/UK	0,03	0,02	0,02	0,52	1,45	0,09	1,42	0,02	2,70	1,88
SA/Aust	3,20	3,56	6,11	2,09	1,12	0,46	0,48	0,52	0,00	1,36
UK/Aust	6,25	0,46	1,45	0,71	1,96	2,13	4,23	0,10	2,38	8,50

Table 5.4.5 CHI-SQUARED VALUES : COMPARISON OF EUMOVATE OINTMENTS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Graded Response										
Occluded										
SA/UK	3,66	2,27	3,07	4,82	7,65	5,89	3,49	0,78	1,24	0,49
SA/Aust	5,88	8,45	6,16	3,66	6,57	11,73	16,78	12,08	3,24	1,06
UK/Aust	11,11	11,81	10,41	9,84	10,98	24,75	21,18	15,25	3,93	0,11
Unoccluded										
SA/UK	2,39	1,10	1,41	0,77	0,82	1,06	1,81	1,87	0,70	0,89
SA/Aust	2,58	2,49	1,35	0,98	2,92	3,34	6,18	5,43	0,02	1,50
UK/Aust	2,18	1,60	1,55	0,63	3,34	1,79	4,80	6,33	0,95	2,57
Paired Comparison										
Occluded										
SA/UK	5,98	3,81	0,81	4,20	1,78	8,01	0,74	1,19	0,03	0,04
SA/Aust	6,15	23,55	15,92	22,50	29,56	41,38	42,45	28,44	2,75	0,15
UK/Aust	5,98	12,96	6,45	10,84	6,78	36,89	21,50	32,51	1,42	0,26
Unoccluded										
SA/UK	0,46	0,00	0,01	1,27	0,20	0,46	0,33	0,00	1,49	0,03
SA/Aust	12,96	6,45	7,62	6,78	1,73	16,70	11,11	18,55	0,63	0,00
UK/Aust	0,01	0,61	0,11	2,74	1,23	0,43	0,05	1,69	0,59	0,02

CHAPTER 6

SENSITIVITY OF DIFFERENT AREAS OF THE FLEXOR ASPECT OF THE FOREARM TO CORTICOSTEROID-INDUCED BLANCHING

Results of studies on regional variation in percutaneous penetration have shown that corticosteroids are absorbed at different rates and to different extents through the stratum corneum at various anatomical sites (section 1.3.2.3). Several comments have appeared in the literature over the years concerning variations in the blanching response on different areas of the forearm. Blanching has been reported to vary from one site to another on forearms after percutaneous absorption through normal or stripped skin (324), to be poor at sites within about 4 cm of the wrist and elbow (397), to be almost invariably more intense on the upper forearm than at the wrist (416) and to be inconsistent on persons with very short or narrow forearms, as well as in persons such as typists who use the forearm muscles consistently throughout the duration of corticosteroid application (179,384).

In studying the vasodilation elicited by topical application of methyl nicotinate, Tur *et al.* (477) found no difference in response between medial and lateral sites of the flexor aspect of the forearm, but found significantly more vasodilation at the proximal position compared to the distal position. McKenzie and Atkinson (135) analysed the combined results of 29 blanching assays and found that there were highly significant differences between sites on the forearm, but reported no further details.

Kirsch *et al.* (376) were the first to attempt to quantify the sensitivity of various sites on the forearm to corticosteroid-induced blanching, and concluded that a gradient exists along the forearm, with the area close to the wrist showing less blanching than the area close to the elbow. This experiment was performed in the occluded mode of application, observations were made only at one reading time and the trial was specifically mounted to assess this variable of the blanching assay. Whilst experience in our laboratories had indicated that blanching was not always consistent along the forearm, the existence of a gradient had not been noticed. It was therefore decided to re-analyse data from

trials already performed in our laboratories to verify the results reported by Kirsch *et al.* (376).

An important difference between the methodology reported by Kirsch *et al.* (376) and that employed in this study is that the objectives of all the trials performed in this study were not to assess a possible gradient, that is to say retrospective analyses were performed. In addition to this, observations were made at ten reading times as opposed to one, and most of the trials were performed in both the occluded and unoccluded modes of application.

6.1 Results and Discussion

6.1.1 Site Variation with the Application of Creams in the Unoccluded Mode (tables 6.1-6.6)

The first three trials that were re-analysed were those discussed in Chapter 3 of this thesis (478). The 12 sites on each arm were divided into six pairs of adjacent sites which were termed wrist, wrist-1, wrist-2, elbow-2, elbow-1 and elbow (see figure 6.1). The results

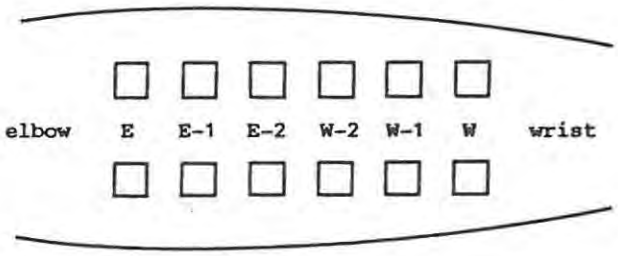


Figure 6.1 Site pair division on the forearm

recorded by all three observers for both arms of all 18 volunteers were pooled. As was explained in section 2.3, four different application patterns were used to ensure that no preparation was applied to the same area of the forearms of all volunteers in any one trial. Both the preparations (Celestoderm-V cream and Betnovate cream) therefore occurred an equal number of times in each site pair for any one trial. A total of 12 960 sites were observed during each trial, allowing the comparison of 6480 site pairs for the retrospective analysis of each trial. Graded response statistical analyses were performed and curves were plotted of AUC values against site pairs (figure 6.2).

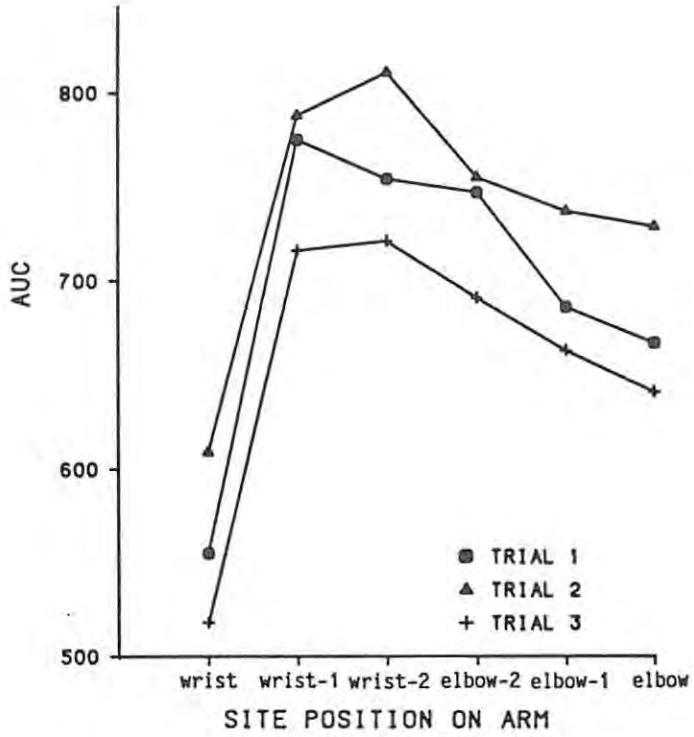


Figure 6.2 Profiles of forearm site variation for trials 1, 2 and 3 of Chapter 3

It can be seen from the graphs that the differences in blanching along the forearm were similar for all three trials. It is evident that the area closest to the wrist produced the lowest blanching response. Much more intense blanching was noted at wrist-1, and in trials 2 and 3 blanching increased to wrist-2 after which blanching decreased along the arm towards the elbow. In trial 1 blanching decreased from wrist-1 to the elbow site pair.

Statistical analyses were performed on the graded responses to ascertain whether significant differences were found between the site pairs, namely wrist and wrist-1, wrist and wrist-2, *etc*. (see appendix for tables). As the curves in figure 6.2 depict AUC values against site pairs, the blanching as a function of time after application cannot be seen. The statistical analyses were, however, performed at each time interval and allowed an assessment of the influence of time on the variable being studied. The results of the statistical analyses were similar in all three trials. In the analysis of the wrist site pair to the other five site pairs, significant differences were found at all reading times, with the exception of 28 and 32 hours after application in all cases, as

well as at the 7 hour reading comparison of wrist to elbow, elbow-1 and elbow-2 in trial 3. In the comparisons of the other site pairs only a few statistically significant chi-squared values were found, and these were generally interspersed throughout the comparisons and three trials. It can therefore be concluded that in this series of three trials, significantly lower blanching responses were observed at the wrists compared to the remainder of the forearm. Whilst intensity of blanching generally decreased along the forearm from wrist-1 to elbow, differences in blanching intensity were not statistically significant.

Although it is expected and acceptable that statistical evaluation (significance or non-significance) may not be consistent throughout all time intervals in a trial (sections 2.4.4 and 5.2), the fact that no significant differences were found at the 28 and 32 hour readings (reported above) prompted a more detailed study of site pairs at each reading time. The actual blanching scores (pooled for the three trials) were plotted against the site pairs (figure 6.3). Combining the results of the three trials allowed the analysis of 1944 site pairs for each observation time.

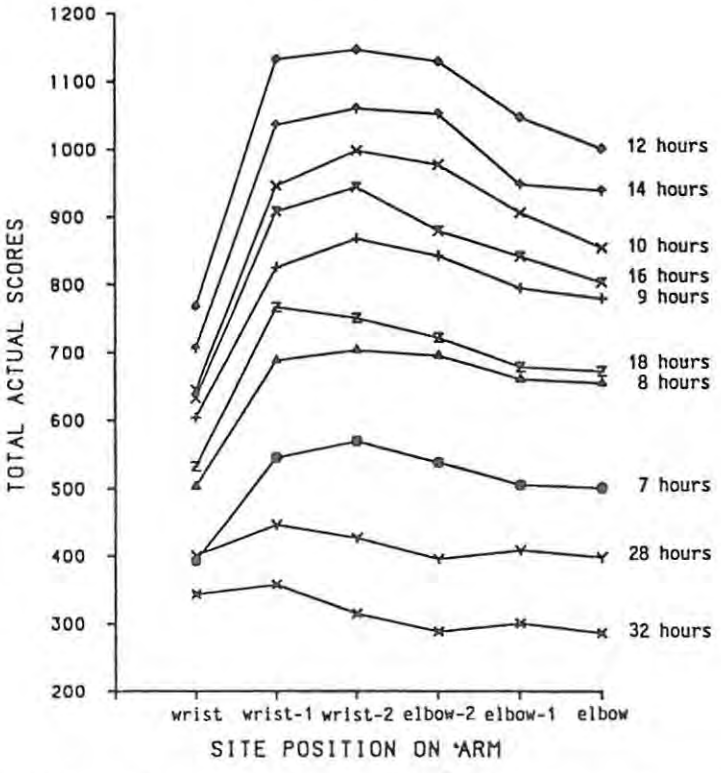


Figure 6.3 Profiles of forearm site variation at each reading time: pooled results for trials 1, 2 and 3 of Chapter 3

It can be seen from the curves that the pattern of site variation along the forearm was similar for the readings from 7 - 18 hours after application. It is worth noting that the decreased blanching intensity close to the wrist compared to the other sites on the forearm becomes more pronounced as the intensity of blanching increases. This may imply that the corticosteroid is less rapidly absorbed, and penetrates to a lesser extent through the stratum corneum close to the wrist compared to the rest of the forearm. The pattern observed at 28 and 32 hours was different to that of the other reading times. This is most likely due to the relatively poor blanching observable at the 28 and 32 hour readings.

The results of the chi-squared analyses of the graded responses of the pooled results are generally similar to those obtained for the individual trials reported above. The comparisons of wrist to the other five site pairs indicated significant differences until 18 hours after application. This statistical significance also occurred at the 28 hour and 32 hour readings in the comparison of wrist to elbow, but no significant differences were found at the 28 hour reading in the remaining four comparisons to the wrist, or at the 32 hour reading of the comparison of wrist to wrist-2 and elbow-1.

As previously mentioned, the comparisons of the remaining site pairs to each other produced similar results to those obtained for the individual trials, although some differences were noted. In the pooled results significant differences were found in the comparison of wrist-1 to elbow at all reading times except the 8 hour and 28 hour reading times. Significant differences were also found for the pooled results in the comparison of wrist-2 to elbow at all except the last two reading times. In the comparison of elbow-2 to elbow, significant differences were found from 8 - 14 hours after application. The remainder of the comparisons were not significant overall, although a few statistically significant differences were scattered throughout the readings.

The analyses of individual trials and pooled results allow some conclusions to be drawn with respect to differences in blanching responses along the forearm when Betnovate and Celestoderm-V creams were applied in the unoccluded mode of application. The lowest intensity of blanching was observed closest to the wrist. The intensity of blanching increased

considerably from this area to the area designated as wrist-1, after which in most cases a moderate increase was noted from wrist-1 to wrist-2. This was followed by a gradual decrease in blanching intensity towards the elbow. The blanching closest to the wrist was statistically significantly lower than at the other sites, and the blanching at the elbow was statistically lower than at wrist-1 and wrist-2, although this was not as obvious in the analysis of individual trials. The results of this study are therefore contradictory to those reported by Kirsch *et al.* (376), where a steady increase in blanching was noted along the forearm from the wrist to the elbow.

The above conclusions were drawn after the study of retrospective analyses of two creams containing the same corticosteroid applied in the unoccluded mode of application. Considering this, as well as the fact that the conclusions drawn from this study and that of Kirsch *et al.* (376) were contradictory, it was decided to study further the site variation along the forearm, and to re-analyse the remainder of the trials reported in this thesis, namely those reported in Chapters 4 and 5. The preparations used were Betnovate and Synalar creams, ointments and lotions, and Locoid and Eumovate creams and ointments. The aim of this was to ascertain whether the same pattern would emerge for creams, ointments and lotions, and whether the pattern is the same in both the occluded and unoccluded application modes. The results of these trials were reorganized in the same way as for the previously discussed three trials, namely pooled results of volunteers, trials and sites to form site pairs. The sites onto which Betnovate cream was applied as a standard preparation in all trials were not excluded in the analysis of ointments and lotions. In these trials Betnovate cream occupied only 25% of the sites and it is felt that this would not influence the final conclusions. The results of this investigation are depicted graphically in figure 6.4. Chi-squared analyses were performed comparing the site pairs at the ten reading times.

The first aspect of this analysis involved the various creams containing different corticosteroids applied in the unoccluded mode. It can be seen from figures 6.2 and 6.4 that the shapes of the curves for the creams applied without occlusive dressings are similar. It is worth noting that in the three trials reported in Chapter 3 the blanching panel consisted

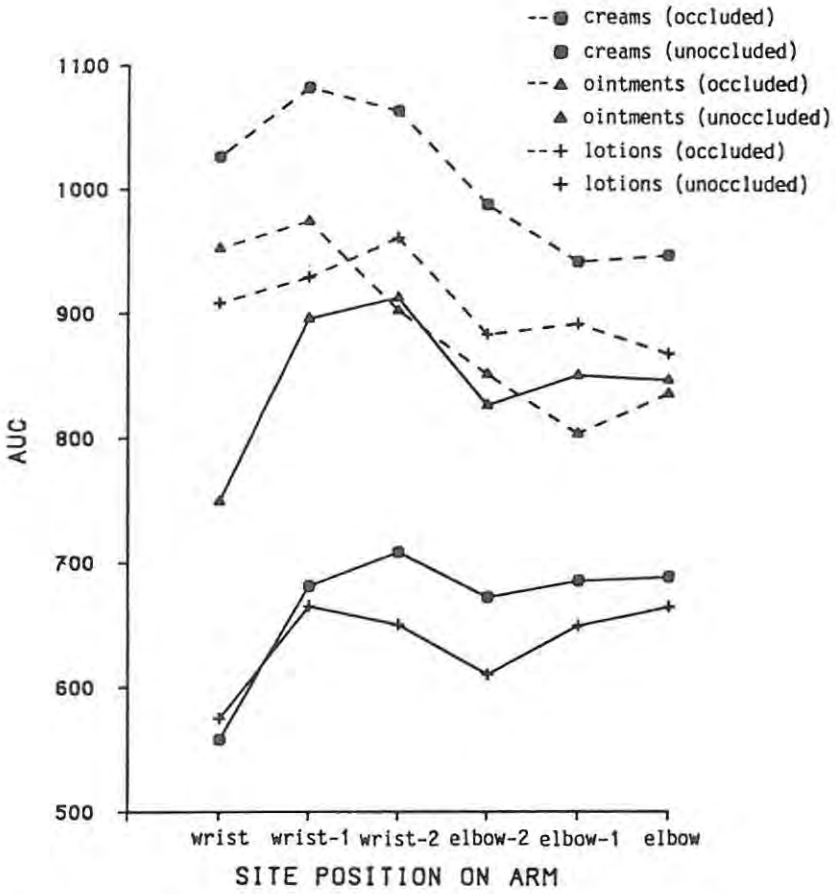


Figure 6.4 Profiles of forearm site variation for the trials of Chapters 4 and 5

of the same 18 subjects for each trial, whilst in the trials reported in Chapters 4 and 5 no attempt was made to employ the same volunteers in any of the trials, the 12 subjects per trial having been drawn from a panel of volunteers known to show a blanching response. It is therefore evident that the constitution of the blanching panels did not influence the results of this investigation. In the comparison of the wrist to the other site pairs, statistically significant differences were found in all but one reading up to and including 18 hours after application. Blanching was found to increase considerably between wrist and wrist-1, with a moderate increase to wrist-2 followed by a small decrease to elbow-2 and a gradual increase towards the elbow. The differences in blanching from wrist-1 to elbow were however not statistically significant. Significant differences were only found at the 28 hour comparison of wrist-1 and elbow, the 7 hour and 8 hour comparisons of wrist-2 and elbow, and the 8 hour comparison of elbow-1 and elbow. It can therefore be concluded that the difference in the intensity of blanching on

various sites of the forearm, namely weaker close to the wrist than on the remainder of the forearm, is not specific to creams containing betamethasone 17-valerate and is a general phenomenon for the topical corticosteroid creams tested in this thesis in the unoccluded application mode.

6.1.2 Site Variation with the Application of Ointments in the Unoccluded Mode (tables 6.5,6.7)

Results for the ointments applied in the unoccluded mode were similar to those reported for the creams (see figure 6.4), although some differences were noted. Statistically significant differences were found in most of the comparisons between the wrist and the other five site pairs, although a few more chi-squared values indicating non-significance were found for the ointments than for the creams. In the comparisons of wrist-1 to wrist-2 and in the region from elbow-2 to elbow all the chi-squared values indicated non-significant differences. Statistical analysis of the apparently large decrease in blanching between wrist-2 and elbow-2 only gave significant differences between 14 and 18 hours after application. In general, although some statistically significant differences were scattered throughout the comparisons along the forearm, these were insufficient to conclude that blanching elicited by ointments in the unoccluded mode of application is different along the forearm from wrist-1 to the elbow, whilst the site pair closest to the wrist produced less blanching than the remainder of the forearm.

6.1.3 Site Variation with the Application of Lotions in the Unoccluded Mode (tables 6.5,6.8)

The profile of site variation of lotions applied in the unoccluded mode was similar to those obtained for creams and ointments, although a slight decrease was noted from wrist-1 to wrist-2 in the case of lotions. Relatively few statistically significant differences were found in the blanching elicited by lotions on different parts of the forearm. Significant differences were found from 8 - 12 hours in the comparison of wrist with wrist-1, but in very few other comparisons of the wrist with the other site pairs. Therefore, although blanching increased from wrist to wrist-1, followed by a decrease from wrist-1 to elbow-2 and an

increase from elbow-2 to elbow, these differences were not statistically significant. It should be noted that creams and ointments were used in more trials than were lotions. The fewer site pairs observed for lotions may account for the lack of statistically significant differences.

6.1.4 Site Variation with the Application of Creams in the Occluded Mode (tables 6.5,6.9)

The profiles of site variation obtained for the cream formulations applied in the occluded mode of application did not indicate the same pattern as those for the unoccluded application mode.

In the cases of occluded creams and ointments (section 6.1.5), blanching increased from wrist to wrist-1, then decreased steadily towards elbow-1 and increased marginally from elbow-1 to elbow, although blanching at elbow-1 and elbow in the occluded creams was essentially the same. It is, however, important to note that whereas in the unoccluded applications the area closest to the wrist produced less blanching than the remainder of the forearm, in the occluded mode the area from elbow-2 to elbow produced less blanching than at the wrist.

Statistically significant chi-squared values were obtained at several places in the analysis of the creams applied under occlusion. Although there were fewer cases where significant differences indicated clear-cut superiority of one site pair over others, significant differences occurred at sufficient reading times to allow conclusions of superiority to be drawn. Wrist-2 was significantly more sensitive to blanching than was wrist. Whilst the difference between wrist and wrist-1 was greater than between wrist and wrist-2, chi-squared values indicating statistical significance were only found at four reading times in the former comparison. Statistically significant differences were found in the comparison of wrist with the site pairs from elbow-2 to elbow, with less intense blanching observed from elbow-2 to elbow. It should be noted that the apparently large difference between wrist-1 and elbow-2 was not statistically significant. This is a similar situation to those reported previously where apparently large differences in blanching responses were found not to be statistically significantly different and *vice versa* (sections 4.3 and 5.2). However, the blanching observed at wrist-1

was significantly superior to that at elbow-1 and elbow. Similarly, the blanching at wrist-2 was significantly greater than that at elbow-1 and elbow. Following the blanching pattern along the forearm, it can be seen that the increase from wrist to wrist-1 was not statistically significant (with statistical significance at only four reading times), the incremental decreases from wrist-1 to elbow-1 and the slight increase from elbow-1 to elbow were not statistically significant. It can thus be concluded that the intensity of blanching decreases up the forearm from the area adjacent to the wrist, namely wrist-1 to the elbow, when creams are applied in the occluded application mode.

6.1.5 Site Variation with the Application of Ointments in the Occluded Mode (tables 6.5,6.10)

Statistical analysis of occluded ointments produced similar results to the occluded creams. Blanching increased from wrist to wrist-1, decreased steadily from wrist-1 to elbow-1 and increased from elbow-1 to elbow, although none of the differences between adjacent site pairs was statistically significant. Statistically significant differences were noted in the blanching between wrist and elbow-1, wrist-1 and elbow-2 to elbow, and wrist-2 and elbow-1. Blanching was thus found to decrease up the forearm between the areas adjacent to the wrist and elbow, namely from wrist-1 to elbow-1.

6.1.6 Site Variation with the Application of Lotions in the Occluded Mode (tables 6.5,6.11)

In the case of occluded lotions, blanching increased from wrist to wrist-2, decreased to elbow-2 and was very similar between elbow-2 and elbow. Blanching at wrist-2 was statistically significantly greater than at the areas closest to the wrist and the elbow. Other statistically significant differences are scattered throughout the trial, but the comparison of the wrist to the elbow gave significant differences at six time intervals. It is, however, clear from the profile in figure 6.4 that the difference between elbow and wrist is small, which emphasizes the importance of taking all factors into consideration and not drawing conclusions from only the statistical analysis.

6.2 Summary and Conclusions

Some interesting conclusions can be drawn from the above analyses of the sensitivity of different areas of the forearm to topical corticosteroid-induced blanching. In general, lower blanching responses were noted at the areas closest to the wrist and elbow than the areas between these regions. This is consistent with the observations of Burdick (397) who reported poor blanching responses within about 4 cm of the wrist and elbow, but inconsistent with the findings of Kirsch *et al.* (376) who reported a gradient along the arm. The reasons for the poor responses at these sites is open to speculation. Some possibilities are that percutaneous penetration is less efficient at these two areas of the forearm, that the number and/or sensitivity of corticosteroid receptors are different at these areas, or that the observation of blanching is in some way being hindered. None of these possibilities appears to have been discussed in the literature. Less efficient percutaneous absorption implies that the skin at these sites is different to the skin on the rest of the flexor aspect of the forearm. It is feasible that the stratum corneum close to the flexures differs to that on the remainder of the forearm. The speculation that the number and/or sensitivity of receptors differs at these areas may be accurate if the thickness of the skin close to the flexures is different to the remainder of the forearm. The third possibility mentioned above is that observation of blanching may be hindered at these sites. The region close to the wrist is rich in blood vessels serving the hand. These vessels may exert pressure on the superficial blood vessels in this region thereby making the comparison of corticosteroid-treated skin with untreated skin more difficult. This however does not explain the lower blanching response observed close to the elbow.

Two aspects of this study are worth emphasizing. Firstly, none of the trials used for this study was designed to assess site variation along the forearm; retrospective analyses were performed and the observers were therefore unbiased towards site variation during the trials. Secondly, scores from a large number of observations were used, with the subsequent analysis of a large number of site pairs. In the analysis of the unoccluded creams studied in the three trials in Chapter 3, 19 440 site pairs were studied, whilst in the studies of the remaining trials,

10 800 site pairs were studied for creams and ointments and 6480 for lotions in each mode of application. In the occluded application mode, more blanching was noted at the wrist than at the elbow, while in the unoccluded mode the converse was noted, with more blanching at the elbow than at the wrist. The highest degree of blanching was generally noted at the site pairs designated as wrist-1 and wrist-2, although this was not obvious in the relatively low blanching observed in the unoccluded creams and lotions. It is however difficult to explain why the patterns of site variation were different between the two modes of application at the areas adjacent to the wrist and elbow. If occlusion masked the effect of site variation, as has been found to occur in comparative bioavailability studies (section 2.2.2), the difference in blanching between the wrist and the rest of the forearm could be expected to have been smaller in the occluded mode of application than in the unoccluded mode, but this does not explain the reversal in rank order between wrist and elbow. It appears as though effects of occlusion may be considerably greater near the wrist, reinforcing the speculation that the structure of the stratum corneum is different near the wrist to other sites on the forearm. The most important practical implication is that blanching is not the same at all areas of the forearm and cognizance should be taken of this when designing blanching trials. Each preparation being assayed should be applied to more than one site on the forearm and the application patterns should ensure that each preparation is distributed along the forearm.

APPENDIX TO CHAPTER 6

The tables in this appendix contain the AUC values and actual scores for the site pairs studied, as well as the chi-squared values for the graded response analyses of site pairs. Chi-squared values greater than 9,49 signify real differences based on the 95% level of significance and assuming 4 degrees of freedom. The following abbreviations are used in the tables: W = wrist, E = elbow, W-1/E-2 = the comparisons of the site pairs at wrist-1 with those at elbow-2, etc.

Table 6.1 AREA UNDER THE CURVE VALUES FOR SITE PAIRS: data from trials 1, 2 and 3 of Chapter 3

	Wrist	Wrist-1	Wrist-2	Elbow-2	Elbow-1	Elbow
Trial 1	555	775	754	747	686	667
Trial 2	609	788	811	755	737	729
Trial 3	518	716	721	691	663	641

Table 6.2 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
data from trials 1, 2 and 3 of Chapter 3

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Trial 1										
W/W-1	9,97	13,29	14,55	28,24	38,66	32,80	41,22	41,30	3,66	3,42
W/W-2	13,23	10,75	21,12	34,81	34,41	34,75	37,68	30,02	5,14	0,22
W/E-2	11,08	17,92	25,32	33,69	42,47	36,28	44,63	36,14	0,62	3,53
W/E-1	10,53	13,02	13,01	17,68	20,79	18,68	30,47	26,21	0,35	3,29
W/E	10,76	22,61	14,45	9,89	17,46	19,64	18,53	20,96	3,17	1,23
W-1/W-2	2,51	0,60	2,53	3,34	0,39	1,41	1,31	2,23	5,10	3,21
W-1/E-2	2,77	0,59	6,78	0,53	0,34	2,45	0,81	3,88	1,85	6,14
W-1/E-1	8,29	2,61	7,50	1,98	4,27	5,62	2,28	14,05	2,19	5,30
W-1/E	8,50	5,43	17,30	8,48	8,63	5,88	8,61	14,77	6,92	4,12
W-2/E-2	1,88	1,82	2,28	2,90	0,83	2,76	2,69	2,30	2,71	2,33
W-2/E-1	5,58	3,44	4,26	4,83	3,02	4,40	3,96	6,74	3,20	2,15
W-2/E	5,47	8,32	12,76	10,55	7,95	6,94	6,77	8,93	1,22	0,45
E-2/E-1	3,34	4,48	7,29	3,43	5,37	5,87	2,41	4,40	0,04	0,04
E-2/E	5,61	6,35	10,42	11,32	11,60	8,65	8,65	5,42	2,35	1,06
E-1/E	3,55	2,09	5,46	3,64	3,46	2,70	4,60	2,65	2,44	1,00
Trial 2										
W/W-1	23,29	26,37	43,50	32,41	38,08	29,88	22,45	15,39	4,53	4,05
W/W-2	27,64	34,03	43,94	44,72	45,07	35,95	32,31	23,22	2,60	2,64
W/E-2	21,03	33,68	38,60	41,07	32,59	37,54	16,49	12,70	6,67	10,51
W/E-1	12,43	29,17	28,43	34,50	27,52	23,10	10,09	12,80	4,31	3,76
W/E	14,85	26,12	27,49	32,29	20,54	22,28	10,40	11,82	7,48	4,19
W-1/W-2	1,58	3,40	5,49	1,62	2,53	5,01	5,57	2,28	1,49	7,24
W-1/E-2	1,71	2,82	0,30	1,33	2,69	4,75	8,26	1,21	4,67	8,63
W-1/E-1	2,69	8,97	5,70	2,59	2,00	10,53	5,05	1,50	2,79	8,48
W-1/E	1,57	3,58	12,57	8,96	5,58	11,73	12,58	0,77	2,40	5,72
W-2/E-2	2,48	3,16	4,48	0,27	1,67	2,86	5,79	3,26	2,54	5,81
W-2/E-1	6,13	9,08	3,15	2,27	3,17	4,46	9,83	3,15	0,73	2,10
W-2/E	4,19	3,85	5,45	12,80	11,91	6,77	11,77	3,40	1,43	1,60
E-2/E-1	2,23	3,92	3,88	1,16	0,56	5,73	2,61	0,10	0,60	4,50
E-2/E	1,18	3,07	9,88	10,91	6,29	6,78	1,30	0,13	1,83	2,04
E-1/E	0,23	8,80	4,04	11,18	3,41	0,62	2,80	0,35	0,97	1,12
Trial 3										
W/W-1	12,39	24,96	17,32	30,99	36,64	34,58	34,83	26,31	2,15	3,01
W/W-2	15,43	23,11	23,77	39,42	38,04	38,78	46,46	25,32	1,87	0,23
W/E-2	9,39	13,88	19,25	32,47	32,43	39,15	29,24	21,60	0,48	2,43
W/E-1	9,03	11,85	14,22	24,11	21,91	22,05	26,67	14,43	1,51	4,05
W/E	6,29	12,15	16,91	17,03	19,79	24,57	20,79	14,81	1,44	9,48
W-1/W-2	0,48	0,28	2,24	3,67	2,52	0,35	2,96	2,29	0,01	2,40
W-1/E-2	0,53	2,48	3,04	3,03	2,21	3,79	7,01	1,04	0,62	4,09
W-1/E-1	3,33	2,88	7,18	8,90	2,59	4,69	3,58	5,50	1,03	4,09
W-1/E	3,80	4,62	2,50	6,35	4,40	2,05	5,27	9,03	1,72	7,94
W-2/E-2	2,00	1,73	0,82	0,43	5,58	2,48	5,06	1,20	0,48	1,24
W-2/E-1	3,54	2,38	5,55	5,12	3,95	4,98	4,38	3,13	0,90	2,41
W-2/E	3,94	3,33	4,38	9,32	3,79	3,60	8,35	4,20	1,59	7,05
E-2/E-1	3,67	1,21	3,52	3,15	2,40	4,50	2,24	2,79	0,93	0,34
E-2/E	4,07	2,50	4,25	6,36	3,85	8,96	3,77	5,57	0,84	3,01
E-1/E	1,20	0,86	4,25	7,51	0,64	4,16	2,92	0,89	3,44	1,49

Table 6.3 ACTUAL SCORES FOR SITE PAIRS
pooled data from trials 1, 2 and 3 of Chapter 3

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Wrist	392	503	604	645	768	706	632	532	400	343
Wrist-1	545	688	825	946	1132	1036	908	766	446	357
Wrist-2	569	703	867	998	1146	1060	944	750	426	314
Elbow-2	538	695	842	977	1129	1052	879	721	395	288
Elbow-1	505	660	794	906	1047	948	841	678	408	300
Elbow	501	655	779	854	1001	939	803	672	398	286

Table 6.4 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from trials 1, 2 and 3 of Chapter 3

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	42,71	56,83	67,61	90,60	103,86	93,57	87,83	74,35	7,54	10,04
W/W-2	53,07	63,03	83,91	116,70	111,19	106,25	110,14	70,56	7,15	2,05
W/E-2	39,12	60,09	73,74	104,37	103,52	101,27	80,88	58,43	3,85	14,05
W/E-1	32,38	43,89	47,91	71,73	65,70	56,60	61,53	41,68	1,44	8,13
W/E	24,02	54,37	47,27	53,15	53,53	62,07	46,14	42,59	11,13	12,57
W-1/W-2	3,29	1,77	7,31	5,15	0,24	1,03	3,19	2,29	1,93	11,35
W-1/E-2	2,11	0,56	2,80	3,11	1,64	0,80	2,71	4,21	5,87	16,89
W-1/E-1	7,09	5,47	16,23	8,94	5,55	7,06	6,62	15,00	3,54	15,71
W-1/E	9,90	8,40	21,32	10,59	15,87	13,22	15,75	19,14	9,40	15,99
W-2/E-2	4,39	3,29	2,57	0,94	2,07	0,23	5,73	2,07	2,86	7,03
W-2/E-1	8,78	7,35	8,99	9,30	7,56	10,07	14,77	8,03	2,31	3,14
W-2/E	10,08	11,47	15,49	25,05	18,60	16,50	23,37	10,94	3,06	5,32
E-2/E-1	3,08	5,48	10,27	5,88	5,58	8,81	2,61	4,30	1,16	1,62
E-2/E	5,88	10,65	12,66	17,95	19,15	16,87	8,75	6,52	3,17	0,38
E-1/E	0,71	9,86	3,78	10,89	5,24	4,95	5,97	1,13	5,50	1,51

Table 6.5 AREA UNDER THE CURVE VALUES FOR SITE PAIRS
data from trials in Chapters 4 and 5

Trial	Wrist	Wrist-1	Wrist-2	Elbow-2	Elbow-1	Elbow
Creams (unoccluded)	558	681	708	672	685	688
Creams (occluded)	1026	1082	1063	987	941	946
Ointments (unoccluded)	749	896	912	826	850	846
Ointments (occluded)	952	974	902	851	803	835
Lotions (unoccluded)	575	665	650	610	649	664
Lotions (occluded)	908	929	960	883	891	867

Table 6.6 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from unoccluded creams studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	11,89	13,46	14,25	19,37	20,43	24,62	12,88	13,64	1,46	0,39
W/W-2	19,44	19,74	22,82	29,78	43,31	28,39	16,27	14,48	9,09	2,98
W/E-2	11,70	22,21	27,40	27,99	36,51	21,39	15,36	10,94	6,45	3,77
W/E-1	25,71	33,35	40,50	39,87	33,88	26,35	14,64	8,70	5,78	4,91
W/E	32,99	34,04	30,68	39,12	32,34	23,01	13,53	12,77	14,77	4,65
W-1/W-2	3,94	1,51	2,91	4,84	4,74	4,20	1,79	1,56	4,71	2,11
W-1/E-2	0,03	2,82	4,86	2,75	5,83	2,15	3,18	0,99	5,31	2,83
W-1/E-1	3,12	8,54	7,70	6,54	2,18	2,59	4,01	2,04	6,29	3,18
W-1/E	8,93	8,17	5,96	7,80	4,50	0,48	4,01	0,18	11,91	4,16
W-2/E-2	3,64	2,65	6,37	4,04	5,62	1,81	4,89	4,23	3,09	1,24
W-2/E-1	2,85	4,83	5,91	6,75	1,72	2,88	4,06	3,43	7,08	2,62
W-2/E	12,03	10,67	4,08	5,48	5,31	2,04	6,92	1,44	4,74	1,13
E-2/E-1	3,21	2,45	4,87	2,09	5,60	0,84	1,25	1,09	1,06	0,76
E-2/E	8,52	8,40	1,96	2,58	0,98	0,98	0,37	1,42	2,35	0,58
E-1/E	7,01	15,00	5,85	5,72	6,10	1,61	1,58	2,48	4,87	2,58

Table 6.7 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from unoccluded ointments studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	16,67	18,21	17,54	33,92	21,24	25,04	14,67	3,84	5,03	3,93
W/W-2	15,74	14,87	20,48	30,13	25,66	34,48	20,96	8,77	3,12	4,19
W/E-2	10,24	10,68	16,39	15,22	14,67	13,90	16,81	14,60	2,26	3,68
W/E-1	9,20	13,74	14,22	22,01	14,67	10,38	10,74	8,11	1,51	1,04
W/E	13,98	12,95	18,44	19,71	14,60	9,99	13,73	15,61	1,95	1,70
W-1/W-2	4,80	4,35	1,58	2,75	2,13	2,74	1,11	1,17	0,55	0,17
W-1/E-2	5,70	2,32	1,81	4,27	3,16	10,86	8,63	11,16	8,84	2,84
W-1/E-1	6,23	7,09	11,48	4,64	4,92	13,30	4,27	2,55	9,70	1,21
W-1/E	1,89	10,11	7,40	4,40	12,08	23,52	7,61	10,18	1,11	0,69
W-2/E-2	0,81	2,87	1,21	4,16	7,27	11,48	10,99	10,78	6,95	4,27
W-2/E-1	1,75	7,09	6,75	4,77	11,02	13,79	6,27	2,16	5,88	1,72
W-2/E	4,45	4,82	5,11	6,90	18,57	24,43	9,88	8,22	0,36	0,64
E-2/E-1	1,12	2,08	7,17	1,89	0,79	0,98	2,61	4,09	5,19	1,44
E-2/E	4,55	4,52	2,44	1,49	3,02	3,53	0,29	1,55	5,96	3,38
E-1/E	5,76	3,55	6,22	1,88	2,47	1,80	1,76	3,39	4,62	0,54

Table 6.8 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from unoccluded lotions studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	8,84	13,21	10,63	12,96	11,56	8,80	7,10	5,27	6,37	7,40
W/W-2	4,29	8,45	6,29	10,79	15,45	7,90	8,87	5,08	6,36	1,76
W/E-2	5,98	5,53	3,97	4,01	8,71	3,49	2,33	1,43	2,85	0,91
W/E-1	4,02	3,66	5,37	8,24	11,64	12,39	8,27	13,10	3,84	1,67
W/E	4,95	5,43	4,71	8,52	11,17	5,52	9,56	5,35	1,88	4,34
W-1/W-2	3,75	4,60	2,40	4,43	2,61	3,87	2,10	2,39	0,05	3,14
W-1/E-2	4,51	4,08	5,16	4,67	3,98	1,52	5,56	6,83	2,54	4,91
W-1/E-1	2,68	5,74	3,38	2,27	3,91	8,86	4,66	4,20	3,27	3,08
W-1/E	6,86	10,08	5,13	3,78	1,45	1,72	2,86	0,51	6,28	12,42
W-2/E-2	3,18	6,37	4,14	6,95	6,34	2,45	3,96	4,22	2,51	0,32
W-2/E-1	2,63	4,37	0,76	7,38	2,88	6,69	5,89	12,16	3,68	0,01
W-2/E	4,03	5,74	1,49	3,49	0,79	1,00	0,13	4,61	6,63	8,15
E-2/E-1	5,50	5,36	3,49	2,20	1,54	7,50	8,76	17,89	1,11	0,37
E-2/E	6,91	9,78	2,56	2,51	4,90	0,83	4,09	8,44	1,83	7,92
E-1/E	1,58	2,40	0,46	2,00	2,43	5,11	5,90	2,41	1,00	7,61

Table 6.9 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from occluded creams studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	6,65	7,03	12,51	19,79	18,08	10,70	6,42	5,65	4,01	7,92
W/W-2	9,15	14,64	19,24	22,41	21,29	12,22	9,44	3,34	15,10	33,88
W/E-2	2,60	3,84	5,06	17,01	13,26	11,95	4,41	3,98	35,03	62,48
W/E-1	10,98	16,35	16,76	13,06	7,40	4,97	2,21	1,23	35,81	51,50
W/E	2,42	6,59	10,52	16,55	10,39	8,88	5,83	13,33	13,74	30,40
W-1/W-2	1,34	7,01	2,62	1,83	4,07	1,01	1,60	1,47	3,93	10,02
W-1/E-2	3,03	5,92	2,65	5,52	5,48	3,14	3,84	6,98	20,65	28,96
W-1/E-1	19,90	23,11	17,03	21,73	14,09	6,21	4,28	5,61	20,06	21,18
W-1/E	8,71	13,96	19,47	21,53	19,04	8,55	13,77	11,31	6,09	8,12
W-2/E-2	3,99	6,09	6,56	7,86	2,26	6,66	2,41	3,18	9,61	10,82
W-2/E-1	21,95	26,79	25,25	22,30	11,33	9,47	5,22	4,33	8,21	6,56
W-2/E	11,75	19,69	31,92	24,85	25,09	14,62	12,40	8,91	3,37	2,52
E-2/E-1	8,62	11,41	9,82	7,99	3,75	3,79	2,28	5,10	0,28	0,66
E-2/E	2,30	5,50	11,28	10,29	14,17	5,59	4,04	5,96	5,05	6,65
E-1/E	4,56	3,25	5,76	11,65	8,75	4,52	5,21	9,56	5,51	3,16

Table 6.10 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from occluded ointments studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	3,91	9,86	4,27	3,56	4,90	6,64	4,10	2,26	3,62	3,92
W/W-2	4,86	7,62	11,56	8,29	5,55	4,77	15,97	11,79	9,96	14,59
W/E-2	7,29	7,07	10,21	6,46	1,79	1,93	6,78	10,23	17,98	16,53
W/E-1	19,31	16,28	10,56	9,40	13,16	5,28	10,51	14,15	16,54	19,32
W/E	5,86	8,85	8,26	6,52	4,79	2,46	11,72	9,58	24,83	15,45
W-1/W-2	1,64	3,79	10,30	7,39	6,39	5,22	7,77	8,60	8,96	4,28
W-1/E-2	10,43	19,47	13,87	11,98	10,75	5,85	6,43	8,89	12,17	6,66
W-1/E-1	28,13	29,57	21,05	22,00	25,91	13,12	13,94	15,02	9,00	8,06
W-1/E	6,41	12,77	15,10	15,67	17,58	11,96	8,84	10,19	16,61	5,26
W-2/E-2	4,71	8,56	2,11	3,35	6,28	7,47	4,34	1,20	2,09	1,38
W-2/E-1	20,14	14,37	12,69	13,89	10,23	9,47	10,11	7,48	3,45	1,26
W-2/E	2,17	3,03	7,44	7,92	8,55	5,90	3,33	7,74	5,39	0,41
E-2/E-1	7,44	2,33	5,05	4,10	9,81	2,76	1,85	2,85	0,77	0,18
E-2/E	1,01	3,03	2,11	1,32	1,87	2,91	0,98	3,63	0,83	0,29
E-1/E	10,39	5,11	1,82	1,17	4,25	1,31	2,56	2,57	1,33	0,33

Table 6.11 CHI-SQUARED VALUES : COMPARISONS OF SITE PAIRS
pooled data from occluded lotions studied in Chapters 4 and 5

Time (hrs)	7	8	9	10	12	14	16	18	28	32
W/W-1	6,10	7,67	3,65	11,38	5,96	9,75	6,61	3,58	12,25	9,39
W/W-2	14,13	11,53	6,31	9,77	6,47	12,16	15,15	7,10	17,48	13,11
W/E-2	2,90	12,14	2,59	5,37	7,48	9,14	9,98	3,67	19,16	15,69
W/E-1	3,02	2,14	4,94	3,77	4,35	7,01	15,66	12,20	17,82	14,05
W/E	7,02	10,22	10,66	6,69	6,93	3,13	16,59	10,34	9,93	10,59
W-1/W-2	4,16	3,88	2,60	7,29	3,98	1,90	8,95	0,80	1,25	1,17
W-1/E-2	2,10	6,29	3,19	6,01	1,33	2,01	2,20	0,17	2,91	2,96
W-1/E-1	5,71	4,69	6,66	5,53	2,68	2,41	2,93	3,56	1,24	2,40
W-1/E	10,68	3,80	12,88	7,51	4,53	4,06	4,74	4,70	1,30	0,50
W-2/E-2	11,15	10,12	3,54	4,28	4,97	1,50	15,86	0,98	2,79	5,52
W-2/E-1	13,38	5,31	5,80	4,32	8,72	3,87	6,17	1,69	0,01	4,54
W-2/E	21,43	12,31	17,38	11,24	14,54	7,81	23,29	4,86	3,65	0,36
E-2/E-1	3,07	6,22	0,74	0,17	2,00	5,49	4,58	3,31	2,52	1,29
E-2/E	5,75	4,39	5,95	2,63	8,04	4,92	2,38	4,91	2,40	5,30
E-1/E	7,03	8,28	4,90	2,36	2,76	3,49	7,12	10,11	3,63	4,18

CHAPTER 7

REPRODUCIBILITY OF THE BLANCHING ASSAY

It was argued in Chapter 2 of this thesis that although the visual assessment of blanching must be acknowledged as being subjective, objective instrumental methods investigated thus far have not been shown to provide more accurate grading of blanching intensities of topical corticosteroids. In view of the subjective nature of the visual assessment of blanching responses, Barry (179) has suggested the inclusion of a standard corticosteroid formulation in all blanching trials. Figure 7.1 contains a set of blanching profiles of Betnovate cream reproduced from reports published from the laboratories of Woodford and Barry (179,384,479). The ten trials from which these curves were extracted were performed over a 3½ year period in the occluded mode of application. The main conclusion drawn from the above study was that for experienced investigators adhering to a strict protocol and using a controlled panel of volunteers, the bioassay is sensitive, accurate and reproducible.

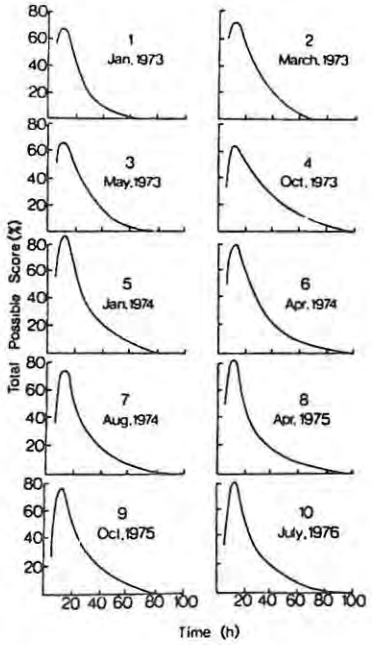


Figure 7.1 Blanching profiles of Betnovate cream from 10 trials performed with occlusion (from Barry and Woodford, ref. 384)

An observation of reproducibility of results of comparative blanching has been reported by Burdick (397) for preparations applied in the unoc-

cluded mode. It was found that fluocinonide in cream base consistently elicited a superior blanching response to betamethasone 17-valerate cream in 23 separate studies. It is worth noting that several new observers were employed over the period of the 23 trials, but each study had at least one experienced observer participating.

Sixteen trials have been reported in this thesis, three in the unoccluded mode of application (Chapter 3) and 13 in both the occluded and unoccluded modes (Chapters 4 and 5). Betnovate cream was utilized in all 16 trials, either as the test preparation or as a standard preparation. The blanching responses of Betnovate cream over the 16 trials are depicted in figure 7.2 and the %TPS values are presented in table 7.1. The key to the letters assigned to each set of profiles is presented in table 7.2 in the appendix to this chapter. It can be seen from the figure that the shapes of the profiles were generally similar for all 16 trials. The mean blanching profiles of Betnovate cream applied in both application modes are depicted in figure 7.3 (see table 7.1 for raw data). The vertical lines in figure 7.3 depict the maximum and minimum scores obtained for Betnovate cream from a combination of the 16 trials. It should be noted that these lines represent range and not standard deviation values. It is interesting to note that the maximum and minimum values did not occur in the same two trials in either application mode. In the occluded mode the maximum values occurred in trial C from 7 - 14 hours after application, whereas in the unoccluded mode the maximum values occurred in trial F from 7 - 14 hours after application. Minimum values were obtained from trial L in the occluded mode from 7 - 12 hours after application, and in the unoccluded mode also from trial L from 7 - 18 hours after application. The other maximum and minimum values were obtained from a number of different trials, although some overlap occurred.

Maximum blanching responses were observed between 12 hours and 14 hours after application in all trials and in both application modes. In two trials (E and F) in the occluded mode, the 12 hour and 14 hour %TPS values were identical, whilst in several other trials in both application modes similar %TPS values were recorded at the 12 hour and 14 hour readings. Maximum blanching responses may, in these cases, have occurred between 12 and 14 hours after application, at which times blan-

Figure 7.2 Blanching profiles of Betnovate cream from the trials reported in this thesis (17 sets of profiles)
(——occluded mode; ----unoccluded mode)

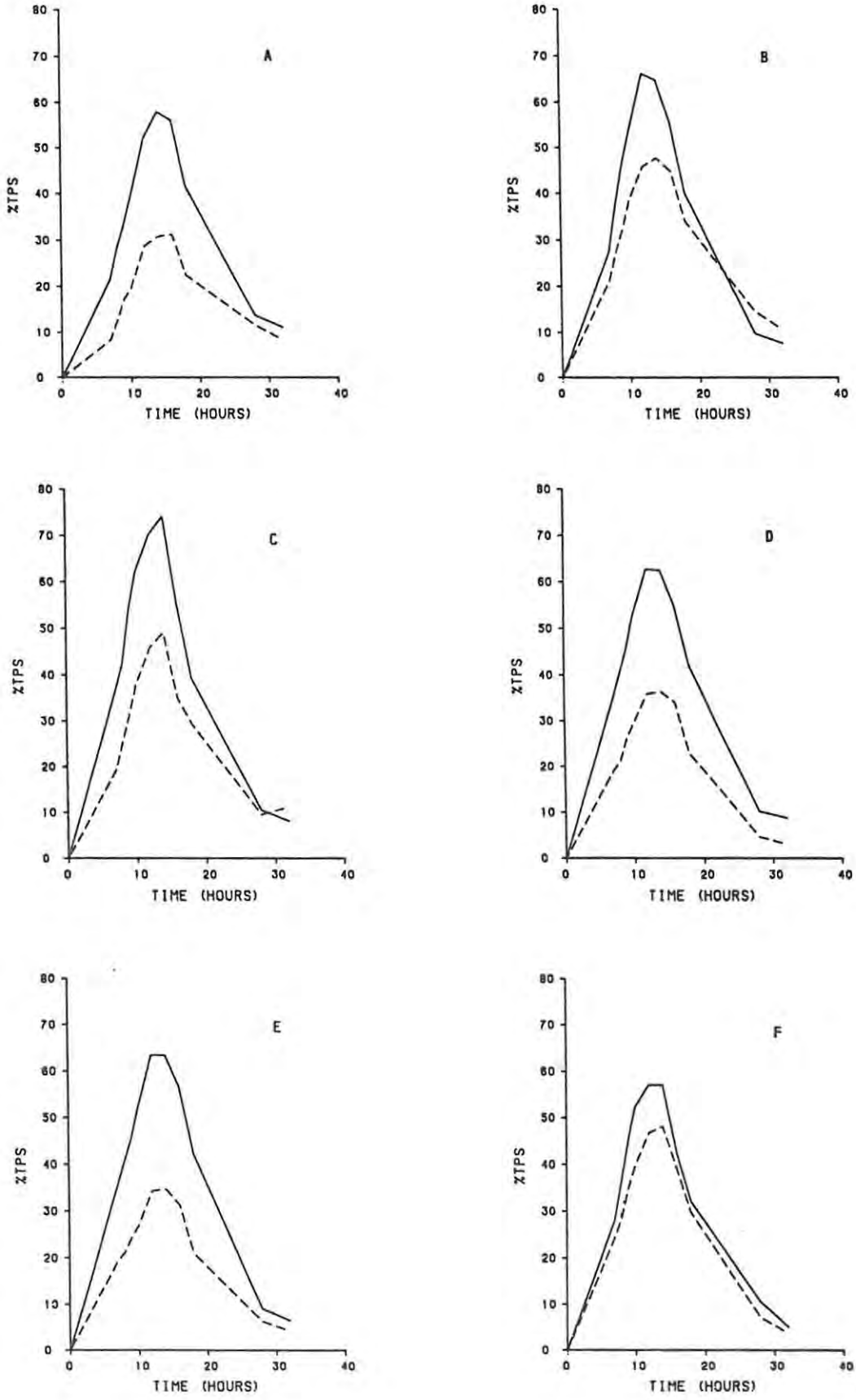
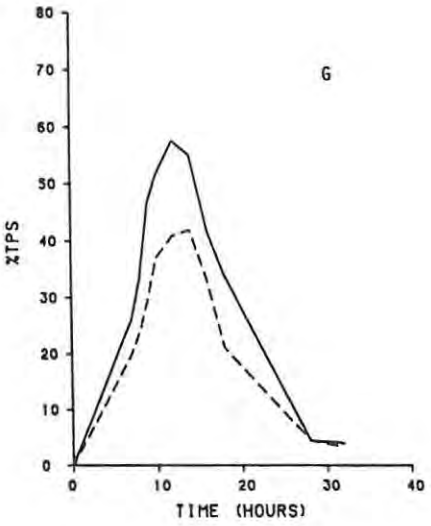
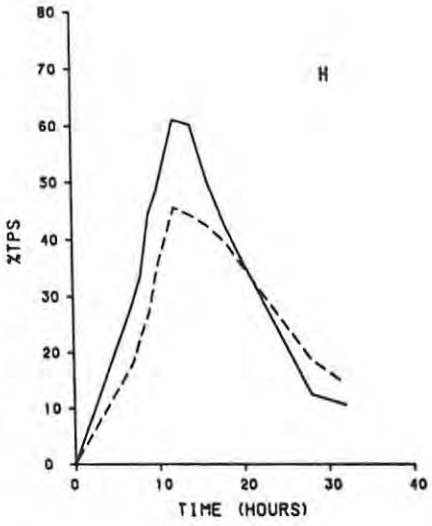
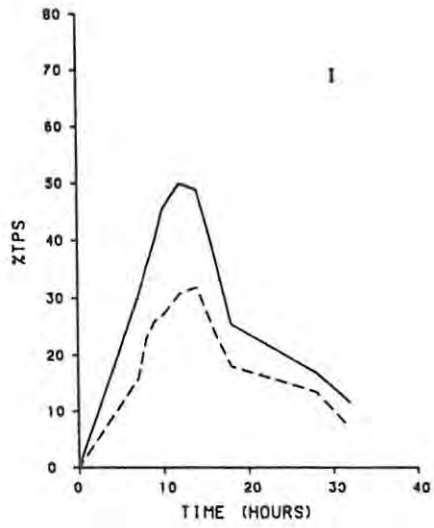
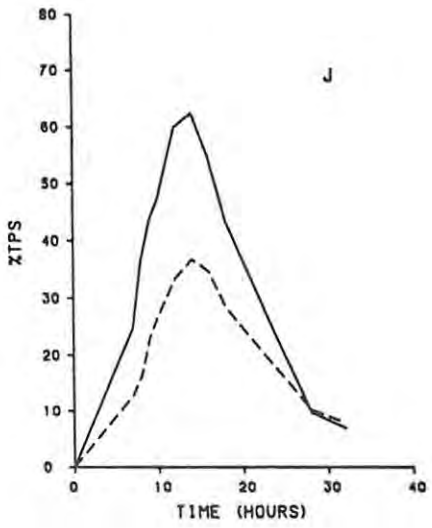


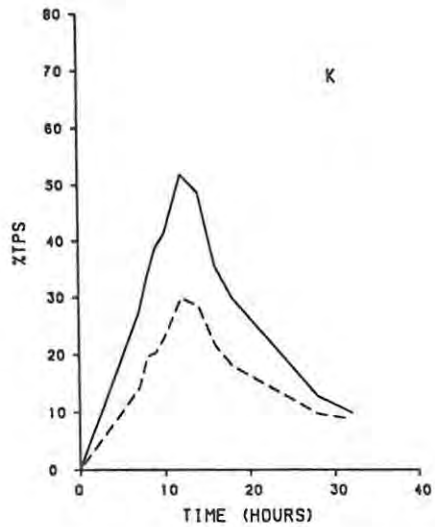
Figure 7.2 (continued)











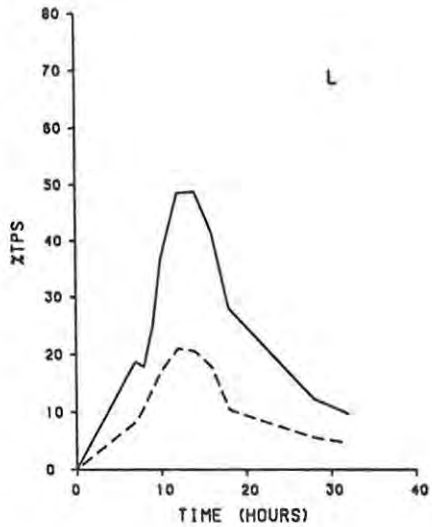
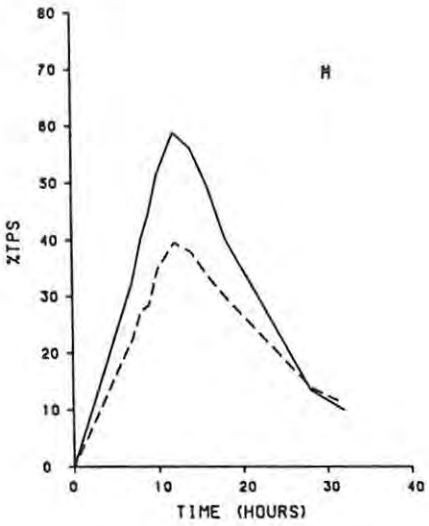
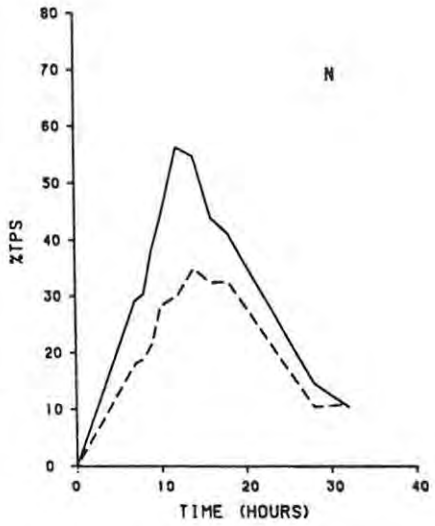
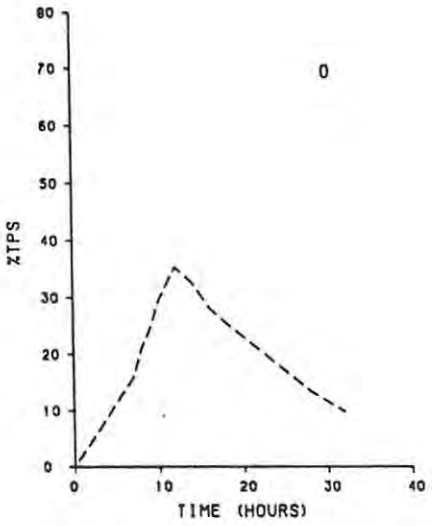
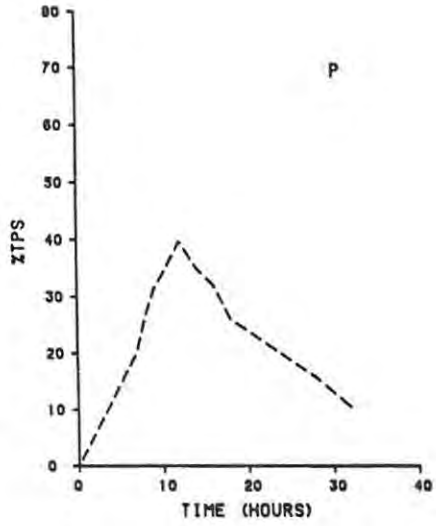


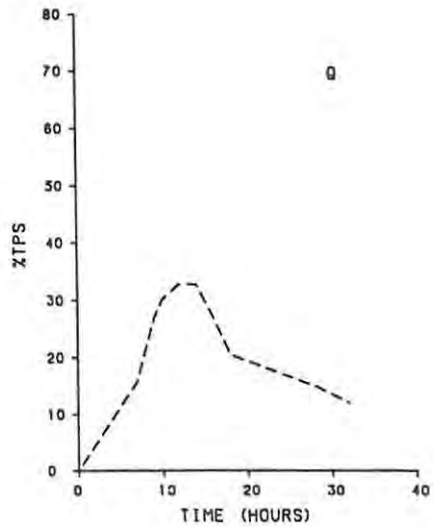
Figure 7.2 (continued)











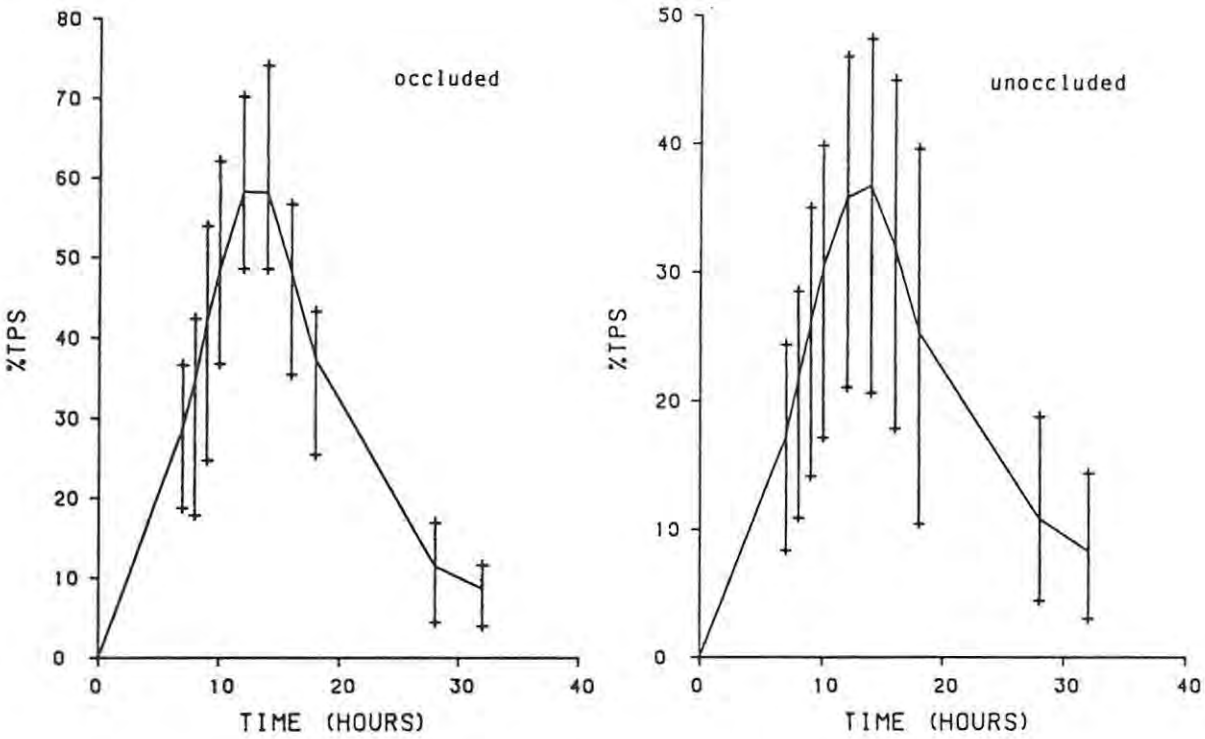


Figure 7.3 Mean blanching responses of Betnovate cream over 16 trials

ching responses were not recorded. The average time after application at which blanching was recorded was 12,71 hours in the occluded mode and 13,18 hours in the unoccluded mode. In the case of the creams reported in Chapter 5 (table 5.5, section 5.2), the average times after application at which maximum blanching was recorded were 12,38 hours and 13,00 hours in the occluded and unoccluded application modes, respectively. It appears therefore that whilst some preparations exhibit more rapid onset of action (for example Eumovate cream; section 5.1.4.1) and others exhibit slower onset of action (for example Synalar cream; section 5.1.2.1) than the average, the time to peak generally did not depend on the corticosteroids used in this thesis. The AUC values of the Betnovate creams ranged from 1059 to 724 in the occluded mode and 838 to 314 in the unoccluded mode. The average AUC values were 914 and 616 in the occluded and unoccluded modes, respectively. This clearly demonstrates the increased blanching responses elicited by corticosteroid preparations applied under occlusive dressings (see section 1.3.2.2).

It is evident that the blanching responses of Betnovate cream were less consistent in the trials reported in this thesis compared to the 10

trials reported by Barry and Woodford (179,384). A possible reason for this is the extent to which the blanching panels varied. The volunteer panel in the laboratories of Barry and Woodford consisted of 16 volunteers, six of whom were employed in all 10 trials as part of the blanching panel of 10 subjects per trial. The volunteer panel in our laboratories consisted of 81 volunteers known to show a blanching response, but no attempt was made to employ the same volunteers in any of the trials, with the exception of those reported in Chapter 3. The same two investigators recorded blanching responses in all 16 trials reported in this thesis, whilst the third observer in each trial was one of two other trained observers in our laboratories. Although it was seen in section 3.1 that the blanching elicited by Betnovate cream differed significantly over three trials notwithstanding the same volunteer and observer panels, the ranges, in terms of %TPS and AUC values (tables 7.1 and 7.3), obtained over the 16 trials reported here were somewhat larger than expected. It can also be seen that the range of minimum and maximum values was greater in the unoccluded mode than in the occluded mode (tables 7.1 and 7.5). This can be expected due to the lower intensity of blanching in the unoccluded mode of application. The ranges were also generally relatively larger at the 28 hour and 32 hour readings, where lower intensity blanching responses occurred.

It is worth noting that in the trials reported by Barry and Woodford, the blanching responses were generally higher than those reported in this thesis (see figure 7.4 and tables 7.4 and 7.5). This may be partly due to observers in different laboratories employing different standards of grading within the arbitrary 0 - 4 scale, but may also be due to the greater blanching response elicited by the UK Betnovate cream compared to the SA Betnovate cream (section 5.1.1.1). Whilst results of comparative blanching studies are generally reproducible in terms of rank order (397,section 3.1), this study of the Betnovate cream profiles from our laboratories and those of Woodford and Barry indicate that the blanching responses *per se* are not necessarily reproducible. However, reproducibility of the assessment of blanching responses in the same trial was clearly demonstrated when Betnovate cream (SA) was incorporated under two different codes in the same trial in section 5.1.1.1 (figure 5.1.1,trial D/E).

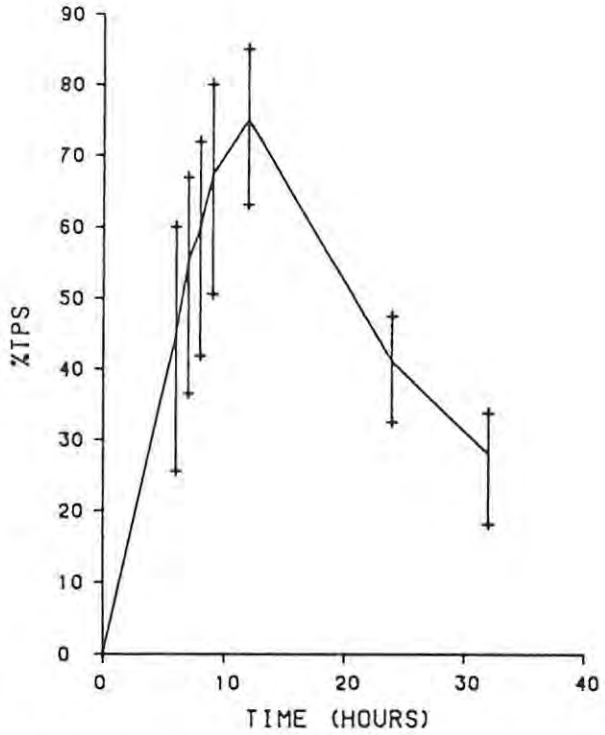


Figure 7.4 Mean blanching responses of Betnovate cream applied under occlusion in 10 trials reported by Barry and Woodford data obtained from reference 479

An important practical consideration gleaned from the wide range of blanching responses discussed above is the unreliability of comparing blanching responses between trials, even if performed in the same laboratory, unless a standard preparation is included and a correction factor employed (68,101,113,138,204-208,309,370). Blanching responses of different corticosteroid preparations should therefore not be compared between trials in which a standard preparation was not included, as conclusions drawn from such comparisons may well be meaningless. Similarly, blanching responses recorded in different laboratories should not be compared unless data are available for the employment of a correction factor.

The similarity in the shapes of the curves (figure 7.2), and the expected lower intensity blanching in the unoccluded mode as opposed to the occluded mode however give credence to the results of the experiments reported in this thesis. In addition to this, the mean curves and ranges of blanching responses (figure 7.3) provide a reference for the blanching intensity of Betnovate cream incorporated as a standard preparation in future trials performed in these laboratories.

APPENDIX TO CHAPTER 7

Table 7.1 %TPS VALUES FOR BETNOVATE CREAMS STUDIED IN THIS THESIS

Time (hrs)	7	8	9	10	12	14	16	18	28	32
Occluded										
Trial										
A	21,53	28,24	33,10	39,12	52,08	57,87	56,02	41,67	13,66	11,11
B	27,55	37,73	46,53	53,47	66,20	64,81	55,32	40,05	9,72	7,64
C	36,57	42,36	53,94	62,04	70,14	74,07	54,86	39,35	10,42	8,10
D	34,49	40,05	45,60	52,78	62,73	62,50	54,86	42,13	10,19	8,80
E	35,42	40,47	45,37	52,08	63,43	63,43	56,71	42,36	9,03	6,48
F	28,01	36,34	45,14	52,31	57,18	57,18	42,36	32,18	10,65	5,09
G	26,16	33,33	46,53	51,39	57,64	55,09	41,90	34,03	4,40	3,94
H	28,70	33,56	44,44	48,61	61,11	60,19	50,00	42,36	12,50	10,65
I	30,09	35,19	39,81	45,60	50,00	48,84	37,73	25,46	16,90	11,57
J	24,77	36,81	43,75	47,45	59,95	62,50	54,63	43,29	9,72	6,94
K	27,31	33,80	38,89	41,20	51,85	48,61	35,42	29,86	12,73	9,95
L	18,75	17,82	24,77	36,81	48,61	48,84	41,44	28,01	12,27	9,72
M	32,41	39,81	44,68	51,39	58,80	56,02	49,31	40,28	13,43	9,95
N	29,17	30,32	38,19	43,52	56,25	54,63	43,98	41,20	14,58	10,42
Mean	28,62	34,70	42,20	48,41	58,28	58,18	48,18	37,30	11,44	8,60
Maximum	36,57	42,36	53,94	62,04	70,14	74,07	56,71	43,29	16,90	11,57
Minimum	18,75	17,82	24,77	36,81	48,61	48,61	35,42	25,46	4,40	3,94
Unoccluded										
Trial										
A	8,33	12,50	17,13	19,44	28,70	30,79	31,25	22,45	11,57	8,33
B	21,30	27,55	32,41	38,66	45,83	47,69	44,91	34,03	14,58	10,65
C	19,21	25,46	31,25	37,96	45,83	49,07	34,95	29,40	9,49	11,11
D	18,98	21,06	26,16	29,40	35,88	36,34	34,03	22,92	4,63	3,01
E	19,44	21,06	24,31	26,85	34,26	34,72	31,25	21,06	6,25	4,17
F	24,31	28,47	34,95	39,81	46,76	48,15	39,12	29,86	7,18	3,70
G	19,91	24,07	29,86	37,04	40,97	41,90	33,10	21,06	4,40	3,24
H	18,06	23,15	27,31	35,42	45,60	44,21	42,36	39,58	18,75	14,35
I	15,74	22,92	25,93	27,08	30,79	31,94	24,31	18,06	13,43	6,94
J	12,73	16,20	23,15	26,85	33,33	36,81	34,72	28,47	10,19	7,87
K	14,21	19,68	20,37	22,92	29,86	28,47	21,53	18,06	9,72	8,80
L	8,33	10,88	14,12	17,13	21,06	20,60	17,82	10,42	5,56	4,63
M	22,45	27,31	28,47	34,72	39,58	37,96	33,56	29,86	13,89	11,11
N	18,06	18,98	21,53	28,24	30,09	34,95	32,41	32,64	10,42	10,88
O	15,86	21,06	24,50	29,32	35,34	32,60	28,16	25,50	13,35	9,80
P	20,22	26,74	31,48	33,95	39,70	35,03	32,10	26,04	15,78	10,53
Q	15,78	21,41	26,81	30,13	32,83	32,72	26,97	20,45	14,97	12,04
Mean	17,23	21,68	25,87	30,29	35,73	36,70	31,91	25,29	10,83	8,30
Maximum	24,31	28,47	34,95	39,81	46,76	48,15	44,91	39,58	18,75	14,35
Minimum	8,33	10,88	14,12	17,13	21,06	20,60	17,82	10,42	4,40	3,01

Table 7.2 KEY TO TRIALS A - Q IN TABLE 7.1

Trial	
A	Influence of mode of transport - Betnovate creams (Chapter 4)
B	Influence of mode of transport - Betnovate ointments (Chapter 4)
C	Influence of mode of transport - Betnovate lotions (Chapter 4)
D	Comparison of preparations from three countries - Betnovate creams (Chapter 5)
E	Comparison of preparations from three countries - Betnovate creams (Chapter 5)
F	Comparison of preparations from three countries - Betnovate ointments (Chapter 5)
G	Comparison of preparations from three countries - Betnovate lotions (Chapter 5)
H	Comparison of preparations from three countries - Synalar creams (Chapter 5)
I	Comparison of preparations from three countries - Synalar ointments (Chapter 5)
J	Comparison of preparations from three countries - Synalar lotions (Chapter 5)
K	Comparison of preparations from three countries - Locoid creams (Chapter 5)
L	Comparison of preparations from three countries - Locoid ointments (Chapter 5)
M	Comparison of preparations from three countries - Eumovate creams (Chapter 5)
N	Comparison of preparations from three countries - Eumovate ointments (Chapter 5)
O	Trial 1, Chapter 3
P	Trial 2, Chapter 3
Q	Trial 3, Chapter 3

Table 7.3 AREA UNDER THE CURVE VALUES FOR BETNOVATE CREAMS STUDIED IN THIS THESIS

Trial	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Occluded	906	971	1059	1003	1004	863	814	971	831	947	804	724	975	930	-	-	-
Unoccluded	506	808	716	524	556	725	585	838	543	598	485	314	718	641	611	691	593

Table 7.4 %TPS VALUES FOR BETNOVATE CREAMS STUDIED BY WOODFORD
all trials were performed in the occluded mode
data obtained from reference 479

Time (hrs)*	6	7	8	9	12	24	32
Trial							
1	56,3	66,9	63,8	64,4	68,1	32,5	18,1
2	60,0	66,3	59,4	66,3	73,1	44,4	30,0
3	50,0	63,1	65,6	70,0	66,3	38,1	26,3
4	25,6	36,9	41,9	50,6	63,1	43,8	33,8
5	55,0	66,3	69,4	80,0	85,0	47,5	27,5
6	50,0	59,4	68,8	73,8	80,0	41,9	31,3
7	36,9	43,1	51,3	60,6	75,6	40,6	28,1
8	53,8	66,9	71,9	76,9	83,8	42,5	25,0
9	26,9	38,8	50,6	63,8	75,6	36,9	30,0
10	34,4	43,8	56,3	66,9	79,4	42,5	29,4
Mean	44,9	55,2	59,9	67,3	75,0	41,1	28,0
Maximum	60,0	66,9	71,9	80,0	85,0	47,5	33,8
Minimum	25,6	36,9	41,9	50,6	63,1	32,5	18,1

* Observations were made up to 96 hours after application

Table 7.5 PERCENTAGE DIFFERENCES OF MAXIMUM AND MINIMUM VALUES FROM MEAN VALUES

Time (hrs)	6	7	8	9	10	12	14	16	18	24	28	32
Occluded												
This Thesis												
Maximum	-	28	22	28	28	20	27	18	16	-	48	35
Minimum	-	34	49	41	24	17	16	26	32	-	62	54
Woodford (479)												
Maximum	34	21	20	19	-	13	-	-	-	16	-	21
Minimum	43	33	30	25	-	16	-	-	-	21	-	35
Unoccluded												
This Thesis												
Maximum	-	41	31	35	31	31	31	41	57	-	73	73
Minimum	-	52	50	45	43	41	44	44	59	-	59	64

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

The human skin blanching assay has become a well accepted method of assessing the bioavailability of corticosteroids from topical dosage forms. Little research has, however, been carried out on the numerous variables of this bioassay.

The good correlation of results of clinical studies and blanching trials has been well documented (section 2.2.8), but cognizance should be taken of the current lack of knowledge of the magnitude of the difference required in blanching studies to reliably predict a difference in clinical efficacy (section 5.2). Research is required to ascertain which methods of statistical analyses are sufficiently insensitive so as to indicate significant differences in blanching studies that are too small to be clinically meaningful. A similar situation pertains to the study of tachyphylaxis to the blanching response (section 1.5). Whilst results obtained thus far have indicated that improved dosage regimens can be suggested from results of blanching trials, statistical methods should, as suggested above, be chosen so that predictions of clinical efficacy are as reliable as possible.

Another aspect that requires further investigation is the mechanism of blanching (section 1.7). Whilst it is generally accepted that vasoconstriction is a major component of the mechanism of blanching, further consideration should be given to the hypothesis of melanolysis proposed by Aiache *et al.* (330). Weak blanching responses have been noted on dark skinned persons in our laboratories, and further studies may enhance the understanding of the mechanism of blanching and the possible relationship between blanching and melanin.

The influence of the formulation of the vehicle on the release of corticosteroids has been thoroughly investigated (section 1.6). Further studies in this area will undoubtedly lead to the discovery of new penetration enhancers (section 1.6.3) and improved formulations, with obvious benefit to patients and clinicians. Whilst the large number of corticosteroid preparations and commercial dilutions on the market makes extemporaneous dilution unnecessary, this practice is still requested by prescribers (section 1.6.4). Manufacturers of corticosteroid prepara-

tions should discourage dilution of their products, but given that the practice will almost certainly continue, names of recommended diluents should be readily available to pharmacists, for example on package inserts or in an easily available reference such as MIMS.

This study involved mainly the critical evaluation of the blanching assay, in the form of the review presented in Chapter 2 as well as the practical work presented in Chapters 3, 6 and 7. Whilst the lack of consensus on the methodology of the assay has been noted previously (15,384), the extent of the variations in size of application areas and amounts applied (table 2.1) and observation times and duration of application (table 2.2) is striking. The influence of these variables on the results of comparative bioavailability studies requires further investigation. The number of times in which a single reading, 7 hours after application, produces different results of comparative bioavailability to the results of extended readings is a cause for concern (sections 2.2.5 and 2.2.8). Conclusions drawn from single observations of blanching apply only to that particular observation time and are of questionable value as they do not provide an overall profile of corticosteroid bioavailability and activity.

Results of the five variables studied in the series of three trials reported in Chapter 3 allowed some suggestions to be made with respect to the methodology of the blanching assay. The first aspect of the assay that is reported in Chapter 3 is the influence of environmental conditions on the blanching response (section 3.2). It appears from the results obtained that ambient temperature and relative humidity could possibly have had some influence on the blanching responses during these three trials. Whilst a correlation was not found between environmental conditions and the intensity of blanching in every case in this study, the possibility of a relationship requires further investigation. The vasodilation due to an increase in ambient temperature could be expected to hasten the removal of the corticosteroid from the site of action. A further consideration is that higher ambient temperatures during the application phase of assays performed in the occluded mode may lead to increased activity of sweat glands and consequently increased hydration of the stratum corneum, with subsequent rapid percutaneous penetration and clearance of the corticosteroid from the site of action.

The effects of extremes in temperature and relative humidity may influence the results of comparative bioavailability studies. Increased hydration of the unoccluded stratum corneum under very humid conditions may mask the influence of the release characteristics of the vehicle in the same way that this has been found to occur when corticosteroid preparations are applied under occlusion (sections 1.3.2.2 and 2.2.2). Similarly, in trials performed in the occluded application mode, elevated ambient temperatures may exacerbate the masking effect of occlusion. Results of blanching trials performed under very hot and/or humid conditions should therefore be interpreted with caution.

A study of the observer-dependent variations in blanching responses (section 3.3) allows some suggestions to be made with respect to the methodology of the assay. It is accepted that the subjective nature of the assessment of the blanching responses demands that observers be trained (179). This was borne out by the study of blanching responses recorded by an inexperienced observer in our laboratories. Preliminary investigation indicates that this observer was able to reliably assess blanching responses after two trials, but it should be noted that training may take longer in laboratories where blanching responses are not recorded at several reading times over an extended period. It was further found, in the study of responses recorded by experienced observers, that conclusions drawn from the scores of a single observer may not always be reliable in assessing comparative bioavailability, whilst pooled results of two observers and three observers produced similar results. It is therefore suggested that the pooled results of at least two experienced observers be utilized in comparative bioavailability studies.

The study of the blanching responses induced on individual volunteers (section 3.4) demonstrated the expected intrapersonal and interpersonal biological variability. An important aspect with respect to the methodology of the assay is that the rank order of the two preparations was not the same in all volunteers, although Celestoderm-V produced more blanching than Betnovate in the majority of volunteers. The number of volunteers utilized in trials was discussed in section 2.2.1 and it was seen that this varied from 4 - 50, with most papers reporting the use of 10 volunteers. It is difficult to ascertain how many volunteers should be employed in a trial for results to be reproducible and statistically

meaningful. In the series of three trials reported in Chapter 3, the same results of comparative bioavailability were obtained from the pooled scores of 18 volunteers, two groups of nine volunteers arranged according to AUC values (table 3.5), two groups of nine volunteers arranged according to sex (section 3.5) and the results of individual arms, which essentially represented nine volunteers (section 3.6). It therefore appears as though the use of nine volunteers was sufficient in this series of trials for the assessment of comparative bioavailability of the two products studied. A study of the minimum number of volunteers required for a trial would have involved comparisons of the results of each volunteer to each of the other volunteers (which was performed for the purposes of section 3.4), as well as analysis of the combined results of all the permutations of two volunteers, three volunteers, *etc*. It was seen that when the results of those volunteers in whom differences in comparative bioavailability were demonstrated on individual analysis were pooled, the pooled results, as expected, also indicated differences in comparative bioavailability of the two preparations. There is no reason to expect that differences would not also have been found had the results of only two such volunteers been pooled. Analysis of the results of all the permutations would therefore have been both an enormous and meaningless exercise. The analysis of these three trials, as well as the results obtained in these and other laboratories do, however, suggest that reliable results are obtained with the use of 10 or 12 subjects. It is however suggested that in trials in which 10 or 12 subjects are employed, use should be made of 12 sites per arm and no more than four preparations should be studied in such trials.

Re-grouping of volunteers in various ways allowed some suggestions to be made with respect to the selection of volunteers. It was seen that male and female subjects produced the same rank order of blanching of the two preparations studied (section 3.5). Blanching panels may therefore be comprised of male and/or female volunteers. Re-grouping of the volunteers according to AUC values indicated that blanching panels can be comprised of both good and average blanchers, although experience in our laboratories has shown that very good blanchers are often poor discriminators especially when potent preparations are being compared. It is therefore suggested that good blanchers be used when weak preparations are being studied, whilst when potent preparations are being studied

blanching panels should be selected so that a range of responses is obtained. It was further ascertained that both arms exhibited similar sensitivity to corticosteroid-induced blanching (section 3.6). It is therefore feasible in trials performed in both modes of application to occlude the same arm in all volunteers. It is suggested that the writing arm be occluded, as the occlusive dressings are less cumbersome than the guards used to protect unoccluded sites.

A further variable of the blanching assay studied in this thesis was the sensitivity of different areas of the forearm to corticosteroid-induced blanching (Chapter 6). Significant differences in blanching responses were found on different areas of the forearm and it was further found that this differed between the two modes of application, but was not dependent on the formulation type. In the occluded mode more blanching was noted at the wrist than at the elbow, whilst in the unoccluded mode more blanching was noted at the elbow than at the wrist. In general, the highest degree of blanching occurred at the sites designated wrist-1 and wrist-2. It was postulated that differences in intensity of blanching are due to structural variation of the stratum corneum near the flexures of the forearm, as opposed to differences in the number or sensitivity of corticosteroid receptors at different areas of the forearm (section 6.2). From the point of view of methodology, it is suggested that each preparation being assayed should be applied to more than one site of the forearm and that application patterns should be designed to ensure that each preparation is distributed along the forearm.

The objective of the work reported in Chapter 4 was to study the influence of international transportation on the release of corticosteroids from three types of formulations. Three Betnovate formulations (cream, ointment and lotion) were purchased in the United Kingdom and transported to South Africa in three different ways, namely in the hold of an aircraft, in the cabin of an aircraft and in the hold of a ship. It was found that the method of transportation had little effect on the blanching abilities of the Betnovate cream applied under occlusion and Betnovate ointment applied in both application modes. The method of transportation was, however, found to influence the blanching abilities of the Betnovate cream applied without occlusion and the Betnovate lotions applied in both application modes. The preparations were all analysed

for betamethasone 17-valerate content and it was found that the content of each formulation type was essentially the same (section 4.4). The abovementioned differences in blanching abilities are therefore almost certainly attributable to alterations in the release characteristics of the vehicles due to extremes in temperature during transportation and/or storage prior to and after transportation. Whilst there seems no reason to expect that the influence of temperature extremes is limited to the release of betamethasone 17-valerate from the products in which differences were found in this study, further research is required to ascertain to what extent this is a general trend. Exportation of the test products is not necessary for future studies, which can be conducted subsequent to the preparations being exposed to artificially induced extremes in temperature. International travellers, as well as manufacturers of products for topical application, should however be aware of the possible alteration of the formulation during transportation where special precautions are not taken to limit extreme fluctuations in temperature.

In Chapter 5 of this thesis, a report is presented on an investigation of the blanching activities of proprietary corticosteroid preparations purchased in three countries, namely South Africa, Australia and the United Kingdom. The products used in this study were Betnovate cream, ointment and lotion, Synalar cream, ointment and lotion, Eumovate cream and ointment and Locoid cream and ointment. A summary of the results of the comparisons and overall conclusions is presented in table 5.4 (section 5.2). It can be seen from the table that there was no definite trend of products from one country consistently exhibiting superior blanching to products from the other two countries, or products from one country consistently exhibiting the lowest degree of blanching. An important aspect of the blanching assay that is discussed in Chapter 5 (section 5.2) is the necessity of drawing conclusions of comparative blanching abilities from the statistical analyses in combination with a study of the blanching profiles and AUC values. Examples are discussed where two forms of statistical analyses did not concur, and where statistical analyses indicated differences between two preparations that appeared to produce similar blanching responses in terms of AUC values and blanching profiles. It is worth reiterating that this is not

possible in blanching studies in which responses are only recorded at a single reading time.

The superior blanching response of Eumovate ointment in the unoccluded mode of application as compared to the occluded mode of application (section 5.1.4.2) requires further investigation. Research is required to ascertain whether this phenomenon exhibited by Eumovate ointment is a characteristic of clobetasone 17-butyrate in all ointment bases, or whether the particular formulation of Eumovate ointment base will influence the release characteristics of other corticosteroids in the same way.

A general observation when studying the results of the 10 trials reported in Chapter 5 was that the time at which maximum blanching was observed is influenced by the formulation type, and that the influence of occlusion on the time-to-peak differed for creams, ointments and lotions. It was explained (section 5.2) that occlusion could be expected to influence the release of corticosteroids from lotions more than from creams due to the relatively simple formulations of lotions compared to creams. On the other hand the influence of occlusion on the release of corticosteroids from ointments could be expected to be less pronounced than from creams due to the inherent occlusivity of ointment bases.

An important conclusion drawn from the results of the study presented in Chapter 5 is that manufacturers may need to consider the reformulation of some products with the objective of obtaining maximum release of the corticosteroids from their vehicles under the specific climatic conditions under which they are to be utilized.

Chapter 7 of this thesis involved a study of the blanching responses of Betnovate cream utilized either as the test preparation or standard preparation in all 16 trials reported in this thesis. Two important observations that emerged from this study of Betnovate cream profiles are that inter-trial comparisons of blanching responses should not be made unless a correction factor is employed, and that whilst results of comparative blanching studies are generally reproducible in terms of rank order, blanching responses *per se* are not necessarily reproducible. It has been recommended by Barry (179) that a standard corticosteroid form-

ulation be included in all blanching trials, and the value of this is evident in this thesis. For example, the weak blanching responses for the Eumovate creams applied without occlusion (section 5.1.4.1) and the anomalous behaviour of the Eumovate ointments applied without occlusion (section 5.1.4.2) may have been a cause for concern had the Betnovate cream standard not elicited the expected blanching responses. In general, the similarity in the shapes of the Betnovate cream profiles (figure 7.2) and the weaker blanching responses observed in the unoccluded mode of application as compared to the occluded mode (figures 7.2 and 7.3) give credence to the results of the trials reported in this thesis.

APPENDIX A

CORTICOSTEROID CONSTITUENTS OF PROPRIETARY PREPARATIONS DISCUSSED IN THIS THESIS

Proprietary name	Corticosteroid constituent
Alphaderm	hydrocortisone
Betnovate	betamethasone 17-valerate
Celestoderm-V	betamethasone 17-valerate
Dermovate	clobetasol 17-propionate
Dioderm	hydrocortisone
Diprosone	betamethasone 17,21-dipropionate
Eumovate	clobetasone 17-butyrate
Kenalog	triamcinolone 16,17-acetonide
Locoid	hydrocortisone 17-butyrate
Metosyn	fluocinonide
Nerisone	diflucortolone 21-valerate
Propaderm	beclomethasone 17,21-dipropionate
Synalar	fluocinolone 16,17-acetonide
Temetex	diflucortolone 21-valerate

APPENDIX B

PREPARATIONS USED IN THIS STUDY

Betnovate cream, ointment and lotion
Eumovate cream, ointment and lotion
Glaxo Australia Pty. Ltd., Boronia, Victoria, Australia
Glaxo (Pty.) Ltd., Wadeville, Transvaal, Republic of South Africa (RSA)
Glaxo Laboratories Ltd., Greenford, England.

Celestoderm-V cream
Scherag (Pty.) Ltd., Isando, Transvaal, RSA.

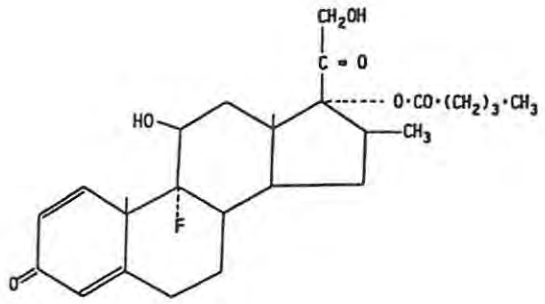
Locoid cream and ointment
Gist-Brocades Australia Pty. Ltd., Artarmom, NSW, Australia*
Riker Laboratories (Pty.) Ltd., Sandton, Transvaal, RSA (under license)
Gist-Brocades Great Britain Ltd., Surrey, England*
*Manufactured by Gist-Brocades nv, Delft, Netherlands.

Synalar cream, ointment and lotion
Imperial Chemical Industries (ICI), Australia Ltd., Villawood, Victoria
(Manufactured in Great Britain - see address below)
ICI South Africa (Pharmaceuticals) Ltd., Braamfontein, Transvaal, RSA
ICI Ltd., Pharmaceutical Division, Cheshire, Great Britain.

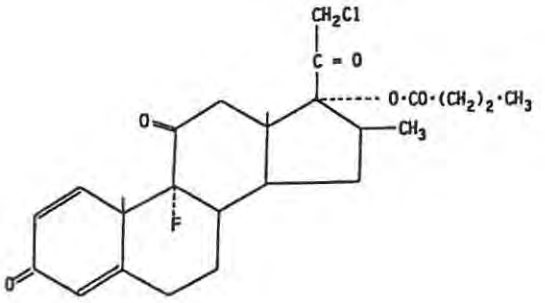
APPENDIX C

STRUCTURES OF CORTICOSTEROIDS STUDIED IN THIS THESIS

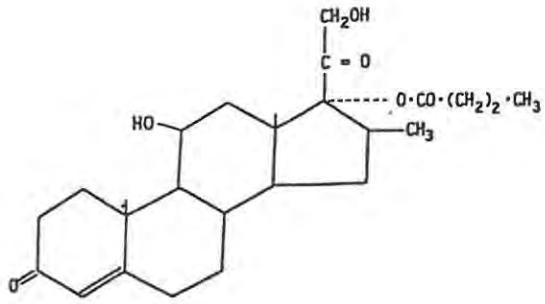
Substituents in the α configuration (---) project below the plane of the molecule; those in the β configuration project above the plane.



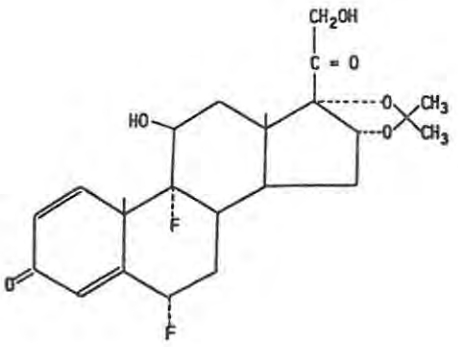
Betnovate
Celestoderm-V
betamethasone 17-valerate



Eumovate
clobetasone 17-butyrate



Locoid
hydrocortisone 17-butyrate



Synalar
fluocinolone 16,17-acetonide

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