THE *IN VIVO* AND QUANTITATIVE ASSESSMENT OF TOPICAL CORTICOSTEROID FORMULATIONS

A thesis submitted to

RHODES UNIVERSITY

in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE

by

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Finally, my parents for their financial assistance, encouragement and help.

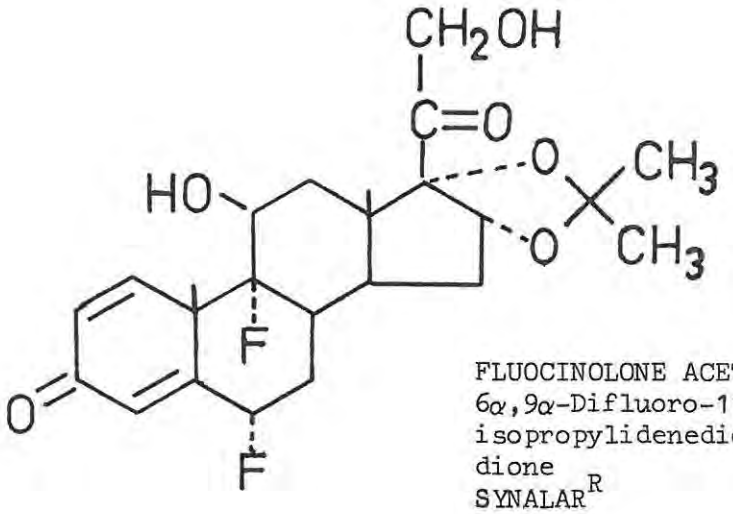
PUBLICATIONS

Parts of this work have already been published as follows:

(1) Comparative bioavailability of some proprietary topical corticosteroid creams. Coleman, G.L., Haigh, J.M. and Kanfer, I. S.A. Med. J. (1977) 52, 414.

(2) Comparative blanching activity of proprietary diflucortolone valerate topical preparations. Coleman, G.L., Kanfer, I. and Haigh, J.M. Dermatologica (in press).

STRUCTURES OF CORTICOSTEROIDS STUDIED IN THIS WORK.



FLUOCINOLONE ACETONIDE
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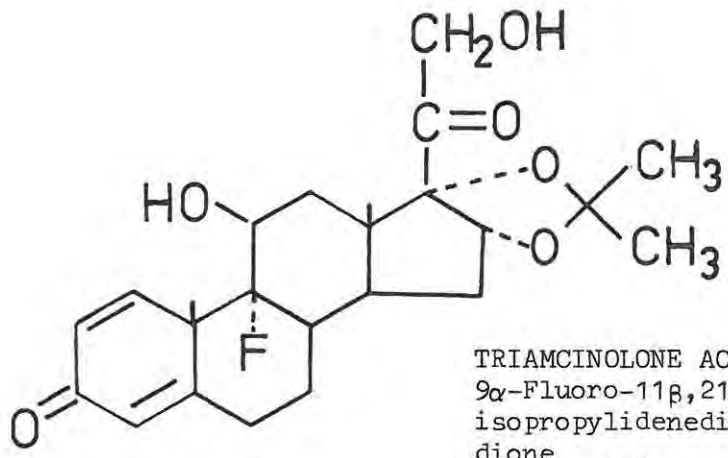
SYNALAR^{R}



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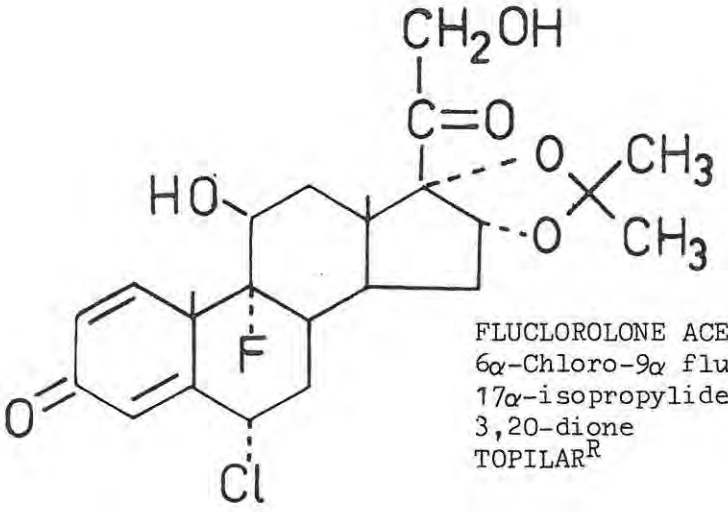
ARISTOCORT^{R}, LEDERCORT-D^{R}



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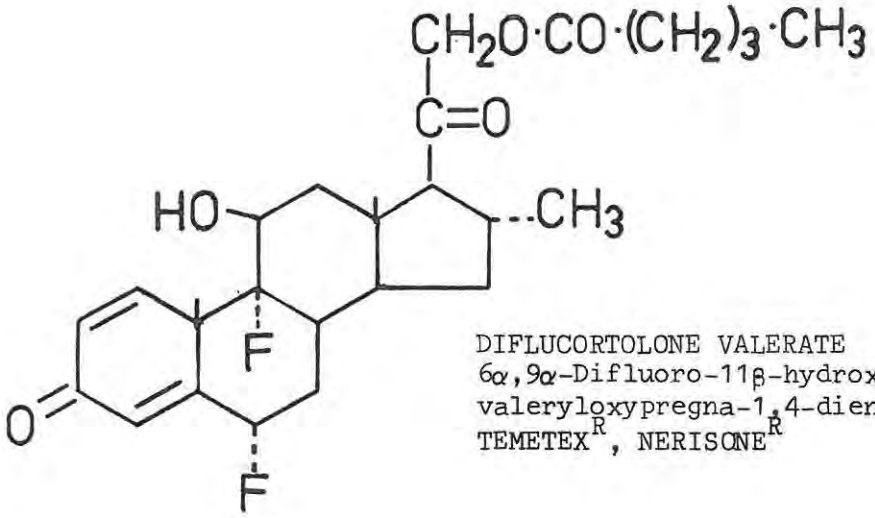

DIFLUCORTOLONE VALERATE
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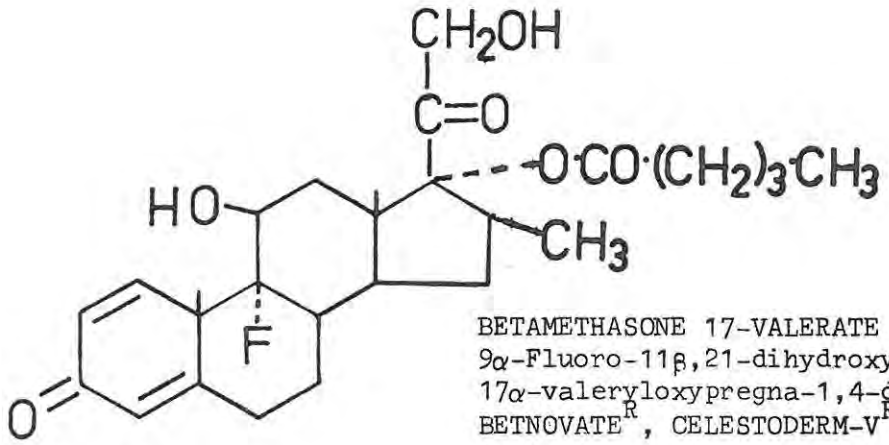
TEMETEX^{R}, NERISONE^{R}



BETAMETHASONE 17-VALERATE
9α-Fluoro-11β,21-dihydroxy-16β-methyl-17α-valeryloxypregna-1,4-diene-3,20-dione
BETNOVATE^{R}, CELESTODERM-V^{R}

INSTRUMENTATION

High Performance Liquid Chromatography

Varian 8500 dual pump system with solvent programmer, 200-800 nm Varichrome detector, and computer data system (CDS III).

Spectrophotometers

Beckman model 25 U.V.-visible recording spectrophotometer.

Beckman Acta VI U.V.-visible recording spectrophotometer.

Balances

Sartorius 4-figure, manual and 5-figure, semi-automatic analytical balances.

Computer

Wang 2200, Basic desk top mini-computer, with graph plotter and print out device.

A. INTRODUCTION

1. THE BLANCHING ASSAY

The profound blanching produced by the topical anti-inflammatory glucocorticosteroids has been studied extensively both as a mechanism and as an assay end-point for evaluating the potency of these compounds.

The blanching assay was first used for assessing percutaneous absorption by McKenzie and Stoughton[1] following their clinical observations that treatment with a topical corticosteroid occluded with a plastic film (saran wrap) produced pallor of the lesion and of the surrounding normal skin. Ashton and Cook[2] observed vasoconstriction in superficial corneal vascularization treated with subconjunctival steroids, and Hollander et al[3] reported that intra-articular steroids produced blanching of the engorged synovial membrane in rheumatoid arthritis.

1.1 MECHANISM OF BLANCHING

The mechanism of blanching is as yet not fully understood. It has been suggested that the adrenocortical steroids may support vascular tone by potentiating the pressor action of noradrenaline[4].

Corticosteroids have been shown to influence the blood vasculature in man and in experimental animals. Using venous occlusion plethysmography Ginsburg and Duff[5] have shown that after intra-articular hydrocortisone administration there is an increase in the vasoconstriction produced by intra-arterial noradrenaline. Thune[6] who also used plethysmography showed that blanching was due to the decongestion of the capillaries and small veins beneath the epidermis.

Reis[7] showed that steroids potentiate noradrenaline while Wolf et al[8] were able to demonstrate that topically applied corticosteroids potentiate catecholamine induced vasoconstriction. This effect is however blocked

by phentolamine, but not by propanolol, and hence it was postulated that the action is mediated via the increased sensitivity of α-adrenergic receptors.

Solomon et al[9] indicated that oral guanethidine, which interferes with the release and distribution of noradrenaline, effectively inhibited the corticosteroid induced blanching in 22 of 23 normotensive subjects and in only 2 of 10 hypertensive subjects. These mechanistic studies suggest that the topical corticosteroids cause blanching by sentizing vascular musculature to noradrenaline.

No evidence of a potentiation was obtained by Juhlin[10] on studying the vascular reactions of iontophoretically and/or intracutaneously administered noradrenaline, metacholine, seretonin and histamine in normal and corticosteroid treated skin and suggested that the blanching be explained as a vasoconstrictor effect of the steroid per se.

Other studies do not implicate noradrenaline exclusively. Frank et al[11] and Altura[12] showed that the corticosteroids do not constrict directly but suppress or block vasodilation induced by numerous agents such as histamine, tetrahydrofurfuryl alcohol and bradykinin and potentiate the effect of noradrenaline and other vasoconstrictors.

Du Vivier and Stoughton[13] have demonstrated indirect activity as evidenced by the release of endogenous noradrenaline from storage vesicles.

Thus the corticosteroids appear to have a virtually universal action and control over the mediators of local vasculature reactivity. Their regulation of the local blood flow involves modulating the responsiveness of vascular smooth muscle to the exogenous and endogenous chemical mediators of inflammation.

1.2 ALCOHOLIC BLANCHING

The first blanching investigations were performed using serial dilutions of the corticosteroids in alcohol (95%) or industrial methylated spirits in order to evaluate topical activity of corticosteroid derivatives in humans. The alcoholic blanching studies, which are simple, easy to perform, reliable and fast are useful in the screening of topical anti-inflammatory corticosteroids for activity[14-22]. Studies of topical corticosteroid blanching involve both percutaneous absorption and inherent vasoconstrictor activity. In order to eliminate the penetration aspect the corticosteroids can be injected intradermally[23]. It is interesting to note that by this method the supposedly non-anti-inflammatory corticosteroids had some blanching activity although the reactions were frequently ill-defined and of a different nature to those produced by the potent glucocorticoids. The final screening should however involve the formulation in which the corticosteroid is to be used clinically.

1.3 BLANCHING OF PROPRIETARY FORMULATED PRODUCTS

The composition of the vehicle may affect the availability of the corticosteroid, and its subsequent rate of release from the base into the skin, and hence alter the degree of blanching produced by that topical corticosteroid when compared to its alcoholic ranking.

The blanching assay has been used to compare the activity of different corticosteroid preparations available on the market, mainly in the United Kingdom, by various workers[24-37].

Several commercially available corticosteroid derivatives were evaluated by Coldman, Lockerbie and Laws[24] who used the blanching test to evaluate

the efficacy of these products. The ranking order, based on approximate area under the curve values was found to be generally the same whether the ointment or cream formulations were considered. Generally the ointments produced a higher degree of blanching than the creams. Also, the ranking order obtained from the blanching produced by the proprietary products was essentially similar to that produced by alcoholic solutions[16,18].

Two ointments both containing the same corticosteroid in the same concentration, but compounded by different manufacturers were included in this trial[24]. One ointment showed a significantly superior rate of release over the other. Microscopic examination showed that the ointment preparation showing greater steroidal bioavailability was more effective because the steroid was present as a solution or as fine particles whereas crystals were visible in the other ointment. This serves to illustrate the importance of good manufacturing protocol.

Woodford and Barry[25] compared various formulations of betamethasone 17-benzoate to selected proprietary formulations of topical corticosteroids using the blanching assay. Blanching studies employing alcoholic solutions indicated betamethasone 17-benzoate to be equivalent to betamethasone 17-valerate[38] in terms of the blanching effect. However, in this trial[25] on formulated betamethasone 17-benzoate cream was found to be significantly less effective than betamethasone 17-valerate in the blanching assay. The betamethasone 17-benzoate gel produced a blanching response almost equivalent to fluocinolone acetonide gel, as would be anticipated from alcoholic blanching studies.

Thirty proprietary corticosteroid creams and gels were evaluated for blanching using an occluded blanching test by Barry and Woodford[27]. In a subsequent trial the same researchers evaluated[28] thirty one proprietary

corticosteroid ointments and were thus able to compare the blanching activities of an extensive range of topical corticosteroid preparations available in the United Kingdom, and by inference the bioavailability of these preparations[30].

The term bioavailability refers to the relative absorption efficacy of a medicament as determined by the rate of release of the corticosteroid from the preparation, followed by its penetration through the epidermis into the dermis to produce the characteristic blanching effect.

The bioavailability may be expressed using the following relationship[31].

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

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

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

1.4 VEHICLE EFFECTS

The therapeutic effect of a corticosteroid applied topically depends not only on the biochemical efficacy of the corticosteroid derivative, but also on the degree to which the drug can reach the diseased layer in the skin. In skin diseases in which the stratum corneum is present, the drug must penetrate through this barrier zone. Although the function of this zone may be altered by the disease, it can still hamper penetration.

Caldwell *et al*[39], in the evaluation of beclomethasone dipropionate, found that beclomethasone dipropionate (0,025%) cream was significantly more effective in the blanching test than 0,025% fluocinolone acetonide cream, while in the ointments, this situation was reversed. Beclomethasone

dipropionate was subsequently prepared in a second ointment base. The second formulation performed better, both in a blanching trial, and clinical comparison. In the second base beclomethasone dipropionate was dissolved in propylene glycol and the resulting solution incorporated into white soft paraffin, while in the original preparation beclomethasone dipropionate was ball-milled in liquid paraffin and the resulting microcrystalline suspension incorporated into white soft paraffin. This shows the importance of correct formulation and manufacturing procedures.

Portnoy[40] has shown that an ointment prepared by first dissolving fluocinolone acetonide in propylene glycol and subsequent incorporation into the base was significantly more effective in the blanching assay than an ointment prepared by suspending microcrystalline fluocinolone acetonide in the base.

The blanching activities of diflorosone diacetate (0,05%) in a cream base containing varying amounts of propylene glycol (2,5-40%) were compared.[41] The results indicated that the cream containing 15% propylene glycol produced the greatest degree of blanching. At this concentration the corticosteroid exists as a saturated solution in the vehicle. With one exception, the differences were not significant. Under occlusion these differences became non significant with the exception of the cream which contained 25% propylene glycol. The 15% propylene glycol containing cream however, always produced the greatest amount of blanching. This serves to emphasize the need to employ unoccluded blanching studies since occlusion tends to mask any differences which are evident in the unoccluded mode of application.

A similar type of study[42] on fluclorolone acetonide creams and ointments using ^{14}C labelled steroid demonstrated enhanced drug release, and superior blanching activity, when the drug was entirely solubilized in the vehicle. Under or over solubilization decreased the rate of release from the ointment. This study also investigated the effect of different manufacturing procedures on the bioavailability. Two creams were prepared to be identical in formulation. In the first cream crystals of fluclorolone acetonide were dispersed in the placebo cream as the final step, whereas in the second cream the corticosteroid was dissolved in the cream during its manufacture. The second cream showed a better rate of release over the first. Thus, not only should formulational considerations be optimized, but so too should manufacturing procedures.

The use of *in vitro* studies[43] have shown that the optimal release of fluocinolone acetonide was obtained from vehicles containing the maximum concentration of propylene glycol required for complete solubilization of the corticosteroid. The poorest release rates were obtained with very high concentrations of propylene glycol. Similar results were obtained for fluocinolone acetonide acetate, but since this corticosteroid is less soluble, a higher concentration of propylene glycol was required to obtain the maximum release rate, and the poorest release rates were obtained with low concentrations of propylene glycol.

The effect of propylene glycol on the release rate of corticosteroids has been established by different methods - *in vivo*[39-41] using the blanching assay and *in vitro*[42-49] using ^{14}C labelled corticosteroids. Both techniques gave similar results.

In one of the earliest studies designed to investigate the role of vehicles in the percutaneous absorption of steroids Sarkany *et al*[50] found that there

was little difference in the blanching behaviour of betamethasone 17-valerate and fluocinolone acetonide in aqueous cream, oily cream or white soft paraffin, but that there was a marked difference in their blanching effect from Carbowax 1500. The area of pallor produced by betamethasone 17-valerate was about 3 times as large as the area of application, while the degree of pallor was similar to that produced from the other vehicles. Fluocinolone acetonide, on the other hand, failed to produce any significant blanching from Carbowax 1500. The above results served to add weight to the observations of Williams[51] that much clinical study by many investigators over many years is required in the selecting of bases for topical application. Furthermore there is no "universal" base for all topical corticosteroids. Each derivative requires individual formulation.

It is well recognized that the degree of hydration of the horny layer of the epidermis affects its permeability for steroids and their transport through the skin. In a trial[24] involving the use of both creams and ointments it was found that the ointments produced a better blanching of the skin than the corresponding creams containing the same steroid. The better response may be due to the high placebo effect of white soft paraffin,[52,53] but it may also be due to the occlusive nature of the ointment preparations tending to increase penetration in the same way as occlusive dressings. In the initial vasoconstrictor assay McKenzie and Stoughton[1] found that the use of occlusion led to a one hundred fold increase in absorption over the non-occluded mode.

Occlusion with plastic film provides the single most effective mechanism for increasing penetration. Maibach[54] has shown that hydrocortisone ($4\ \mu g/cm^2$) is absorbed in ten fold greater amounts with plastic occlusion compared to unoccluded. This difference could be clinically significant - many corticoid unresponsive dermatoses could become responsive due to this factor alone.

Sultzberger and Witten[55] showed that triamcinolone acetonide ointment under plastic occlusion was clinically as effective in obstinate psoriasis as an intralesional injection of steroid. They attributed the enhanced activity to better contact between the ointment and skin, more accurate localization of the ointment and increased percutaneous absorption as a result of epidermal maceration and increased skin temperature.

There is a real and direct relationship between temperature and skin permeability. However the practical importance of temperature effects in topical therapy is likely to be of minor importance. An occlusive covering of the skin will tend to increase the skin temperature by a few degrees by preventing evaporation of sweat and also by reducing loss of heat by radiation. The permeability change induced by this small increase in temperature is probably slight relative to that produced by the increased hydration of the stratum corneum.

Hydration results from water diffusing from underlying epidermal layers or from perspiration accumulating after application of an occlusive vehicle or covering on the surface. Under occlusive conditions the stratum corneum is changed from a tissue that normally contains very little water (5-15%) to one that may contain as much as 50% water and permeability increases in the order of four to five times.[56] Hydration apparently opens up the compact substance of the stratum corneum and not only increases the rate of percutaneous absorption, but also creates a depot effect in the stratum corneum.[57]

Approximately 1% of 4 $\mu g/cm^2$ hydrocortisone is absorbed from the normal forearm,[54] consequently attention has been focussed on methods of increasing the rate of absorption of topically applied drugs.

The enhancement of percutaneous absorption of many drugs from solutions containing more than 50% dimethylsulphoxide (DMSO) is a well known phenomenon,[58,59] and this increased penetration has been extensively studied for corticosteroids.[57,60-64]

DMSO non-selectively reduces the diffusional resistance of intact skin. The penetration enhancing effect of DMSO may relate to its own unusual and solvating characteristics. When DMSO is applied considerable swelling and distortion of the skin takes place, which would tend to open up its dense, compact structure and increase its permeability. In addition relatively high concentrations of DMSO are achieved in the stratum corneum and DMSO may in effect become the continuous membrane phase. Since most drugs are much more soluble in DMSO than in water it is possible that relatively high concentrations of the drug could be attained in the stratum corneum.

Following topical and oral administration of DMSO lens changes have been observed in the rat eye. DMSO application to human skin often produces a burning sensation and local irritation. In addition foul breath and taste accompany topical administration.

Tetrahydrofurfuryl alcohol (THFA), N,N-dimethylacetamide (DMA) and N,N-dimethylformamide (DMF) have also been used to produce permeability changes in the skin.

Sarkany et al[50] found that the addition of THFA and DMA to cream and ointment bases facilitated the absorption of hydrocortisone and the production of blanching was comparable to that produced by betamethasone 17-valerate and fluocinolone acetonide.

In another investigation[65] an ointment base containing DMA was chosen as the base for comparing the blanching response of hydrocortisone, triamcinolone

acetonide and fluocinolone acetonide with clinical efficacy. The DMA containing base was shown experimentally to be associated with more blanching than the other bases tested. Blanching ranking order correlated well with clinical efficacy.

The existence in the skin of a depot or reservoir for topical corticosteroids was suggested by Malkinson and Ferguson.[66] In experiments with topical corticosteroids Vickers[57] demonstrated that a reservoir of considerable capacity exists in the stratum corneum. In this work[57] small quantities of either triamcinolone acetonide or fluocinolone acetonide in 95% ethanol were applied to the surface of the skin and occluded with a non-permeable dressing. Blanching followed the removal of the occlusive dressing. The blanching faded in 10-16 hours. When the areas were reoccluded, blanching reappeared for as long as 14 days after the original application without the further application of more corticosteroid.

A series of stripping experiments[57] using the stripping technique described by Wolf[67] showed that a reservoir could not be established on skin stripped of stratum corneum before the application of steroid, and that a reservoir established on normal skin could be destroyed by stripping. The existence of the reservoir has also been shown for formulated products by Barry and Woodford when they compared the bioavailability of proprietary creams[27] and ointments.[28]

1.5 COMPARISON OF BLANCHING WITH CLINICAL RESULTS

Reid and Brookes[65] correlated the blanching activity of three widely used corticosteroid ointments; hydrocortisone (1%), fluocinolone acetonide (0,025%) and triamcinolone acetonide (0,025%), with their clinical efficacy in a group of patients with eczema. Clinically, they found that fluocinolone acetonide was slighter better than triamcinolone acetonide, which was in turn slightly better than

hydrocortisone. This data mirrored their blanching activity. In the above trial clinical efficacy and blanching activity were assessed using a common ointment base which allowed for good percutaneous absorption and which was also suitable for application to the diseased skin.

Hydrocortisone in a concentration of 0,1% was incorporated[38] into a two phase cream system and compared to 1% hydrocortisone cream (B.P.C.) using the human blanching bioassay. The new formulation produced blanching in 9 out of 10 patients, while with the official formulation only one blanching response out of 10 patients was observed. The clinical efficacy correlated well with the ability of the 0,1% concentration to produce blanching.

Diflorasone diacetate was found to be generally more potent than three high potency reference corticosteroid standards when the compounds were dissolved in 95% alcohol and ranked using the blanching test on healthy volunteers. In a double blind comparison in patients with dermatoses, 0,05% diflorasone diacetate was found to be as effective as 0,05% fluocinonide cream in the therapy of psoriasis.[41]

The strong degree of parallelism between blanching activity and clinical efficacy has been the subject of a number of papers,[31,36,68-75] and hence blanching activity would appear to be a quick and reliable method of screening new corticosteroid derivatives for activity, and for comparing existing formulations. The human blanching bioassay has the added advantages of using healthy skin and permitting the evaluation of numerous compounds simultaneously.

2. OTHER BIOASSAYS USED TO ASSESS TOPICAL CORTICOSTEROID POTENCY

The human blanching assay is not the only means of assessing the efficacy of topical corticosteroids. A number of biological assays are available to establish the relative activity of corticosteroids.

2.1 RAT THYMOLYTIC ANTI-INFLAMMATORY ACTIVITY

This assay involves the subcutaneous administration of the corticosteroid to rats or mice. The test substance is administered over a period of days. Twenty four hours after the last dose the animal is sacrificed, the thymus removed and weighed. The degree of involution caused by the steroid is determined as an indication of its potency.

In general, good agreement is shown between the ranking order obtained by the in vivo human blanching bioassay and that produced by the systemic involution assay.[17]

2.2 ANTIGRANULOMA ASSAY

Adrenolectomized rats are used in this assay.[76] Granulomas are induced by subcutaneous implantation of cotton pellets on either side of the thorax. The degree of granuloma inhibition achieved by a corticosteroid reflects its potency.

This assay, as well as the thymus involution assay measures systemic rather than topical activity. Systemic assays afford a preliminary indication of the possible value of a topical corticosteroid. As the potencies become enhanced the correlation between rat and man become less reliable.

2.3 FIBROBLAST ASSAY

The effects of anti-inflammatory corticosteroids on fibroblasts were demonstrated *in vivo* by Dougherty and Schneebeli,[77] who showed that physiological doses of cortisol caused morphological changes which consisted of the formation of globular forms of fibroblasts *in vivo*. The same morphological effects have been shown to occur when fibroblasts in tissue culture were treated with physiological amounts of anti-inflammatory corticosteroids. Although it is difficult to correlate this change in cell morphology with the effectiveness of corticosteroids as anti-inflammatory agents, it may be possible that this globular form could enable the fibroblast to resist the chain of reactions of cell destruction initiated by cytotoxic products liberated during inflammation. This permits the body's natural resources to clear up the inflammed area and repair the damaged tissue.

In spite of this difficult correlation there is good agreement between the ranking orders obtained by the fibroblast assay and the blanching assay for fluocinonide.[78]

2.4 RAT EAR ASSAY

A fixed volume of a mixture of pyridine, water, diethylether and croton oil, with or without the test substance is inucted into the ears of rats. After a period of time the ears are removed and uniform areas of the ear are punched out and weighed.

In this method each rat serves as its own control and the inhibition in the increase in weight of the treated ear is calculated.

There is a good correlation between the results obtained by this technique

and by using the blanching assay,[78] even though the assays were performed by different investigators.

The above techniques use animals to measure biological activity and do not take into account vehicle effects, as the pure corticosteroid is used in a solution in the investigations.

2.5 MITOSIS INHIBITION BIOASSAYS

The two actions most commonly responsible for the cutaneous therapeutic effects of corticosteroids are inhibition of epidermal mitosis and lysosome stabilization.

The antimitotic effect has been studied by various workers.[79-81] In the work performed by Fisher and Maibach[79] topically administered fluocinolone acetonide (0,025% cream) had no effect on mitotic activity, suggesting that this is not a sensitive screening procedure, as fluocinolone acetonide is considered to be a grade II or potent corticosteroid preparation.[82]

A reduction in skin thickness is thought to reflect antimitotic activity, and a correlation has been shown between thinning of the epidermis and reduction in mitotic activity,[83] and thus the thinning action of corticosteroids on the epidermis has been used as a screening test for these compounds.[83,84] The measurement of changes in skin thickness have demonstrated the activity of corticosteroids in very dilute concentrations. Blanching studies have also demonstrated the activity of these compounds in very dilute concentrations, and they too have shown similar logarithmic dose-response curves.

2.6 STANDARDIZED SKIN-SURFACE TRAUMA

The keratin layer may be removed from the skin surface by repeated stripping

with an adhesive tape. Corticosteroid preparations inhibit the vasodilation due to the erythema.

This method has been known for some twenty years, after being developed by Wells,[86] but only one topical steroid, halcinonide, has been investigated using this test.

The removal of the keratin scales increases the permeability of the epidermis and hence this method does not necessarily take into account vehicle effects. In addition the technique is painful to the subject, while weak or placebo preparations can cause distress to the volunteers.

2.7 INFLAMMATION SUPPRESSION STUDIES

2.7.a. Ultraviolet Erythema Suppression

This assay assesses corticosteroid activity at a different locus than blanching. This locus is presumably the lysosome membrane.

It partially corrects a criticism of the blanching test in that the influence of blanching *per se* on the diseased state is probably not clinically significant.

Each individual's minimum exposure dose (M.E.D.) to ultraviolet light must be determined. Exposure to over 3 M.E.D.'s is found to completely override the corticosteroid effect, which results in pain to the volunteer, while 2 or 3 M.E.D.'s results in less discrimination than 1 M.E.D. The time of application of the test medicament is critical.

Close correlation between the results of the blanching assay and U.V. erythema suppression assay have been shown.[87] Rankings obtained by these methods parallel clinical experience.[88]

2.7.b. Croton Oil Suppression[88,89]

The inherent variability of response among volunteers and the longer occlusion time make the croton oil suppression assay less sensitive than its counterpart, the ultraviolet suppression assay.

These suppression assays have the disadvantages of not being able to utilize placebo preparations, as painful uncomfortable blistering may arise. Compounds showing low potency may also cause discomfort to the volunteers.

2.8 WEAL REDUCTION BIOASSAY

Weals are induced in volunteers when the skin is pricked to a standard depth with a needle through a histamine solution.

This assay, like the blanching assay, is non-clinical. It would appear not to be as sensitive as other methods as its potency ratio is rather compressed. Beclomethasone dipropionate has a potency ratio of 23 relative to hydrocortisone, while betamethasone 17-valerate's potency ratio is 24,[90] compared to the potency ratio's of 5000 and 3600 respectively obtained from the blanching assay.[39]

This assay is claimed to possess close similarity to the process involved in the development of inflammed dermatoses.

Kaidbey and Kligman[91] have assayed cream and ointment formulations of potent steroids using the suppression of Rhus dermatitis as the criterion for assessment. Vesicular dermatitis is induced following patch application of Rhus oleoresin.

The Rhus assay is a severe test and hence weak and intermediate strength

corticosteroids should be avoided as they do not hasten the rate of regression of the dermatitis to any great extent. This assay is sufficiently sensitive enough to detect differences between regular and high strength preparations, as does the blanching assay.

2.9 SCHOLTZ AND DUMAS PSORIASIS ASSAY[92]

This uses psoriasis as the experimental model. The test is carried out on a human dermatosis which is one of the most important indications for the use of corticosteroids.

It requires that appropriate patients be available, only chronic, stabilized patients are used. Furthermore these patients must be selected by trained personnel. In the determination of the potency of fluclorolone acetonide the results obtained from the psoriasis assay and animal assays correlated well.[93]

It can be seen from the above that no one method fulfils the requirements of an ideal assay system and hence it is important to seek corraborative evidence from several assay systems before a clinical trial, which should form the final assessment, is undertaken.

The blanching assay is the determination which is most often used to assess the ranking order of formulated topical corticosteroid preparations.

Corticosteroids may be compared in terms of their onset of action, maximum response achieved and duration of action. The area under the curve (A.U.C.) values obtained from the graph of response plotted against time may be used to calculate bioavailability.

The effect of vehicle composition may be monitored using the blanching assay.

The rates of release of the same corticosteroid derivative in vehicles of differing composition may be assessed.

The blanching test is performed on healthy volunteers and causes little discomfort to them, hence permitting the evaluation of placebo and corticosteroids of weak or moderate potency.

3. ANALYTICAL METHODS

Quantitative assays were performed on the formulated fluocinolone acetonide preparations used in the blanching assays.

The two assay systems most often used for assessing purity of corticosteroids are the ultraviolet and colorimetric methods.

3.1 ULTRAVIOLET (U.V.) ASSAY

The ultraviolet assay is the method adopted by the United States Pharmacopoeia XIX[94] (U.S.P.). The extraction procedure is non-specific for fluocinolone acetonide and hence the extract must be passed through a column in order to eliminate those excipients which will contribute to the absorbance of the steroid. Accordingly the protocol is tedious and time consuming.

3.2 COLORIMETRIC ASSAY

The colorimetric assay is the method of analysis for corticosteroids in the British Pharmacopoeia (1974).[95] Tetrazolium salts in alkaline solution yield an orange/red colour, which obeys Beer-Lamberts Law in low enough concentrations. This reaction is widely used for the quantitative determination of corticosteroids. In this assay it is not essential to pretreat the extract by passing it through a silicaceous column, but the reaction requires a considerable length of time for the colour to develop. Colour formation is subject to many variables. Temperature, structural qualities of the corticosteroid, influence of oxygen, stability of the tetrazolium salt, presence of water and base concentration may all affect the reaction. It is essential, therefore,

to define the conditions under which the reaction will take place.

3.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (H.P.L.C.)

In recent years high performance liquid chromatography (H.P.L.C.) has been shown[96-102] to be a suitable assay method for the quantitative determination of formulated corticosteroids. This system has the advantage of having reduced analysis time and high sensitivity. Perhaps its main advantage lies in the fact that the corticosteroid and excipients from the formulation are separated out on the column. Correct choice of solvent system, column packing material and flow rate is necessary to obtain separation of the component peaks. The conditions for separation for cream formulations are more stringent than ointments, due to the inclusion of preservatives in the former.

B. EXPERIMENTAL

1. BLANCHING ASSAY

1.1 Formulated Corticosteroid Preparations

Proprietary formulated preparations were purchased from local pharmacies. Manufacturers samples were not used. The first gram of each tube was rejected, in case of any interaction between the closure and the formulation. Non-commercial formulations were prepared in bulk and stored in amber bottles at room temperature.

1.2 Volunteers

Only healthy male and female Caucasians who demonstrated a positive response to a standard preparation (Synalar ointment, occluded) were included in the investigation. None had received either topical or systemic corticosteroid therapy for at least six weeks before the studies.[13]

1.3 Mode of Application

The flexor aspects of the forearms were masked to produce twelve 7 mm square application sites. The squares were punched out of adhesive labels. For each volunteer both arms were used, one set of applications being occluded, using a non-porous plastic film (Blenderm^{R}), and the other left unoccluded, but protected with a cardboard frame in order to prevent spreading. Three 7 mm stripes, approximating to 5 mg, of each preparation were applied to the application sites in a random manner. The standard mass was extruded from a 1 ml disposable tuberculin syringe, the needle of which had been cut to 5 mm in order to facilitate the extrusion of the formulated product. The syringes were filled immediately prior to use so as to minimize any possible interaction between the corticosteroid

derivative and the plastic matrix of the syringe barrel. The syringes were discarded after use. The mass of preparation applied was determined by differential weighing. Seven millimeter stripes of product were extruded onto weighing paper and weighed. The average of 20 stripes was 1,71 mg. The lowest limit was 1,23 mg, while 2,43 mg was the upper limit.

1.4 Reading of Results

Six hours after application the maskings and frames were removed and the forearms washed with soap and warm water. The arms were evaluated independently using a double blind technique by 3 observers at various time intervals adequate to establish a blanching profile. There were no oblique light sources. Standard lighting by overhead fluorescent lamps was used. The averaged readings of all the observers were used to analyse the data.

1.5 Statistical Evaluation

Three methods were used to evaluate the results.[37]

Method 1. Number of sites exhibiting blanching.
This method involved a yes/no determination of whether or not blanching was evident at each application site. These data are reported in terms of total number of sites responding to a given formulation.

Method 2. Intensity of blanching.
A visual determination of the degree of blanching produced at each application site was made with this method. An arbitary response scale was defined as follows:

0 No blanching, application site appeared identical to surrounding area of skin.

1 Faint pallor observed, clear outline of application site was not seen.

2 Moderate blanching, square outline of application site was clearly visible.

3 Intense pallor was observed over the entire application site.

Method 3. Paired comparisons of adjacent sites.

This method involved the direct comparison of different formulations applied as pairs. For each pair the following decision was required: one site exhibited a greater degree of blanching, both sites showed equal blanching or blanching was not observed at either site.

The data obtained by Methods 1 and 3 (relative number of sites responding and paired comparisons, respectively) were analysed by χ^2 techniques. A 2x2 contingency table was examined employing Yates' correction factor for continuity in Method 1, whereas the χ^2 procedure of McNemar[103] was adopted for Method 3. The data obtained by Method 2 (relative intensity response) were tabulated for each product and application mode. Since a four point (0,1,2,3) intensity score was used a 2x4 contingency table was obtained for each of the product comparisons.

1.6 Calculation of Percent Total Possible Score (% T.P.S.)

Maximum score per site = 3. For 10 volunteers each having Z sites per arm and 3 independent observers, total possible score (T.P.S.) = 3 x 10 x 3 x Z

= 90Z

E.g. the 10 hr occluded mode score for preparation A was 203, calculated from the 4-point intensity score. It was applied to 3 sites per arm.

The T.P.S. = 90 x 3

= 270 and

$$\% \text{ T.P.S.} = \frac{203}{270} \times 100$$

= 75,2

1.7 Determination of Area Under the Curve (A.U.C.) Values

Area values are calculated by means of the trapezoidal rule. The parameters used are response (% T.P.S.) scores and time values (in hours) and hence the A.U.C. is reported in terms of % x hr.

In most cases a response was still evident at the terminal 28 hr. reading time, and hence it was necessary to calculate a correction factor.[104]

$$(A.U.C.)_{0-\infty} = (A.U.C.)_{0-28hr.} + (A.U.C.)_{28hr.-\infty}$$

and $$(A.U.C.)_{28hr.-\infty} = \frac{(R)_{28hr}}{k_e}$$

where (R) is the response at the 28 hr. reading time, and k_e is the elimination constant determined from the slope of the terminal (18-28 hr.) portion of the semi-log plot of % T.P.S. vs. time.

The area from the correction factor was added to the area obtained from the trapezoidal rule to yield a corrected A.U.C. value.

2. ANALYTICAL METHODS

2.1 COLOURIMETRIC ASSAY ON FLUOCINOLONE ACETONIDE (0,025%) CREAM AND OINTMENT FORMULATIONS

2.1.a. Reagents

(i) Cyclohexane: Reagent grade.

(ii) Methanol: Reagent grade.

(iii) Saturated Sodium Chloride Solution: Prepare 10 ml saturated sodium chloride solution.

(iv) Methanolic Sodium Chloride Solution: Transfer 20 ml of 10% sodium chloride solution to a 100 ml volumetric flask. Make up to volume with methanol and mix.

(v) Aluminium Potassium Sulphate Solution: Prepare a 1 in 10 solution of aluminium potassium sulphate in water to give 100 ml solution.

(vi) Chloroform: Reagent grade.

(vii) Anhydrous Sodium Sulphate: Reagent grade.

(viii) Absolute Ethanol: Freshly prepared by the following procedure. One litre of ethanol (95%) was refluxed for 4-6 hours over 25 g zinc dust and 25 g KOH. After standing overnight the absolute ethanol was collected by distillation.

(ix) Blue Tetrazolium Solution, 0,35%: Dissolve 175 mg of blue tetrazolium in 50 ml absolute ethanol by shaking. Prepare fresh solutions daily and protect from light.

(x) Tetramethylammonium Hydroxide Solution, 5% v/v: Dilute 2,0 ml of the 25% aqueous reagent to 10,0 ml with absolute ethanol. Prepare fresh solutions daily.

(xi) Glacial Acetic Acid: Reagent grade.

(xii) Fluocinolone Acetonide Standard Solution: Dissolve about 20 mg of fluocinolone acetonide (B.P. standard - Authentic Specimen) accurately weighed, in about 80 ml of absolute ethanol. Make up to 100,0 ml in a volumetric flask with additional absolute ethanol. Prepare fresh daily.

(xiii) Fluocinolone Acetonide Working Standard Solution: Transfer 5,0 ml of fluocinolone acetonide standard solution to a 50,0 ml volumetric flask. Make up to volume with absolute ethanol.

2.1.b. Procedure

The sample is either extruded, if a proprietary formulation, or mixed and then filled into a 10 ml syringe which has been previously accurately weighed. A quantity of sample, equivalent to about 2 mg of fluocinolone acetonide is extruded into a 250 ml separating funnel. The amount transferred is then

determined by accurately re-weighing the syringe. Fifty ml of cyclohexane, 25 ml of methanol and 1 ml saturated sodium chloride solution are then added to the separating funnel, if a cream formulation. In the case of an ointment 50 ml of cyclohexane, 25 ml methanol and 4 ml water are added to the separating funnel. The stoppered separating funnel is then shaken until the sample is dispersed (5 minutes). The layers are allowed to separate and the lower methanolic layer is transferred to a second 250 ml separating funnel. The cyclohexane layer remaining in the first separating funnel is extracted with 15 ml methanolic sodium chloride. The methanolic layer is added to the second separating funnel.

The combined methanolic extracts in the second separating funnel are diluted with 100 ml of aluminium potassium sulphate solution. The resulting solution is extracted with four 20 ml portions of chloroform, filtering each extract through 10 g anhydrous sodium sulphate. The sodium sulphate is finally washed through with 20 ml chloroform. The combined chloroform extracts are cautiously evaporated to dryness under reduced pressure.

The residue is taken up in exactly 50,0 ml absolute ethanol. Ten ml of this solution is transferred into a 25,0 ml volumetric flask. One (1,0) ml of a 0,35% blue tetrazolium solution and 1,0 ml of a 5% tetramethylammonium hydroxide solution are added. This solution is made up to volume with absolute ethanol, mixed, and allowed to stand for 1 hour at 30°C, protected from light. The solution is shaken intermittantly during this period. One (1,0) ml of glacial acetic acid is pipetted into the solution in the volumetric flask and mixed.

The absorbance of the solution was determined at 485 nm in 1 cm cells, using as a blank a solution prepared in exactly the same manner as above, but utilizing instead of the extract, 10,0 ml of absolute ethanol.

Ten ml of the fluocinolone acetonide working standard solution was transferred to a 25,0 ml volumetric flask and treated as above. The absorbance of this solution is likewise determined.

2.1.c. Calculation

$$\frac{Au}{As} \times \frac{Ps}{Pu} \times 10 = \% \text{ Fluocinolone Acetonide}$$

where:-

Au = absorbance of sample

Pu = weight in grams of sample

As = absorbance of standard solution

Ps = weight in grams of standard in 100 ml

2.2 ULTRAVIOLET ASSAY ON FLUOCINOLONE ACETONIDE (0,025%) CREAM AND OINTMENT FORMULATIONS

2.2.a. Reagents

(i) Cyclohexane: Reagent grade.

(ii) Methanol: Reagent grade.

(iii) Saturated Sodium Chloride Solution: Prepare 10 ml of saturated sodium chloride solution.

(iv) Methanolic Sodium Chloride: Transfer 20 ml of 10% sodium chloride solution to a 100 ml volumetric flask. Make up to volume with methanol and mix.

(v) Aluminium Potassium Sulphate Solution: Prepare a 1 in 10 solution of aluminium potassium sulphate in water to give 100 ml solution.

(vi) Chloroform: Reagent grade is used for extraction, otherwise spectroscopic grade when used to prepare mobile phase.

(vii) Anhydrous Sodium Sulphate: Reagent grade.

(viii) Absolute Ethanol: Freshly prepared by the following procedure. One litre of ethanol (95%) was refluxed for 4-6 hours over 25 g zinc dust and 25 g KOH. After standing overnight the absolute ethanol was collected by distillation.

(ix) Hexane: Redistilled reagent grade.

(x) Dioxane: Redistilled reagent grade.

(xi) Internal Phase: For each column used, 180 ml hexane, 20 ml chloroform, 80 ml dioxane and 10 ml water was shaken in a separating funnel. The lower layer is the internal phase.

(xii) Mobile Phase: The upper layer of the mixture of hexane, chloroform, dioxane and water is the mobile phase. Its suitability is determined as follows. Evaporate a 20,0 ml portion to dryness, on a vacuum pump. Dissolve the residue in 5,0 ml absolute ethanol, and determine the absorbance at 238 nm, using alcohol as the blank. This is suitable as the mobile phase if the absorbance is not more than 0,050.

(xiii) Silicaceous Earth: Acid washed Celite 545.

(xiv) Standard Preparation: Transfer approximately 10 mg of B.P. Fluocinolone Acetonide Authentic Specimen, accurately weighed to a 100 ml volumetric flask, dissolve in absolute ethanol, add absolute ethanol to volume and mix. Prepare fresh daily.

2.2.b. Chromatographic Columns

Fifteen grams of chromatographic silicaceous earth mixed with 10 ml of internal phase packed to a height of 18 to 19 cm in a glass chromatographic column, measuring about 50 cm x 1,9 cm, fitted with a glass scinter plate (number 2 porosity) and a glass tap at the bottom. Wash the column with 75 ml of mobile phase, leaving $\pm$ 1 mm of it above the top of the column bed.

2.2.c. Procedure

Weigh accurately a quantity of Fluocinolone acetonide cream, equivalent to about 2 mg of fluocinolone acetonide, by differential weighing, into a 250 ml separating funnel. Fifty ml of cyclohexane, 25 ml methanol and 1 ml saturated sodium chloride solution are then added to the separating funnel. In the case of the ointment preparation 50 ml of cyclohexane, 25 ml methanol and 4 ml water are added to the separating funnel. The stoppered separating funnel is then shaken until the sample is dispersed (5 min). The layers are allowed to separate and the lower methanolic layer is transferred to a second 250 ml separating funnel. The cyclohexane layer remaining in the first separating funnel is extracted with 15 ml methanolic sodium chloride. The methanolic layer is then added to the second separating funnel.

The combined methanolic extracts in the second separating funnel are diluted with 100 ml potassium aluminium sulphate solution. The resulting solution is extracted with four 20 ml portions of chloroform, filtering each extract through 10 g anhydrous sodium sulphate into a 100 ml volumetric flask. The sodium sulphate is finally washed through with 20 ml chloroform. The extract is made up to 100 ml with chloroform.

Transfer 25,0 ml of the extract and 5,0 ml of the standard preparation to separate round bottomed flasks and evaporate to dryness under reduced pressure. Dissolve the residues in 2 ml portions of internal phase by warming on a steam bath and mix with 3 g portions of chromatographic silicaceous earth. Add the two mixtures to separate chromatographic columns and elute the columns with mobile phase, discarding the first 100 ml of eluate, and collecting the second 100,0 ml. Pipette 20,0 ml of the eluates from the assay and standard preparations, respectively, into

100 ml round bottomed flasks, and cautiously evaporate to dryness under reduced pressure, heating if required. Dissolve the residues in 5,0 ml of absolute ethanol and determine the absorbances of the solutions in 1 cm cells at 238 nm, using absolute ethanol as the blank.

2.2.d. Calculation

$\frac{0,5\ C}{V}\left(\frac{Au}{As}\right)$ = quantity, in mg, of Fluocinolone Acetonide in the portion of preparation taken.

where:-

C = concentration in $\mu g/ml$ of the standard solution.

V = volume, in ml, of the assay preparation used.

Au = absorbance of the assay preparation.

As = absorbance of the standard preparation.

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ON FLUOCINOLONE ACETONIDE (0,025%) CREAM AND OINTMENT PREPARATIONS

2.3.a. Reagents

(i) Water: Deionized then distilled.

(ii) Methanol: Reagent grade.

(iii) Iso-octane: Reagent grade.

(iv) Toluene: Analytical reagent.

(v) Standard Preparation: Accurately weigh approximately 2,5 mg of Fluocinolone Acetonide Authentic Specimen (British Pharmacopoeia) and transfer to a 50 ml volumetric flask. Add 200 μl toluene, then make up to volume with methanol.

2.3.b. Conditions

(i) Solvent: Synalar cream and Cream B: 48% methanol, 52% water.

Synalar ointment and Ointments A and B: 59% methanol, 41% water

(ii) Flow rate: Cream B: 100 ml/hr.
Synalar cream: 60 ml/hr
Synalar ointment and Ointments A and B: 80 ml/hr.

(iii) Column: CH-10 MICROPAK (Varian).

(iv) Detector: 240 nm.

(v) Sensitivity: 0,0032 AUFS - i.e. Detector: 0,2
C.D.S.: 8
Recorder: 2 mV

2.3.c. Procedure

Accurately weigh approximately 10 g of formulation into a 50 ml separating funnel. The amount transferred is determined by differential weighing.

Add 15 ml of a 1:4 water:methanol mixture to the separating funnel if the formulation being extracted is an ointment. In the case of a cream 15 ml methanol is added.

The formulation is dispersed with the aid of gentle heat and agitation. Add 50 ml iso-octane to the separating funnel and shake. The lower methanolic layer is run off into a second separating funnel after the two phases have separated.

Fifteen ml methanol is added to the iso-octane layer remaining in the first separating funnel. The separating funnel is shaken, and on separation the methanolic layer is transferred to the second separating funnel. Gentle heat may be necessary to cause the layers to separate.

The bulked methanolic layers are allowed to stand for approximately 15

minutes. This achieves further separation of iso-octane. The lower methanolic layer is run off into a 50 ml volumetric flask. Toluene (200 μl) is added as an internal standard. The solution is made up to volume with methanol.

An aliquot (5 μl) of this solution is injected and the peak areas are determined by the instrument. This is followed by an equivalent injection volume of the standard solution. Peak area values are determined. The average area per cent for the fluocinolone acetonide and toluene peaks in both the sample and standard solutions are calculated.[97,100]

2.3.d. Calculation

$$\frac{\text{peak area \% sample}}{\text{peak area \% standard}} \times \frac{\text{peak area \% toluene in standard}}{\text{peak area of toluene in sample}} \times \frac{\text{wt. standard}}{\text{wt. sample}} \times 100$$

= % fluocinolone acetonide in sample

where:

wt. standard is in mg/50 ml

wt. sample is in mg/50 ml.

C. RESULTS

In the case of the yes/no and paired comparison forms of statistical treatment, χ^2 values greater than 3,84 are real differences, based on the 95% level of significance.

In the case of the intensity of blanching form of statistical treatment, χ^2 values greater than 7,82 are real differences, based on the 95% level of significance.

1. THE BLANCHING ASSAY

TABLE 1. TRIAL A.

VARIOUS % P.G. IN CREAMS.

UNOCCLUDED APPLICATION MODE.

No propylene glycol in cream base.

TIME(HOURS)

RESPONSE	7	8	10	12	14	16	18	24	28
0	23	21	15	14	10	16	14	40	42
1	31	33	33	29	27	23	28	14	12
2	0	0	6	10	11	12	10	0	0
3	0	0	0	1	6	3	2	0	0
TOTAL	31	33	45	52	67	56	54	14	12
T.P.S.	162	162	162	162	162	162	162	162	162
% T.P.S.	19,1	20,4	27,8	32,1	41,4	34,6	33,3	8,6	7,4

$7\frac{1}{2}$% Propylene glycol in cream base.

RESPONSE	7	8	10	12	14	16	18	24	28
0	21	12	6	3	1	1	5	29	38
1	33	41	39	33	33	36	34	25	16
2	0	1	9	18	15	11	12	0	0
3	0	0	0	0	5	6	3	0	0
TOTAL	33	43	57	69	78	76	67	25	16
T.P.S.	162	162	162	162	162	162	162	162	162
% T.P.S.	20,4	26,5	35,2	42,6	48,1	46,9	40,7	15,4	9,9

15% Propylene glycol in cream base.

RESPONSE	7	8	10	12	14	16	18	24	28
0	20	17	9	6	4	3	3	23	32
1	32	33	30	25	22	25	26	29	20
2	2	4	14	19	18	20	22	2	2
3	0	0	1	4	10	6	3	0	0
TOTAL	36	41	61	75	88	83	82	33	24
T.P.S.	162	162	162	162	162	162	162	162	162
% T.P.S.	22,2	25,3	37,7	46,3	54,3	51,2	50,6	20,4	13,6

TABLE 2. TRIAL A.

TABLE USED IN CALCULATING χ^2 VALUES FOR YES/NO STATISTICAL METHOD.

UNOCCLUDED APPLICATION MODE.

No Propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in cream.

TIME(HOURS)

	7	8	10	12	14	16	18	24	28
YES	31 33	33 42	39 48	40 51	44 53	38 53	40 49	14 25	12 16
NO	23 21	21 12	15 6	14 3	10 1	16 1	14 5	40 29	42 38
χ^2 VALUE	0,04	2,79	3,78	6,98	6,48	13,68	4,09	4,01	0,43

$7\frac{1}{2}$% Propylene glycol in cream vs. 15% propylene glycol in cream.

	7	8	10	12	14	16	18	24	28
YES	33 34	42 37	48 45	51 48	53 50	53 51	49 51	25 31	16 22
NO	21 20	12 17	6 9	3 6	1 4	1 3	5 3	29 23	38 32
χ^2 VALUE	0,00	0,75	0,31	0,49	0,84	0,26	0,14	0,93	1,02

TABLE 3. TRIAL A.

TABLE USED IN CALCULATING χ^2 VALUES FOR INTENSITY OF BLANCHING STATISTICAL METHOD.

UNOCCLUDED APPLICATION MODE.

No propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in cream.

TIME(HOURS)

	7	8	10	12	14	16	18	24	28
0	23 21	21 12	15 6	14 3	10 1	16 1	14 5	40 29	42 38
1	31 33	33 41	33 39	29 33	27 33	23 36	28 34	14 25	12 16
2	0 0	0 1	6 9	10 18	11 15	12 11	10 12	0 0	0 0
3	0 0	0 0	0 0	1 0	6 5	3 6	2 3	0 0	0 0
χ^2 VALUE	0,15	4,32	4,96	10,66	8,70	17,14	5,23	4,86	0,77

$7\frac{1}{2}$% propylene glycol in cream vs. 15% propylene glycol in cream.

	7	8	10	12	14	16	18	24	28
0	21 20	12 17	6 9	3 6	1 4	1 3	5 3	29 23	38 32
1	33 32	41 33	39 30	33 25	33 22	36 25	34 26	25 29	16 20
2	0 2	1 4	9 14	18 19	15 18	11 20	12 22	0 2	0 2
3	0 0	0 0	0 1	0 4	5 10	6 6	3 3	0 0	0 0
χ^2 VALUE	2,04	3,53	3,86	6,13	5,94	5,60	4,51	2,99	2,99

TABLE 4. TRIAL A.

TABLE USED IN CALCULATING χ^2 VALUES FOR PAIRED COMPARISON STATISTICAL METHOD.

UNOCCLUDED APPLICATION MODE.

No propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in cream.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
>	19	18	16	14	14	13	8	5	8
<	14	31	33	37	34	40	42	23	6
=	10	5	6	7	12	7	8	2	5
0	17	6	5	2	0	0	2	30	41
χ^2 VALUE	0,49	2,94	5,22	9,49	7,52	12,76	21,78	10,32	0,07

$7\frac{1}{2}$% propylene glycol in cream vs. 15% propylene glycol in cream.

	7	8	10	12	14	16	18	24	28
>	14	22	17	20	17	15	14	8	10
<	30	30	29	36	33	38	35	22	18
=	3	4	8	3	10	7	10	8	2
0	13	4	6	1	0	0	1	22	30
χ^2 VALUE	5,11	0,94	2,63	4,02	4,50	9,13	8,16	5,63	1,75

TABLE 5. TRIAL A.

OINTMENTS, UNOCCLUDED APPLICATION MODE, % T.P.S.

TIME(HOURS)

	7	8	10	12	14	16	18	24	28
No propylene glycol in ointment base (D)	13,0	13,6	18,5	28,4	32,1	27,8	25,0	7,4	5,0
7½% propylene glycol in ointment base (E)	21,6	25,3	36,4	38,3	48,1	46,3	43,2	14,8	13,0
15% propylene glycol in ointment base (F)	24,7	26,0	32,1	40,7	48,1	47,5	42,6	13,6	10,5

CREAMS, OCCLUDED APPLICATION MODE.

	7	8	10	12	14	16	18	24	28
No propylene glycol in cream base (A)	18,5	27,2	38,3	46,9	46,3	41,4	36,4	8,0	5,6
7½% propylene glycol in cream base (B)	29,0	32,1	43,2	51,2	51,2	49,4	41,4	11,1	5,6
15% propylene glycol in cream base (C)	30,9	33,3	42,6	43,2	56,8	52,5	43,8	14,8	4,9

..... Table 5 Continued/

TABLE 5 Continued/

OINTMENTS, OCCLUDED APPLICATION MODE.

TIME(HOURS)

	7	8	10	12	14	16	18	24	28
No propylene glycol in ointment base (D)	11,7	15,4	22,8	27,8	28,4	24,7	23,5	8,0	4,3
$7\frac{1}{2}$% propylene glycol in ointment base (E)	25,9	26,5	40,7	45,1	48,1	44,4	38,9	9,9	4,9
15% propylene glycol in ointment base (F)	21,0	25,9	40,7	44,4	46,3	49,4	40,7	10,5	5,6

TABLE 6. TRIAL A.

χ^2 RESULTS FOR VARIOUS % P.G. IN FORMULATIONS.

UNOCCLUDED APPLICATION MODE.

No propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in cream.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
YES/NO	0,04	2,79	3,78	6,98	6,48	13,68	4,09	4,01	0,43
INTENSITY OF BLANCHING	0,15	4,32	4,96	10,66	8,70	17,14	5,23	4,86	0,77
PAIRED COMPARISONS	0,49	2,94	5,22	9,49	7,52	12,76	21,78	10,32	0,07

$7\frac{1}{2}$% propylene glycol in cream vs. 15% propylene glycol in cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,00	0,75	0,31	0,49	0,84	0,26	0,14	0,93	1,02
INTENSITY OF BLANCHING	2,04	3,53	3,86	6,13	5,94	5,98	4,51	2,99	2,96
PAIRED COMPARISONS	5,11	0,94	2,63	4,02	4,50	9,13	8,16	5,63	1,75

No propylene glycol in ointment vs. $7\frac{1}{2}$% propylene glycol in ointment.

	7	8	10	12	14	16	18	24	28
YES/NO	5,33	8,37	11,26	6,12	18,28	10,38	19,75	5,37	5,83
INTENSITY OF BLANCHING	6,28	10,11	14,09	7,36	22,62	13,24	22,76	9,06	7,28
PAIRED COMPARISONS	4,22	10,02	16,56	14,79	10,86	28,31	26,32	5,26	11,52

..... Table 6 Continued/

TABLE 6 Continued/

$7\frac{1}{2}$% propylene glycol in ointment vs. 15% propylene glycol in ointment.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	0,65	0,00	0,19	0,09	0,13	0,09	0,99	0,00	1,07
INTENSITY OF BLANCHING	1,04	3,52	6,90	3,40	1,99	8,68	1,03	2,70	1,47
PAIRED COMPARISONS	1,76	1,11	1,48	3,38	5,02	4,68	6,35	0,76	0,05

$7\frac{1}{2}$% propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in ointment.

	7	8	10	12	14	16	18	24	28
YES/NO	3,89	0,75	1,66	0,99	0,26	3,37	0,13	0,15	0,37
INTENSITY OF BLANCHING	2,06	3,52	6,90	3,40	1,99	8,68	1,03	2,70	1,47
PAIRED COMPARISONS	3,18	0,00	1,84	0,35	0,08	0,00	0,00	0,76	5,26

TABLE 7. TRIAL A.

χ^2 RESULTS FOR VARIOUS % P.G. IN FORMULATIONS.

OCCLUDED APPLICATION MODE.

No propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in cream.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
YES/NO	3,93	0,00	0,00	0,09	0,66	0,60	0,31	0,72	0,07
INTENSITY OF BLANCHING	6,99	2,93	3,33	0,72	2,02	2,59	1,31	1,13	0,00
PAIRED COMPARISONS	20,89	4,17	7,20	11,76	8,89	2,88	3,20	0,17	0,00

$7\frac{1}{2}$% propylene glycol in cream vs. 15% propylene glycol in cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,05	0,05	0,00	0,09	0,49	0,09	0,09	0,69	0,00
INTENSITY OF BLANCHING	0,63	1,33	0,36	2,44	1,93	0,58	1,84	1,06	0,07
PAIRED COMPARISONS	0,20	0,02	1,84	0,02	0,31	0,02	0,09	0,15	0,08

No propylene glycol in ointment vs. $7\frac{1}{2}$% propylene glycol in ointment.

	7	8	10	12	14	16	18	24	28
YES/NO	12,00	5,38	6,09	10,85	9,24	7,25	11,17	0,19	0,00
INTENSITY OF BLANCHING	13,56	7,51	10,73	14,30	14,92	10,66	12,93	0,42	0,08
PAIRED COMPARISONS	26,26	21,84	23,67	36,98	27,38	37,53	23,67	1,44	2,12

..... Table 7 Continued/

TABLE 7 Continued/

$7\frac{1}{2}$% propylene glycol in ointment vs. 15% propylene glycol in ointment.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	1,39	0,00	0,05	0,00	0,08	0,00	0,07	0,00	0,00
INTENSITY OF BLANCHING	3,59	0,51	2,66	0,09	1,17	4,74	1,35	0,04	0,10
PAIRED COMPARISONS	0,03	1,84	0,03	0,08	0,54	0,03	1,64	0,45	0,90

$7\frac{1}{2}$% propylene glycol in cream vs. $7\frac{1}{2}$% propylene glycol in ointment.

	7	8	10	12	14	16	18	24	28
YES/NO	0,18	0,18	0,00	0,00	0,00	0,08	0,08	0,04	0,00
INTENSITY OF BLANCHING	0,58	2,23	0,19	2,61	0,39	6.64	0,44	0,17	0,07
PAIRED COMPARISONS	15,56	2,25	6,25	4,36	8,21	5,63	0,11	0,19	0,09

TABLE 8. TRIAL A.

A.U.C. VALUES FOR VARIOUS % P.G. CREAMS.

UNOCCLUDED APPLICATION MODE.

	Area	Relative to $7\frac{1}{2}$% cream
No propylene glycol	618,6	0,78
$7\frac{1}{2}$% propylene glycol	796,6	1,00
15% propylene glycol	940,9	1,18

CREAMS, OCCLUDED APPLICATION MODE.

	Area	Relative to $7\frac{1}{2}$% cream
No propylene glycol	687,3	0,84
$7\frac{1}{2}$% propylene glycol	814,4	1,00
15% propylene glycol	845,1	1,04

OINTMENTS, UNOCCLUDED APPLICATION MODE.

	Area	Relative to $7\frac{1}{2}$% ointment
No propylene glycol	464,1	0,55
$7\frac{1}{2}$% propylene glycol	843,6	1,00
15% propylene glycol	809,0	0,96

OINTMENTS, OCCLUDED APPLICATION MODE.

	Area	Relative to $7\frac{1}{2}$% ointment
No propylene glycol	445,2	0,60
$7\frac{1}{2}$% propylene glycol	738,5	1,00
15% propylene glycol	739,2	1,00

TABLE 9. TRIAL B.

% T.P.S. SYNALAR CREAM, SYNALAR OINTMENT, FLUOCINOLONE ACETONIDE CREAM A AND FLUOCINOLONE ACETONIDE OINTMENT A.

UNOCCLUDED APPLICATION MODE.

	TIME (HOURS)					
	8	10	12	14	24	32
SYNALAR CREAM	20	23	35	42	30	9
FLUOCINOLONE ACETONIDE CREAM	23	35	60	60	35	16
FLUOCINOLONE ACETONIDE CREAM BASE	5	0	7	0	7	2
FLUOCINOLONE ACETONIDE OINTMENT BASE	10	2	0	0	2	1
FLUOCINOLONE ACETONIDE OINTMENT	25	30	50	63	33	10
SYNALAR OINTMENT	30	33	58	67	33	13

% TPS SYNALAR CREAM, SYNALAR OINTMENT, FLUOCINOLONE ACETONIDE CREAM A, AND FLUOCINOLONE ACETONIDE OINTMENT A.

OCCLUDED APPLICATION MODE.

	8	10	12	14	24	32
SYNALAR CREAM	53	67	70	72	30	7
FLUOCINOLONE ACETONIDE CREAM	43	50	58	57	27	7
FLUOCINOLONE ACETONIDE CREAM BASE	7	5	2	2	2	0
FLUOCINOLONE ACETONIDE OINTMENT BASE	3	3	0	0	5	2
FLUOCINOLONE ACETONIDE OINTMENT	45	53	62	63	38	7
SYNALAR OINTMENT	38	43	60	60	27	4

TABLE 10. TRIAL B.

χ^2 VALUES.

UNOCCLUDED APPLICATION MODE.

Synalar cream vs. Fluocinolone acetonide cream A.

	TIME (HOURS)					
	8	10	12	14	24	32
YES/NO	0,00	8,02	2,50	0,53	0,53	0,00
INTENSITY OF BLANCHING	5,26	10,00	8,53	4,29	6,12	2,30

Synalar ointment vs. Fluocinolone acetonide ointment A.

	8	10	12	14	24	32
YES/NO	0,12	2,01	0,00	0,00	0,00	0,00
INTENSITY OF BLANCHING	0,58	4,00	3,75	0,20	0,36	0,36

OCCLUDED APPLICATION MODE.

Synalar cream vs. Fluocinolone acetonide cream A.

	8	10	12	14	24	32
YES/NO	0,00	0,00	0,00	0,00	0,00	0,00
INTENSITY OF BLANCHING	5,50	4,51	12,70	3,15	1,67	1,70

Synalar ointment vs. Fluocinolone acetonide ointment A.

	8	10	12	14	24	32
YES/NO	0,00	0,00	0,00	0,00	1,22	0,00
INTENSITY OF BLANCHING	1,18	0,93	5,20	3,80	3,80	0,78

Note: The trial design did not permit the paired comparison method of statistical evaluation.

TABLE 11. TRIAL B.

A.U.C. VALUES.

CREAMS UNOCCLUDED APPLICATION MODE.

	Area	Relative to cream A
SYNALAR CREAM	833,8	0,69
FLUOCINOLONE ACETONIDE CREAM A	1207,5	1,00
CREAM A BASE	122,8	0,10

CREAMS OCCLUDED APPLICATION MODE.

	Area	Relative to cream A
SYNALAR CREAM	1307,5	1,20
FLUOCINOLONE ACETONIDE CREAM A	1085,5	1,00
CREAM A BASE	78,0	0,07

OINTMENTS UNOCCLUDED MODE.

	Area	Relative to ointment A
SYNALAR OINTMENT	1192,6	1,12
FLUOCINOLONE ACETONIDE OINTMENT A	1067,0	1,00
OINTMENT A BASE	87,5	0,08

OINTMENTS OCCLUDED MODE.

	Area	Relative to ointment A
SYNALAR OINTMENT	1021,8	0,83
FLUOCINOLONE ACETONIDE OINTMENT A	1236,1	1,00
OINTMENT A BASE	90,5	0,07

TABLE 12. TRIAL C.

% T.P.S. SYNALAR CREAM, SYNALAR OINTMENT, FLUOCINOLONE ACETONIDE CREAM A AND FLUOCINOLONE ACETONIDE OINTMENT A.

UNOCCLUDED APPLICATION MODE.

	8	10	12	15	27
SYNALAR CREAM	2,1	14,6	18,8	16,7	16,7
FLUOCINOLONE ACETONIDE CREAM	2,2	16,1	25,0	32,2	26,1
SYNALAR OINTMENT	2,2	19,4	21,1	37,2	37,8
FLUOCINOLONE ACETONIDE OINTMENT	6,3	30,6	41,7	47,2	47,9

% T.P.S. SYNALAR CREAM, SYNALAR OINTMENT, FLUOCINOLONE ACETONIDE CREAM A AND FLUOCINOLONE ACETONIDE OINTMENT A.

OCCLUDED APPLICATION MODE.

	8	10	12	15	27
SYNALAR CREAM	10,0	18,3	25,0	26,1	17,8
FLUOCINOLONE ACETONIDE CREAM	16,0	29,2	37,5	38,9	19,4
SYNALAR OINTMENT	6,1	15,6	26,7	35,0	22,2
FLUOCINOLONE ACETONIDE OINTMENT	9,7	34,0	39,6	45,8	21,5

TABLE 13. TRIAL C.

χ^2 VALUES.

UNOCCLUDED APPLICATION MODE.

Synalar cream vs. Fluocinolone acetonide cream A.

TIME(HOURS)

	8	10	12	15	27
YES/NO	7,63	2,50	1,70	15,07	15,98
INTENSITY OF BLANCHING	0,08	6,01	5,33	25,07	20,43
PAIRED COMPARISONS	0,00	4,26	0,69	13,22	17,33

Synalar ointment vs. Fluocinolone acetonide ointment A.

	8	10	12	15	17
YES/NO	2,14	2,98	1,81	2,61	6,50
INTENSITY OF BLANCHING	3,25	6,93	2,91	3,29	8,25
PAIRED COMPARISONS	0,00	1,09	3,03	0,00	12,40

OCCLUDED APPLICATION MODE.

Synalar cream vs. Fluocinolone acetonide cream A.

	8	10	12	15	17
YES/NO	0,00	0,01	0,66	1,33	1,40
INTENSITY OF BLANCHING	3,86	6,22	2,13	7,70	1,52
PAIRED COMPARISONS	0,64	0,48	0,02	0,55	0,00

Synalar ointment vs. Fluocinolone acetonide ointment A.

	8	10	12	15	17
YES/NO	0,15	3,51	1,66	0,00	0,04
INTENSITY OF BLANCHING	3,05	8,71	1,42	2,16	2,17
PAIRED COMPARISONS	2,76	9,75	0,12	0,43	0,00

TABLE 14. TRIAL C.

A.U.C. VALUES.

CREAMS, UNOCCLUDED APPLICATION MODE.

	Area	Relative to Cream A
SYNALAR CREAM	321,3	0,91
FLUOCINOLONE ACETONIDE CREAM A	354,6	1,00

CREAMS, OCCLUDED APPLICATION MODE.

	Area	Relative to Cream A
SYNALAR CREAM	452,6	0,71
FLUOCINOLONE ACETONIDE CREAM A	641,0	1,00

OINTMENTS, UNOCCLUDED APPLICATION MODE.

	Area	Relative to Ointment A
SYNALAR OINTMENT	578,6	0,69
FLUOCINOLONE ACETONIDE OINTMENT A	840,5	1,00

OINTMENTS, OCCLUDED APPLICATION MODE.

	Area	Relative to Ointment A
SYNALAR OINTMENT	524,9	0,76
FLUOCINOLONE ACETONIDE OINTMENT A	689,1	1,00

TABLE 15. TRIAL D.

% T.P.S. SYNALAR CREAM, SYNALAR OINTMENT, FLUOCINOLONE ACETONIDE CREAM B, FLUOCINOLONE ACETONIDE OINTMENT B, CREAM B BASE AND OINTMENT B BASE.

UNOCCLUDED APPLICATION MODE.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
SYNALAR CREAM	12,8	14,8	17,3	18,1	19,3	14,8	11,5	7,0	4,5
FLUOCINOLONE ACETONIDE CREAM B	14,0	17,3	23,9	33,3	37,9	37,0	28,8	16,0	14,4
CREAM B BASE	4,9	2,5	0,0	1,2	1,2	0,0	0,0	0,0	0,0
SYNALAR OINTMENT	15,0	17,1	18,8	23,9	24,8	23,5	17,9	11,5	10,7
FLUOCINOLONE ACETONIDE OINTMENT B	19,8	24,7	28,4	31,3	35,4	31,7	24,3	17,3	15,6
OINTMENT B BASE	4,9	3,7	3,7	2,5	2,5	2,5	0,0	0,0	0,0

OCCLUDED APPLICATION MODE.

	7	8	10	12	14	16	18	24	28
SYNALAR CREAM	24,3	28,8	35,8	41,2	43,6	40,3	32,9	14,0	9,9
FLUOCINOLONE ACETONIDE CREAM B	30,5	33,7	38,7	47,7	49,8	44,9	33,3	16,9	12,3
CREAM B BASE	4,9	0,0	2,4	0,0	1,2	0,0	0,0	2,4	2,4
SYNALAR OINTMENT	21,0	28,8	29,6	40,3	41,8	39,9	31,7	16,0	16,5
FLUOCINOLONE ACETONIDE OINTMENT B	21,8	31,8	36,2	42,4	46,1	43,6	33,3	19,3	16,5
OINTMENT B BASE	4,9	4,9	4,9	6,1	4,9	3,7	6,1	1,2	0,0

TABLE 16. TRIAL D.

χ^2 VALUES.

UNOCCLUDED APPLICATION MODE.

Synalar ointment vs. Fluocinolone acetonide ointment B.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	1,07	3,28	3,90	2,79	9,25	3,52	3,27	3,53	3,07
INTENSITY OF BLANCHING	4,73	8,54	7,66	4,31	11,31	5,77	4,21	4,80	3,67
PAIRED COMPARISONS	3,18	10,58	16,36	6,45	7,35	9,45	12,80	1,44	5,28

Synalar cream vs. Fluocinolone acetonide cream B.

	7	8	10	12	14	16	18	24	28
YES/NO	0,03	0,10	1,59	6,58	18,08	34,03	28,82	12,04	16,06
INTENSITY OF BLANCHING	0,39	1,38	4,90	18,56	25,55	42,29	32,03	13,21	17,49
PAIRED COMPARISONS	0,24	5,11	8,80	16,02	28,02	43,89	25,33	20,48	32,03

Fluocinolone acetonide ointment B vs. Ointment B base.

	7	8	10	12	14	16	18	24	28
YES/NO	11,25	22,90	24,03	31,25	50,99	37,18	32,12	19,93	17,54
INTENSITY OF BLANCHING	12,96	25,18	26,51	33,94	54,48	48,52	34,69	22,03	19,54
PAIRED COMPARISONS	7,56	18,05	15,43	15,43	22,04	16,41	18,05	15,06	17,05

..... Table 16 Continued/

Table 16 Continued/

Fluocinolone acetonide cream B vs. Cream B base.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
YES/NO	4,50	11,25	26,45	33,57	55,43	68,06	45,44	18,31	15,34
INTENSITY OF BLANCHING	5,69	12,94	28,80	36,25	57,24	72,00	48,52	20,35	17,26
PAIRED COMPARISONS	1,45	2,72	16,06	19,05	20,05	23,04	21,04	13,07	11,08

TABLE 17. TRIAL D.

χ^2 VALUES.

OCCLUDED APPLICATION MODE.

Synalar ointment vs. Fluocinolone acetonide ointment B.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	0,10	0,30	3,89	0,05	0,81	1,43	0,05	1,21	0,0
INTENSITY OF BLANCHING	1,82	0,77	4,86	0,47	11,30	7,96	0,87	1,60	1,06
PAIRED COMPARISONS	0,00	1,25	7,88	0,06	0,38	4,06	3,52	0,96	0,83

Synalar cream vs. Fluocinolone acetonide cream B.

	7	8	10	12	14	16	18	24	28
YES/NO	2,69	0,15	0,20	0,70	0,09	0,09	0,00	0,89	0,69
INTENSITY OF BLANCHING	5,00	3,85	4,56	3,60	4,01	2,05	0,38	1,22	1,00
PAIRED COMPARISONS	10,26	8,16	1,64	6,68	1,13	1,73	3,41	2,53	4,32

Fluocinolone acetonide ointment B vs. Ointment B base.

	7	8	10	12	14	16	18	24	28
YES/NO	14,29	31,47	36,60	36,65	65,33	59,86	36,65	21,11	19,11
INTENSITY OF BLANCHING	16,19	34,42	39,95	40,71	70,07	64,00	39,98	23,22	21,18
PAIRED COMPARISONS	14,06	15,04	19,05	18,05	23,04	20,35	16,96	7,69	10,08

.....Table 17 Continued/

Table 17 Continued/

Fluocinolone acetonide cream B vs. Cream B base.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
YES/NO	36,60	51,12	48,72	81,06	72,53	81,06	68,06	14,03	7,16
INTENSITY OF BLANCHING	39,62	54,41	52,18	85,50	76,79	85,50	72,00	15,78	8,53
PAIRED COMPARISONS	14,06	18,05	17,39	17,39	18,38	22,04	19,36	5,81	6,13

TABLE 18. TRIAL D.

A.U.C. VALUES.

UNOCCLUDED APPLICATION MODE, FLUOCINOLONE ACETONIDE CREAMS.

	Area	Relative to Cream B
SYNALAR	350,4	0,45
CREAM B	778,0	1,00
CREAM B BASE	28,2	0,04

OCCLUDED APPLICATION MODE, FLUOCINOLONE ACETONIDE CREAMS.

	Area	Relative to Cream B
SYNALAR	766,1	0,85
CREAM B	896,6	1,00
CREAM B BASE	43,6	0,05

UNOCCLUDED APPLICATION MODE, FLUOCINOLONE ACETONIDE OINTMENTS.

	Area	Relative to Ointment B
SYNALAR	626,2	0,66
OINTMENT B	946,8	1,00
OINTMENT B BASE	47,6	0,05

OCCLUDED APPLICATION MODE, FLUOCINOLONE ACETONIDE OINTMENTS.

	Area	Relative to Ointment B
SYNALAR	905,2	0,92
OINTMENT B	986,8	1,00
OINTMENT B BASE	96,6	0,10

TABLE 19. TRIAL E.

% T.P.S.

UNOCCLUDED APPLICATION MODE.

Triamcinolone acetonide creams.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
ARISTOCORT HP	0,00	2,0	11,1	12,1	13,1	10,1	6,0	5,0	4,0
ARISTOCORT RP	0,8	3,4	11,1	11,9	14,5	13,6	9,4	9,4	3,4
ARISTOCORT LP	3,0	7,1	11,1	11,1	17,1	6,1	3,0	2,0	2,0
LEDERCORT	2,7	7,4	13,8	15,7	18,5	12,9	9,2	7,4	3,7
SYNALAR	5,1	14,5	17,9	20,5	27,3	22,2	17,0	14,5	10,0

OCCLUDED APPLICATION MODE.

Triamcinolone acetonide creams.

	7	8	10	12	14	16	18	24	28
ARISTOCORT HP	25,0	43,5	50,9	55,6	52,8	36,1	28,7	23,1	15,7
ARISTOCORT RP	30,3	35,4	42,4	45,5	43,4	29,3	22,2	17,2	6,1
ARISTOCORT LP	25,6	34,2	40,2	38,5	34,2	21,4	12,8	10,3	5,1
LEDERCORT	31,3	38,4	47,5	45,5	42,4	27,3	19,2	12,1	10,1
SYNALAR	29,9	42,7	51,3	53,8	53,0	39,3	30,8	26,5	12,0

TABLE 20. TRIAL E.

χ^2 VALUES.

UNOCCLUDED APPLICATION MODE. TRIAMCINOLONE ACETONIDE CREAMS.

Synalar cream vs. Aristocort H.P. cream.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	3,71	11,10	1,67	2,81	4,45	2,43	2,64	4,61	6,07
INTENSITY OF BLANCHING	5,54	12,96	2,87	4,15	7,14	6,59	5,72	6,03	7,61
PAIRED COMPARISONS	1,33	2,29	0,75	8,64	13,07	9,09	5,82	8,64	7,11

Synalar cream vs. Aristocort R.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	2,51	9,38	1,89	4,18	3,29	0,46	0,52	0,91	8,14
INTENSITY OF BLANCHING	3,92	11,07	3,21	5,32	6,96	6,51	5,36	2,11	9,85
PAIRED COMPARISONS	3,13	10,32	10,32	13,14	18,38	16,06	14,06	9,33	16,06

Synalar cream vs. Aristocort L.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,20	3,08	1,67	3,74	1,48	7,12	6,73	9,87	9,87
INTENSITY OF BLANCHING	0,65	4,03	2,87	5,15	4,60	10,16	9,00	11,71	11,71
PAIRED COMPARISONS	0,80	4,27	3,50	5,88	13,47	11,08	8,10	5,82	7,63

..... Table 20 Continued/

Table 20 Continued/

Synalar cream vs. Ledercort cream.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	0,34	2,95	1,19	1,05	2,18	1,79	1,00	2,24	7,11
INTENSITY OF BLANCHING	0,88	3,85	2,73	1,65	5,53	3,13	2,96	3,51	8,74
PAIRED COMPARISONS	0,13	1,23	0,56	1,90	4,05	5,06	10,56	5,79	8,10

Aristocort L.P. cream vs. Aristocort R.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,47	0,92	0,06	0,06	0,16	3,39	3,04	4,52	0,05
INTENSITY OF BLANCHING	1,45	1,66	0,00	0,88	0,40	4,40	4,17	5,92	0,41
PAIRED COMPARISONS	1,33	2,29	2,13	4,17	7,11	0,00	0,50	0,00	0,00

Aristocort L.P. cream vs. Aristocort H.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	1,40	2,06	0,07	0,00	0,56	0,74	0,58	0,64	0,18
INTENSITY OF BLANCHING	3,14	3,22	0,00	0,07	1,04	1,32	1,16	1,44	0,73
PAIRED COMPARISONS	0,50	3,20	0,00	0,17	0,00	0,50	0,00	0,25	0,00

..... Table 20 Continued/

Table 20 Continued/

Aristocort **R.P.** cream vs. Aristocort H.P. cream.

TIME (HOURS)

	7	8	10	12	14	16	18	24	28
YES/NO	0,07	0,05	0,06	0,00	0,03	0,49	0,52	1,09	0,02
INTENSITY OF BLANCHING	0,86	0,41	0,00	1,04	0,21	0,89	1,00	1,76	0,06
PAIRED COMPARISONS*									

Aristocort cream L.P. vs. Ledercort cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,10	0,04	0,00	0,48	0,01	1,34	2,03	2,44	0,10
INTENSITY OF BLANCHING	0,01	0,01	1,90	1,58	1,59	3,05	3,31	3,63	0,55
PAIRED COMPARISONS	0,50	1,13	0,13	2,29	4,08	4,00	6,13	3,13	0,25

*The design of the trial did not permit the paired comparison form of statistical treatment for Aristocort R.P. cream vs. Aristocort H.P. cream.

TABLE 21. TRIAL E.

χ^2 VALUES FOR TRIAMCINOLONE ACETONIDE CREAMS.

OCCLUDED APPLICATION MODE.

Synalar cream vs. Aristocort H.P. cream.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	1,50	0,52	0,01	0,19	0,09	0,59	0,03	0,22	0,58
INTENSITY OF BLANCHING	6,14	4,40	0,43	0,60	3,00	2,52	1,63	1,38	0,99
PAIRED COMPARISONS	5,26	1,04	2,45	1,04	1,57	2,56	5,26	0,04	0,24

Synalar cream vs. Aristocort R.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,18	0,02	0,39	0,04	0,41	1,55	0,24	4,03	1,98
INTENSITY OF BLANCHING	1,47	1,32	1,84	2,07	3,49	3,72	5,02	5,63	2,80
PAIRED COMPARISONS	0,84	1,44	0,59	1,75	0,64	0,27	1,56	3,77	3,13

Synalar cream vs. Aristocort L.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,46	1,51	2,18	3,81	7,24	11,54	7,46	14,91	3,30
INTENSITY OF BLANCHING	1,39	2,17	5,87	6,32	13,04	13,78	13,54	17,05	4,30
PAIRED COMPARISONS	4,50	4,76	13,14	11,17	17,63	20,83	16,41	15,04	15,06

..... Table 21 Continued/

Table 21 Continued/

Synalar cream vs. Ledercort cream.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	0,49	0,02	0,07	1,06	2,89	3,25	1,03	10,49	0,06
INTENSITY OF BLANCHING	1,79	0,65	0,71	2,28	4,53	5,35	7,94	12,44	0,25
PAIRED COMPARISONS	5,26	3,37	0,06	1,14	5,88	4,05	8,45	3,06	0,00

Aristocort L.T. cream vs. Aristocort R.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	0,21	2,93	0,23	1,61	9,60	3,34	3,58	2,39	0,00
INTENSITY OF BLANCHING	3,55	2,08	4,33	2,94	7,51	4,92	5,25	3,20	0,10
PAIRED COMPARISONS	8,64	2,77	10,56	15,43	16,41	14,45	9,09	2,77	0,50

Aristocort L.P. cream vs. Aristocort H.P. cream.

	7	8	10	12	14	16	18	24	28
YES/NO	1,66	4,59	2,80	3,30	13,01	5,57	8,71	9,71	7,49
INTENSITY OF BLANCHING	8,41	8,90	8,11	6,95	10,13	7,04	12,40	11,20	8,92
PAIRED COMPARISONS	8,47	3,05	10,32	12,19	16,41	16,41	18,05	18,05	9,60

..... Table 21 Continued/

Table 21 Continued/

Aristocort R.P. Cream vs. Aristocort H.P. cream.

	TIME (HOURS)								
	7	8	10	12	14	16	18	24	28
YES/NO	0,25	1,17	0,73	0,01	0,02	0,04	0,58	1,63	5,29
INTENSITY OF BLANCHING	2,20	3,72	1,83	2,38	2,09	2,29	2,31	2,32	6,53
PAIRED COMPARISONS*									

Aristocort L.P. cream vs. Ledercort cream.

	7	8	10	12	14	16	18	24	28
YES/NO	2,43	0,57	0,64	0,30	1,76	1,67	1,91	0,06	1,52
INTENSITY OF BLANCHING	5,51	1,45	2,35	1,02	2,57	2,76	2,62	0,25	2,30
PAIRED COMPARISONS	3,27	4,92	6,67	4,27	14,47	6,75	5,82	0,10	0,80

*The design of the trial did not permit the paired comparison form of statistical treatment for Aristocort R.P. cream vs. Aristocort H.P. cream.

TABLE 22. TRIAL E.

A.U.C. VALUES FOR TRIAMCINOLONE ACETONIDE CREAMS.

UNOCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
ARISTOCORT H.P.	251,85	0,48
ARISTOCORT R.P.	235,33	0,45
ARISTOCORT L.P.	188,78	0,36
LEDERCORT	265,52	0,50
SYNALAR	526,85	1,00

A.U.C. VALUES FOR TRIAMCINOLONE ACETONIDE CREAMS.

OCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
ARISTOCORT H.P.	998,98	1,01
ARISTOCORT R.P.	750,92	0,76
ARISTOCORT L.P.	590,62	0,60
LEDERCORT	822,92	0,84
SYNALAR	985,46	1,00

TABLE 23. TRIAL F.

% T.P.S. CREAMS.

UNOCCLUDED APPLICATION MODE.

	TIME (HOURS)						
	7	8	10	12	14	24	26
BETNOVATE	40,0	50,2	61,9	69,3	61,1	27,4	17,0
CELESTODERM-V	35,2	47,4	58,0	67,9	58,0	25,9	13,6
TOPILAR	25,0	44,4	52,8	63,9	58,3	29,6	13,9
SYNALAR	13,9	25,6	32,4	54,6	55,6	29,6	19,4

% T.P.S. CREAMS.

OCCLUDED APPLICATION MODE.

	7	8	10	12	14	24	26
BETNOVATE	59,3	67,6	76,7	68,9	59,3	19,6	5,9
CELESTODERM-V	58,0	70,4	74,1	67,9	59,9	18,5	5,6
TOPILAR	33,3	58,9	64,8	63,9	56,5	14,8	5,5
SYNALAR	41,7	43,3	55,6	54,6	49,1	21,3	10,2

% T.P.S. OINTMENTS.

UNOCCLUDED APPLICATION MODE.

	7	9	$11\frac{1}{2}$	13	$14\frac{1}{2}$	16	25	28
BETNOVATE	12,0	22,7	33,3	38,4	38,4	31,0	6,0	1,4
CELESTODERM-V	25,0	34,0	49,3	45,8	44,4	31,9	11,8	5,2
TOPILAR	48,0	52,8	63,9	54,2	45,8	36,1	7,0	2,1
SYNALAR	14,4	21,8	36,0	36,1	36,0	31,0	13,9	9,0

% T.P.S. OINTMENTS.

OCCLUDED APPLICATION MODE.

	7	9	$11\frac{1}{2}$	13	$14\frac{1}{2}$	16	25	28
BETNOVATE	24,1	37,5	54,2	49,5	49,4	43,5	7,4	2,8
CELESTODERM-V	37,5	49,3	59,0	53,8	44,4	43,1	10,4	3,1
TOPILAR	58,0	64,6	72,9	59,7	45,8	44,4	3,5	0,0
SYNALAR	25,9	36,1	48,6	46,0	44,4	39,8	13,0	4,9

TABLE 24. TRIAL F.

χ^2 VALUES: CREAMS.

UNOCCLUDED APPLICATION MODE.

Synalar cream vs. Betnovate cream.

	TIME (HOURS)						
	7	8	10	12	14	24	26
YES/NO	20,38	3,70	14,32	3,72	2,18	0,05	0,18
INTENSITY OF BLANCHING	23,07	15,59	24,47	11,27	5,07	0,31	0,40
PAIRED COMPARISONS	21,04	14,09	18,58	14,81	2,72	0,06	0,13

Synalar cream vs. Celestoderm-V cream.

	7	8	10	12	14	24	26
YES/NO	21,47	3,08	16,06	7,15	3,28	0,18	1,59
INTENSITY OF BLANCHING	23,70	12,75	23,03	11,93	7,90	0,74	2,18
PAIRED COMPARISONS	12,04	15,43	17,93	5,33	0,41	1,89	8,64

Synalar cream vs. Topilar cream.

	7	8	10	12	14	24	26
YES/NO	3,62	4,01	8,10	4,55	1,64	0,00	1,41
INTENSITY OF BLANCHING	4,59	9,45	11,80	9,14	10,12	1,19	2,19
PAIRED COMPARISONS	4,92	12,50	13,14	4,76	2,04	0,00	6,13

..... Table 24 Continued/

Table 24 continued/

Celestoderm-V cream vs. Betnovate cream.

	TIME (HOURS)						
	7	8	10	12	14	24	26
YES/NO	0,38	0,02	1,10	1,10	0,00	0,02	0,91
INTENSITY OF BLANCHING	7,60	0,94	8,56	3,95	2,10	0,19	1,28
PAIRED COMPARISONS	3,03	0,77	0,02	0,02	1,56	3,23	5,88

TABLE 25. TRIAL F.

χ^2 VALUES: CREAMS.

OCCLUDED APPLICATION MODE.

Synalar cream vs. Betnovate cream.

	TIME (HOURS)						
	7	8	10	12	14	24	26
YES/NO	1,35	0,21	2,18	4,38	2,27	0,04	1,79
INTENSITY OF BLANCHING	8,80	15,53	9,14	13,76	5,44	1,52	2,49
PAIRED COMPARISONS	13,88	16,41	19,36	16,69	16,41	1,77	0,57

Synalar cream vs. Celestoderm-V cream.

	7	8	10	12	14	24	26
YES/NO	5,36	0,04	5,51	3,94	3,94	0,55	1,67
INTENSITY OF BLANCHING	8,46	6,52	11,56	8,94	8,26	1,75	2,41
PAIRED COMPARISONS	20,35	12,19	16,96	12,00	14,81	5,88	0,00

Synalar cream vs. Topilar cream.

	7	8	10	12	14	24	26
YES/NO	0,00	0,00	3,44	2,38	2,38	2,01	1,23
INTENSITY OF BLANCHING	3,27	1,35	5,44	4,74	6,99	2,74	1,93
PAIRED COMPARISONS	0,21	3,04	5,50	4,32	0,84	7,69	6,13

..... Table 25 Continued/

Table 25 continued/

Celestoderm-V cream vs. Betnovate cream.

	TIME (HOURS)						
	7	8	10	12	14	24	26
YES/NO	0,00	0,00	1,10	0,07	0,11	0,03	0,00
INTENSITY OF BLANCHING	5,80	1,69	9,24	2,31	3,08	0,13	2,90
PAIRED COMPARISONS	4,69	1,17	7,20	0,63	0,24	1,63	0,27

TABLE 26. TRIAL F.

χ^2 VALUES: OINTMENTS.

UNOCCLUDED APPLICATION MODE.

Synalar ointment vs. Betnovate ointment.

	TIME (HOURS)							
	7	9	11½	13	14½	16	25	28
YES/NO	0,00	0,03	2,17	1,11	0,77	0,00	1,42	6,77
INTENSITY OF BLANCHING	5,53	0,13	3,13	2,51	1,32	1,66	9,00	8,45
PAIRED COMPARISONS	0,00	1,83	9,30	6,61	0,36	0,10	17,39	8,10

Synalar ointment vs. Celestoderm-V ointment.

	7	9	11½	13	14½	16	25	28
YES/NO	3,96	5,35	5,77	2,53	2,50	0,00	0,12	0,53
INTENSITY OF BLANCHING	6,21	8,47	8,69	7,70	4,08	0,01	0,87	1,39
PAIRED COMPARISONS	12,12	13,23	36,69	8,51	6,56	0,20	1,71	5,14

Synalar ointment vs. Topilar ointment.

	7	9	11½	13	14½	16	25	28
YES/NO	47,06	28,80	12,22	8,85	7,21	0,87	4,12	3,47
INTENSITY OF BLANCHING	69,65	37,38	30,67	20,07	8,89	1,98	5,24	4,77
PAIRED COMPARISONS	65,01	54,39	58,38	37,79	10,87	1,31	4,65	5,14

..... Table 26 Continued/

Table 26 continued/

Celestoderm-V ointment vs. Betnovate ointment.

	TIME (HOURS)							
	7	9	11½	13	14½	16	25	28
YES/NO	3,96	4,09	1,04	0,24	0,41	0,02	3,75	1,89
INTENSITY OF BLANCHING	14,18	7,24	2,39	3,53	2,20	1,29	4,63	3,16
PAIRED COMPARISONS	7,04	17,93	17,63	8,83	4,97	6,76	3,76	0,17

TABLE 27. TRIAL F.

χ^2 VALUES: OINTMENTS.

OCCLUDED APPLICATION MODE.

Synalar ointment vs. Betnovate ointment.

	TIME (HOURS)							
	7	9	11½	13	14½	16	25	28
YES/NO	0,03	0,00	1,07	5,14	0,48	0,45	3,96	15,24
INTENSITY OF BLANCHING	1,09	1,77	2,04	6,51	1,72	2,69	4,71	2,65
PAIRED COMPARISONS	0,12	2,38	9,76	10,32	4,97	0,52	6,72	1,33

Synalar ointment vs. Celestoderm-V ointment.

	7	9	11½	13	14½	16	25	28
YES/NO	3,34	0,84	2,62	4,57	0,06	0,36	0,44	10,24
INTENSITY OF BLANCHING	10,31	6,74	4,69	6,61	0,84	1,82	0,73	1,66
PAIRED COMPARISONS	7,53	17,33	17,31	0,13	0,00	1,09	1,79	3,20

Synalar ointment vs. Topilar ointment.

	7	9	11½	13	14½	16	25	28
YES/NO	23,67	11,45	6,34	8,76	0,32	0,19	10,33	26,99
INTENSITY OF BLANCHING	50,16	24,87	20,31	12,46	0,92	2,81	11,71	6,62
PAIRED COMPARISONS	61,13	57,02	52,02	24,60	5,33	3,89	14,09	3,20

..... Table 27 Continued/

Table 27 continued/

Celestoderm-V ointment vs. Betnovate ointment.

	TIME (HOURS)							
	7	9	11½	13	14½	16	25	28
YES/NO	0,33	1,16	0,27	0,01	0,00	0,00	0,80	0,06
INTENSITY OF BLANCHING	12,89	4,27	1,15	1,38	0,15	0,54	1,23	1,50
PAIRED COMPARISONS	12,00	11,12	8,26	3,70	1,80	12,04	3,50	0,50

TABLE 28. TRIAL F.

A.U.C. VALUES: CREAMS.

UNOCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
BETNOVATE	1205,16	1,18
CELESTODERM-V	1093,22	1,07
TOPILAR	1057,64	1,04
SYNALAR	1019,70	1,00

A.U.C. VALUES: CREAMS.

OCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
BETNOVATE	1139,78	1,18
CELESTODERM-V	1125,96	1,17
TOPILAR	940,58	0,98
SYNALAR	962,64	1,00

A.U.C. VALUES: OINTMENTS.

UNOCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
BETNOVATE	493,14	0,77
CELESTODERM-V	703,38	1,10
TOPILAR	856,14	1,34
SYNALAR	640,82	1,00

A.U.C. VALUES: OINTMENTS.

OCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
BETNOVATE	738,80	0,98
CELESTODERM-V	862,69	1,15
TOPILAR	964,50	1,28
SYNALAR	752,95	1,00

TABLE 29. TRIAL G.

% T.P.S. DIFLUCORTOLONE VALERATE CREAMS.

UNOCCLUDED APPLICATION MODE.

TIME (HOURS)

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
TEMETEX CREAM	21,1	24,4	31,9	30,0	29,3	24,4	10,4	1,5	0,7
NERISONE CREAM	23,7	28,1	33,7	33,0	30,4	26,0	16,3	4,1	1,5
SYNALAR CREAM	21,9	18,5	43,3	44,1	47,4	41,4	34,4	9,6	5,9
NERISONE OINTMENT	39,3	45,9	53,0	48,9	42,2	37,4	19,6	4,8	3,3

% T.P.S. DIFLUCORTOLONE VALERATE CREAMS.

OCCLUDED APPLICATION MODE.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
TEMETEX CREAM	52,2	64,8	68,6	58,1	49,3	37,4	19,6	2,6	1,0
NERISONE CREAM	47,4	64,1	69,6	58,9	47,4	40,4	25,2	4,8	2,6
SYNALAR CREAM	38,5	53,3	62,9	63,3	64,4	56,7	43,3	13,3	7,0
NERISONE OINTMENT	47,0	60,0	65,6	51,5	44,4	36,3	20,7	4,8	3,0

TABLE 30. TRIAL G.

χ^2 VALUES: DIFLUCORTOLONE VALERATE CREAMS.

UNOCCLUDED APPLICATION MODE.

Temetex cream vs. Nerisone cream.

	TIME (HOURS)								
	7	8½	10½	11½	13½	15	17	25½	28
YES/NO	0,10	0,11	0,14	0,03	0,04	0,24	5,21	2,62	0,17
INTENSITY OF BLANCHING	2,81	3,58	4,58	1,13	0,60	0,43	5,93	3,56	0,69
PAIRED COMPARISONS	0,02	0,06	0,39	1,16	2,15	3,21	1,76	0,44	0,25

Nerisone cream vs. Synalar cream.

	7	8½	10½	11½	13½	15	17	25½	28
YES/NO	0,02	0,00	0,14	1,96	3,64	8,16	17,70	5,11	6,81
INTENSITY OF BLANCHING	1,36	0,03	8,05	14,71	28,93	24,19	38,31	6,83	8,10
PAIRED COMPARISONS	0,15	0,14	8,45	18,28	38,88	46,08	57,14	13,14	8,10

Synalar cream vs. Temetex cream.

	7	8½	10½	11½	13½	15	17	25½	28
YES/NO	0,00	0,24	0,04	3,08	3,64	12,04	55,10	15,27	10,43
INTENSITY OF BLANCHING	0,35	3,66	19,81	18,92	35,15	26,84	62,25	17,09	12,10
PAIRED COMPARISONS	4,98	3,71	16,12	24,99	59,07	70,12	68,01	17,93	4,92

..... Table 30 Continued/

Table 30 continued/

Nerisone ointment vs. Nerisone cream.

	TIME (HOURS)								
	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
YES/NO	17,65	16,44	13,80	13,80	12,66	9,50	6,36	0,00	1,33
INTENSITY OF BLANCHING	26,43	29,75	27,07	24,60	19,33	17,00	4,30	1,00	2,07
PAIRED COMPARISONS	49,92	33,33	47,04	25,96	36,05	12,32	3,52	1,46	3,20

TABLE 31. TRIAL G.

χ^2 VALUES: DIFLUCORTOLONE VALERATE CREAMS.

OCCLUDED APPLICATION MODE.

Temetex cream vs. Nerisone cream.

TIME (HOURS)

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
YES/NO	0,00	0,00	0,08	0,08	0,51	0,39	2,80	1,41	1,87
INTENSITY OF BLANCHING	1,61	0,58	2,33	1,51	2,68	1,36	3,82	2,03	2,92
PAIRED COMPARISONS	0,00	0,66	0,70	0,23	6,30	1,41	3,38	0,00	0,50

Nerisone cream vs. Synalar cream.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
YES/NO	13,80	5,35	0,51	1,36	0,51	0,82	25,08	15,53	5,44
INTENSITY OF BLANCHING	21,42	11,57	2,60	10,27	32,11	41,62	33,73	14,05	4,47
PAIRED COMPARISONS	26,94	16,12	4,01	0,00	18,55	26,65	48,35	16,96	5,79

Synalar cream vs. Temetex cream.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$13\frac{1}{2}$	15	17	$25\frac{1}{2}$	28
YES/NO	16,26	5,35	0,00	0,26	0,51	3,27	42,79	22,64	13,80
INTENSITY OF BLANCHING	20,23	12,62	3,00	11,20	30,75	46,46	53,62	24,46	15,58
PAIRED COMPARISONS	16,83	11,68	0,06	1,48	33,52	37,87	68,01	20,35	12,07

..... Table 31 Continued/

Table 31 continued/

Nerisone ointment vs. Nerisone cream.

	7	8½	10½	11½	13½	15	17	25½	28
YES/NO	2,65	0,51	1,36	1,36	0,51	0,80	4,48	0,05	0,00
INTENSITY OF BLANCHING	5,90	3,42	3,33	6,69	4,03	2,59	5,15	0,00	0,07
PAIRED COMPARISONS	9,01	10,78	5,16	8,45	2,76	8,35	0,02	0,90	0,00

TABLE 32. TRIAL G.

A.U.C. VALUES: DIFLUCORTOLONE VALERATE CREAMS.

UNOCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
TEMETEX CREAM	386,74	0,55
NERISONE CREAM	462,40	0,66
SYNALAR CREAM	700,73	1,00
NERISONE OINTMENT	694,99	0,99

A.U.C. VALUES: DIFLUCORTOLONE VALERATE CREAMS.

OCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
TEMETEX CREAM	798,83	0,80
NERISONE CREAM	833,40	0,83
SYNALAR CREAM	998,77	1,00
NERISONE OINTMENT	775,91	0,78

TABLE 33. TRIAL G.

% T.P.S. DIFLUCORTOLONE VALERATE OINTMENTS.

UNOCCLUDED APPLICATION MODE.

	TIME (HOURS)								
	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
TEMETEX	34,7	41,3	51,6	57,3	51,6	37,3	27,6	9,3	5,3
NERISONE	40,9	44,9	59,6	58,6	47,5	33,3	26,3	7,1	6,1
TEMETEX FATTY	22,2	31,7	48,1	49,7	40,7	35,4	23,8	8,5	4,8
NERISONE FATTY	25,9	32,1	45,1	46,3	40,7	34,0	25,3	6,2	3,7
SYNALAR	17,2	19,7	26,3	26,8	26,8	28,3	27,3	16,7	9,6

% T.P.S. DIFLUCORTOLONE VALERATE OINTMENTS.

OCCLUDED APPLICATION MODE.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
TEMETEX	54,2	56,0	69,8	70,2	69,9	47,6	30,7	11,6	5,8
NERISONE	49,2	53,6	68,1	67,1	58,5	44,9	28,5	10,1	2,9
TEMETEX FATTY	39,4	46,1	65,0	62,2	55,6	44,4	28,3	9,4	3,3
NERISONE FATTY	35,8	46,9	59,9	64,2	54,3	46,3	31,5	9,3	4,3
SYNALAR	14,1	23,2	27,8	32,8	34,3	38,9	31,8	18,7	14,1

TABLE 34. TRIAL G.

χ^2 VALUES FOR DIFLUCORTOLONE VALERATE OINTMENTS.

UNOCCLUDED APPLICATION MODE.

Nerisone ointment vs. Temetex ointment.

TIME (HOURS)

	7	8½	10½	12	14	16	18	24	28
YES/NO	0,10	0,00	0,57	0,40	1,30	2,06	2,50	0,54	0,01
INTENSITY OF BLANCHING	2,14	1,99	5,14	2,43	4,32	3,26	1,47	0,87	0,12
PAIRED COMPARISONS	10,62	9,26	3,51	0,19	0,21	0,02	0,11	0,05	0,00

Nerisone fatty ointment vs. Temetex fatty ointment.

	7	8½	10½	12	14	16	18	24	28
YES/NO	0,87	0,02	0,00	0,62	0,23	0,00	0,03	0,45	0,06
INTENSITY OF BLANCHING	1,38	1,40	2,13	3,76	1,01	0,21	1,01	0,80	0,26
PAIRED COMPARISONS	2,07	0,69	0,03	0,10	0,23	1,94	0,03	1,07	0,90

Synalar ointment vs. Nerisone ointment.

	7	8½	10½	12	14	16	18	24	28
YES/NO	13,82	8,63	12,47	15,57	12,60	1,71	0,00	10,71	1,52
INTENSITY OF BLANCHING	2,40	26,49	33,39	35,68	17,80	3,23	0,07	11,93	2,07
PAIRED COMPARISONS	12,26	18,37	20,42	32,22	19,15	2,88	1,84	1,76	1,07

..... Table 34 Continued/

Table 34 continued/

Temetex ointment vs. Synalar ointment.

	TIME (HOURS)								
	7	8½	10½	12	14	16	18	24	28
YES/NO	11,43	10,42	7,73	20,81	23,87	8,77	0,27	6,29	2,64
INTENSITY OF BLANCHING	15,51	24,37	23,99	41,06	33,14	10,38	1,55	7,19	3,35
PAIRED COMPARISONS	15,37	30,02	36,16	41,95	21,12	12,02	2,33	0,00	1,39

Nerisone fatty ointment vs. Nerisone ointment.

	7	8½	10½	12	14	16	18	24	28
YES/NO	2,34	0,72	0,47	0,00	0,01	0,06	0,14	0,02	0,68
INTENSITY OF BLANCHING	9,16	7,23	10,88	6,56	3,85	0,97	2,61	0,13	1,17
PAIRED COMPARISONS	13,08	10,45	13,08	5,30	6,88	3,69	0,15	4,27	1,50

Temetex fatty ointment vs. Temetex ointment.

	7	8½	10½	12	14	16	18	24	28
YES/NO	5,69	1,40	0,00	3,10	3,10	0,48	0,02	0,02	0,00
INTENSITY OF BLANCHING	8,90	4,43	2,24	7,27	6,99	3,67	3,64	0,12	0,08
PAIRED COMPARISONS	5,30	1,23	0,25	3,84	1,69	0,57	0,97	0,76	0,10

TABLE 35. TRIAL G.

χ^2 VALUES FOR DIFLUCORTOLONE VALERATE OINTMENTS.

OCCLUDED APPLICATION MODE.

Nerisone ointment vs. Temetex ointment.

	TIME (HOURS)								
	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	0,01	1,59	0,21	1,01	0,79	0,03	0,07	0,13	1,64
INTENSITY OF BLANCHING	1,64	2,91	3,44	2,38	1,88	1,69	0,55	0,29	2,34
PAIRED COMPARISONS	0,03	0,00	0,21	0,09	0,02	0,08	0,54	1,57	0,00

Nerisone fatty ointment vs. Temetex fatty ointment.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	0,11	0,09	0,02	0,41	2,42	0,03	1,74	0,02	0,04
INTENSITY OF BLANCHING	0,53	0,41	1,11	2,02	6,16	1,16	2,90	0,01	0,25
PAIRED COMPARISONS	0,78	1,09	0,43	1,29	1,03	0,39	5,30	0,00	0,45

Synalar ointment vs. Nerisone ointment.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	39,46	12,84	15,35	12,23	10,41	4,04	0,02	7,08	18.62
INTENSITY OF BLANCHING	50,29	36,02	41,17	35,64	20,73	5,18	2,71	8,59	20,37
PAIRED COMPARISONS	20,90	19,22	30,19	26,56	23,56	1,02	8,82	5,63	13,14

..... Table 35 Continued/

Table 35 continued/

Temetex ointment vs. Synalar ointment.

	TIME (HOURS)								
	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	43,29	24,66	21,55	21,02	18,32	3,82	0,04	4,85	9,34
INTENSITY OF BLANCHING	58,70	44,88	49,89	45,80	28,21	4,81	1,46	6,34	10,72
PAIRED COMPARISONS	39,45	32,81	28,92	39,05	27,16	5,78	0,08	22,13	13,89

Nerisone fatty ointment vs. Nerisone ointment.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	5,27	0,00	0,35	1,66	0,57	0,04	0,87	0,02	0,22
INTENSITY OF BLANCHING	5,83	5,43	7,08	5,89	1,14	0,63	1,36	0,10	0,58
PAIRED COMPARISONS	5,03	3,51	6,56	2,44	1,73	0,43	1,64	0,00	0,80

Temetex fatty ointment vs. Temetex ointment.

	7	$8\frac{1}{2}$	$10\frac{1}{2}$	12	14	16	18	24	28
YES/NO	3,97	2,44	0,23	0,04	0,08	0,06	0,50	0,36	0,94
INTENSITY OF BLANCHING	9,90	6,30	3,80	3,45	2,32	4,67	0,85	0,62	1,48
PAIRED COMPARISONS	19,11	20,02	4,69	2,75	2,75	4,36	9,82	0,04	1,45

TABLE 36. TRIAL G.

A.U.C. VALUES: DIFLUCORTOLONE VALERATE OINTMENTS.

UNNOCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
TEMETEX	790,01	1,29
NERISONE	814,83	1,33
TEMETEX FATTY	682,51	1,12
NERISONE FATTY	646,36	1,06
SYNALAR	610,77	1,00

A.U.C. VALUES: DIFLUCORTOLONE VALERATE OINTMENTS.

OCCLUDED APPLICATION MODE.

	Area	Relative to Synalar
TEMETEX	1020,93	1,29
NERISONE	923,41	1,16
TEMETEX FATTY	846,45	1,07
NERISONE FATTY	841,89	1,06
SYNALAR	793,31	1,00

2. ANALYTICAL RESULTS

2.1 COLOURIMETRIC ASSAY

This assay was performed on Synalar cream, Synalar ointment, Cream A and Ointment A. These preparations were used in Trials B and C.

Four 10 ml aliquots of the resulting ethanolic extracts and fluocinolone acetonide working standard solution were treated with the colour reagents.

	Fluocinolone Acetonide	Ointment A	Synalar Ointment	Cream A	Synalar Cream
Mass (g)	0,0228	9,5534	9,5701	9,4708	10,0346
Absorbance at 485 nm	0,314 0,318 0,309 0,313	0,327 0,331 0,324 0,326	0,321 0,307 0,319 0,314	0,354 0,352 0,354 0,353	0,364 0,362 0,360 0,358
Average A_{485}	0,314	0,327	0,316	0,353	0,361
% Fluocinolone acetonide	-	0,0248	0,0240	0,0271	0,0261
% Purity		99,2	96,0	108,4	104,3

2.2 ULTRAVIOLET ASSAY

This assay was performed on Synalar cream, Synalar ointment, Cream A and Ointment A. These preparations were used in trials B and C.

Four 20,0 ml aliquots of the eluates were evaporated to dryness and dissolved in 5,0 ml absolute ethanol. The absorbance of these four solutions was determined at 238 nm.

	Fluocinolone acetonide	Ointment A	Synalar Ointment	Cream A	Synalar Cream
Mass (g)	0,00913	8,3628	8,8430	8,50182	8,38599
A_{238}	0,690 0,659 0,668 0,673	0,783 0,757 0,768 0,768	0,685 0,714 0,697 0,720	0,777 0,776 0,767 0,772	0,735 0,738 0,744 0,747
Average	0,672	0,769	0,704	0,773	0,741
mg in sample		2,089	1,913	2,100	2,013
% Purity		100,0	97,6	98,8	96,1

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY

This assay was performed on Synalar cream, Synalar ointment, Cream B and Ointments A and B.

(a) Toluene (37,5 µl) was added to the resulting methanolic extracts (25,0 ml) and to the fluocinolone acetonide standard solution.

(b) Toluene (200 µl) was added to the resulting methanolic extracts (50,0 ml) and to the fluocinolone acetonide standard solution.

The average area of the fluocinolone acetonide and toluene peaks was determined from the data obtained from five injections.

(a) Results for Synalar ointment and Ointment A, used in Trials B and C.

	Fluocinolone acetonide	Ointment A	Synalar ointment
Mass[+]	0,00347	9,39090	9,47027
Average area Fluocinolone acetonide peak	88,74	84,48	84,24
Average area toluene peak	11,26	15,52	15,76
% Fluocinolone acetonide		0,0255	0,0248
% Purity		102,0	99,2

[+] in 25,0 ml.

(b) Results for Synalar ointemnt, Synalar cream, Cream B and Ointment B, used in Trial D.

	Fluocinolone Acetonide	Ointment[a] B	Synalar[a] Ointment	Cream[a] B	Synalar[b] Cream
Mass	(a) 0,0031 (b) 0,00269	8,7256	8,9820	9,8259	9,46469
Average area Fluocinolone acetonide peak	(a) 54,98 (b) 47,37	48,11	48,45	49,18	44,11
Average area Toluene peak	(a) 45,02 (b) 52,63	51,89	51,55	50,82	55,89
% Fluocinolone acetonide		0,0269	0,0246	0,0250	0,0249
% Purity		107,9	98,4	100,0	99,6

a = 0,0031 g in 50,0 ml.

b = 0,00269 g in 50,0 ml.

D. DISCUSSION

1. THE BLANCHING ASSAY

TRIAL A. The effect of propylene glycol concentration on blanching activity.

The effect of vehicle composition on the release rate of corticosteroids from topical formulations has been widely studied. It was decided to assess the *in vivo* effect of propylene glycol on the release rate of fluocinolone acetonide from cream and ointment formulations, since this additive appears to be one of the most critical vehicle components influencing the release of corticosteroids from topical preparations.[41-49] Ostrenga, Steinmetz and Poulsen[46] compared the *in vivo* release of 0,025% fluocinolone acetonide, monitored as blanching, to *in vitro* release of ^{14}C labelled fluocinolone acetonide through cadaver skin into isopropyl myristate. A gel containing varying amounts of propylene glycol - water was used as the delivery system. They found that the *in vitro* data indicated that the greatest release rate was given by the gel which contained 30% propylene glycol. A gel containing 15% propylene glycol performed poorly. The cumulative amount of fluocinolone acetonide penetrating through the membrane was approximately half that amount which penetrated from the gel which contained 30% propylene glycol. However, an *in vivo* analysis utilizing a modified McKenzie-Stoughton blanching assay showed that the 15% and 30% propylene glycol containing gels produced a similar amount of blanching. However the fluocinolone acetonide gels which produced the maximum amount of blanching were those which contained 5, 10 and 20% propylene glycol.

The *in vivo* blanching assay has been used to optimize propylene glycol concentration for other corticosteroids. Bluefarb, *et al*[41] used the

blanching assay to determine the appropriate propylene glycol concentration required to optimize the release of diflorasone diacetate in a 0,05% concentration from a cream vehicle to the skin. They found that occlusion tended to mask any significant differences which occurred in the unoccluded application mode between the various propylene glycol containing creams.

For the present trial nine volunteers were used. Both forearms were masked to produce 12 application sites per arm. One forearm was occluded, the other being left unoccluded. The arms were evaluated by three observers.

Cream and ointment formulations, varying in propylene glycol concentration, containing 0,025% fluocinolone acetonide were compared to Synalar cream and ointment. The Synalar preparations were locally manufactured and contained 0,025% fluocinolone acetonide.

The cream and ointment formulations were prepared (see formulae 1 and 2) to contain no propylene glycol, 7½% and 15% propylene glycol.

CREAM A	
Liquid paraffin	90,00 g
Cetyl alcohol	90,00 g
Lanette wax SX	30,00 g
Nipasept	2,50 g
Thiomersal	0,02 g
Fluocinolone acetonide	0,25 g
Propylene glycol	q.s.
Purified water to	1000,00 g

FORMULA 1

OINTMENT A	
White soft paraffin	590,00 g
Lanette wax SX	35,00 g
Fluocinolone acetonide	0,25 g
Propylene glycol	q.s.
Liquid paraffin to	1000,00 g

FORMULA 2

In formulae 1 and 2 the propylene glycol content was either 0,0 g, 75,0 g or 150,0 g, depending upon the desired percent propylene glycol required in the final formulation.

Cream A (formula 1) was prepared as follows. Lanette wax, cetyl alcohol and liquid paraffin were melted together. Fluocinolone acetonide was dissolved in propylene glycol and incorporated into the melted lanette wax, cetyl alcohol and liquid paraffin, while still molten. When no propylene glycol was present fluocinolone acetonide was dispersed into the melted portion of the base. Nipasept and thiomersal were dissolved in the required amount of water to bring the final preparation up to mass. This solution, after being gently heated to the same temperature as the melted portion was mixed with the latter.

Ointment A (formula 2) was prepared in a similar manner to Cream A. White soft paraffin, liquid paraffin and lanette wax were heated together at a low temperature. A solution of fluocinolone acetonide in propylene glycol was then added to the melted components, if propylene glycol was to be included in the formulation, and mixed until cool. When no propylene glycol was incorporated into the ointment, fluocinolone acetonide was dispersed into the melted components as the final step.

Creams

The results are depicted graphically in figures 1 and 2.

In the unoccluded mode statistical differences in the blanching response were observed 12-24 hours after application between the creams which contained no propylene glycol and $7\frac{1}{2}\%$ propylene glycol in favour of the latter. When the creams containing $7\frac{1}{2}\%$ and 15% propylene glycol were

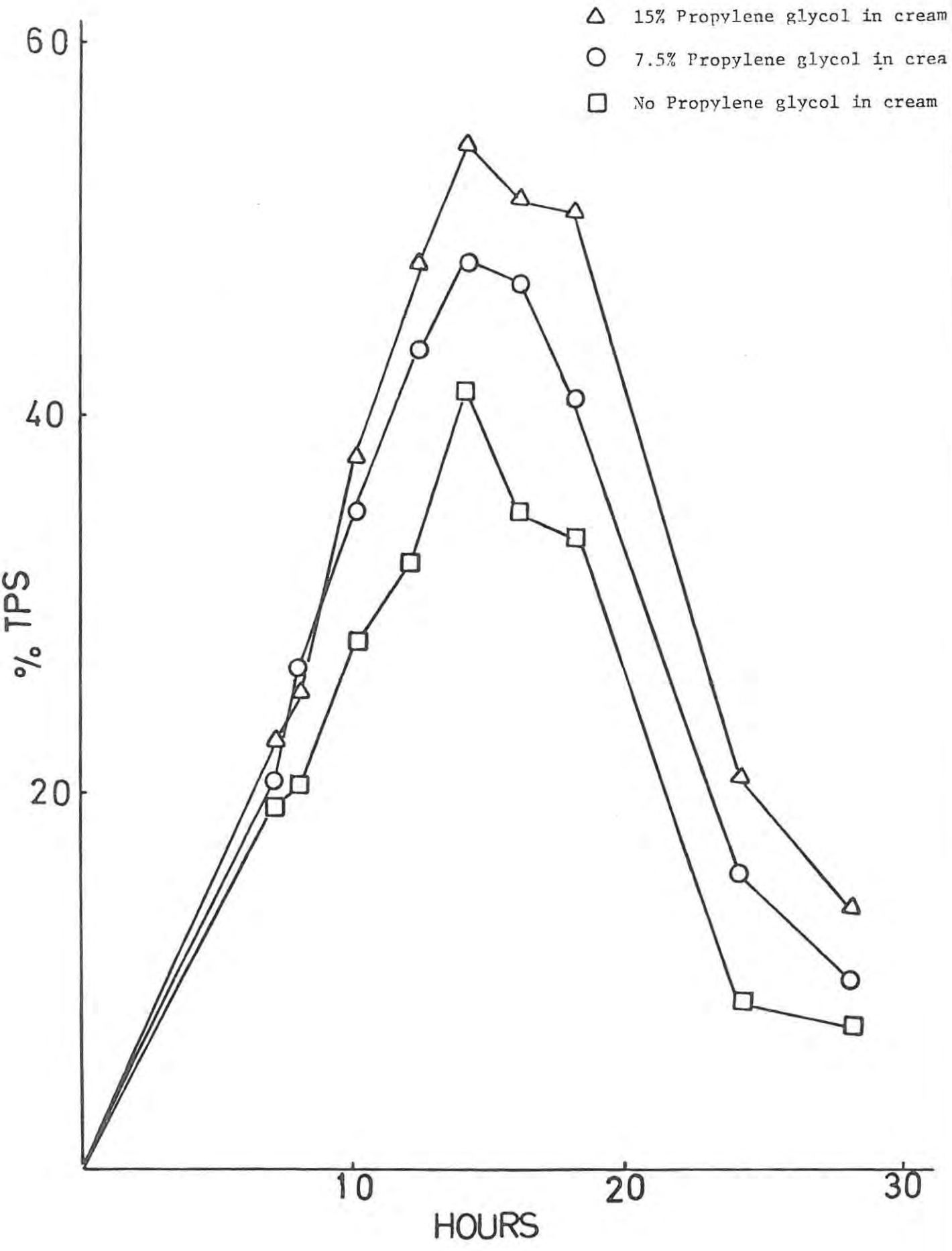


fig 1. Blanching profiles obtained from Trial A. (unoccluded creams)

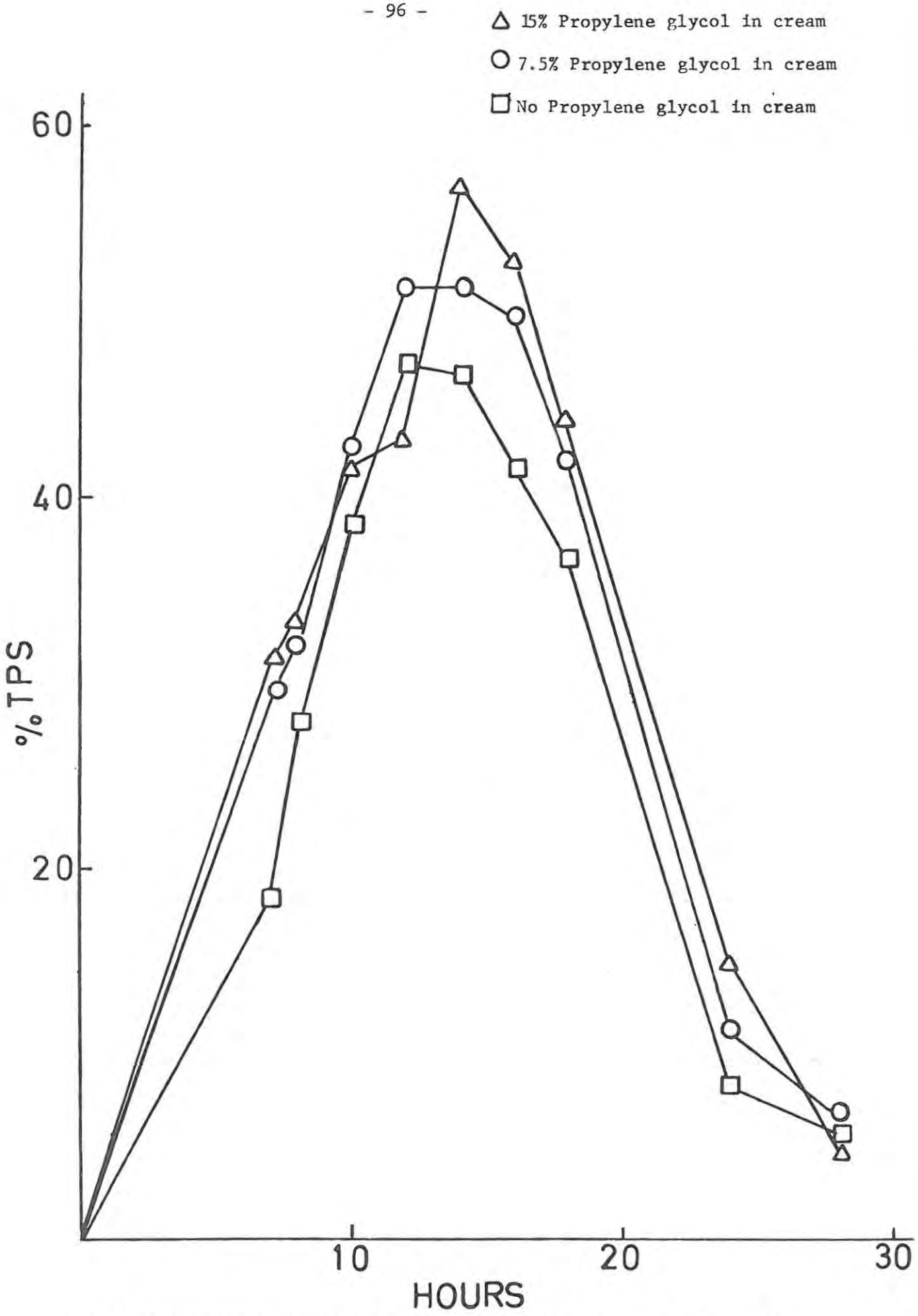


fig 2. Blanching profiles obtained from Trial A. (occluded creams)

compared few statistically significant differences were noted (table 6). Under occlusion fluocinolone acetonide appeared to be released from the cream containing no propylene glycol at a rate essentially similar to that of the two propylene glycol containing creams. In this application mode few statistically significant differences were observed between the 0% and 7½% propylene glycol containing creams, while no statistically significant differences were observed between the 7½% and 15% propylene glycol containing creams (table 7). Hence occlusion tends to suppress any differences evident in the unoccluded application mode.

This is not unexpected. Occlusion tends to favour the poorly designed vehicle.[105] The occlusive dressing hydrates the stratum corneum thereby promoting percutaneous absorption. It has been well established[1,54,55,108,109] that the degree of hydration of the horny layer of the epidermis affects its affinity for steroids and their transport through the skin. Knowledge of this phenomenon has given rise to the use of occlusive dressings in dermatology.

Ointments

The results are depicted graphically in figures 3 and 4.

In the unoccluded mode the ointment containing 7½% propylene glycol was significantly superior to the ointment containing no propylene glycol throughout the whole time span of the trial. No statistically significant differences were observed between the ointments containing 7½% and 15% propylene glycol in the unoccluded application mode (table 6). In fact almost identical blanching profiles were obtained for these two ointments.

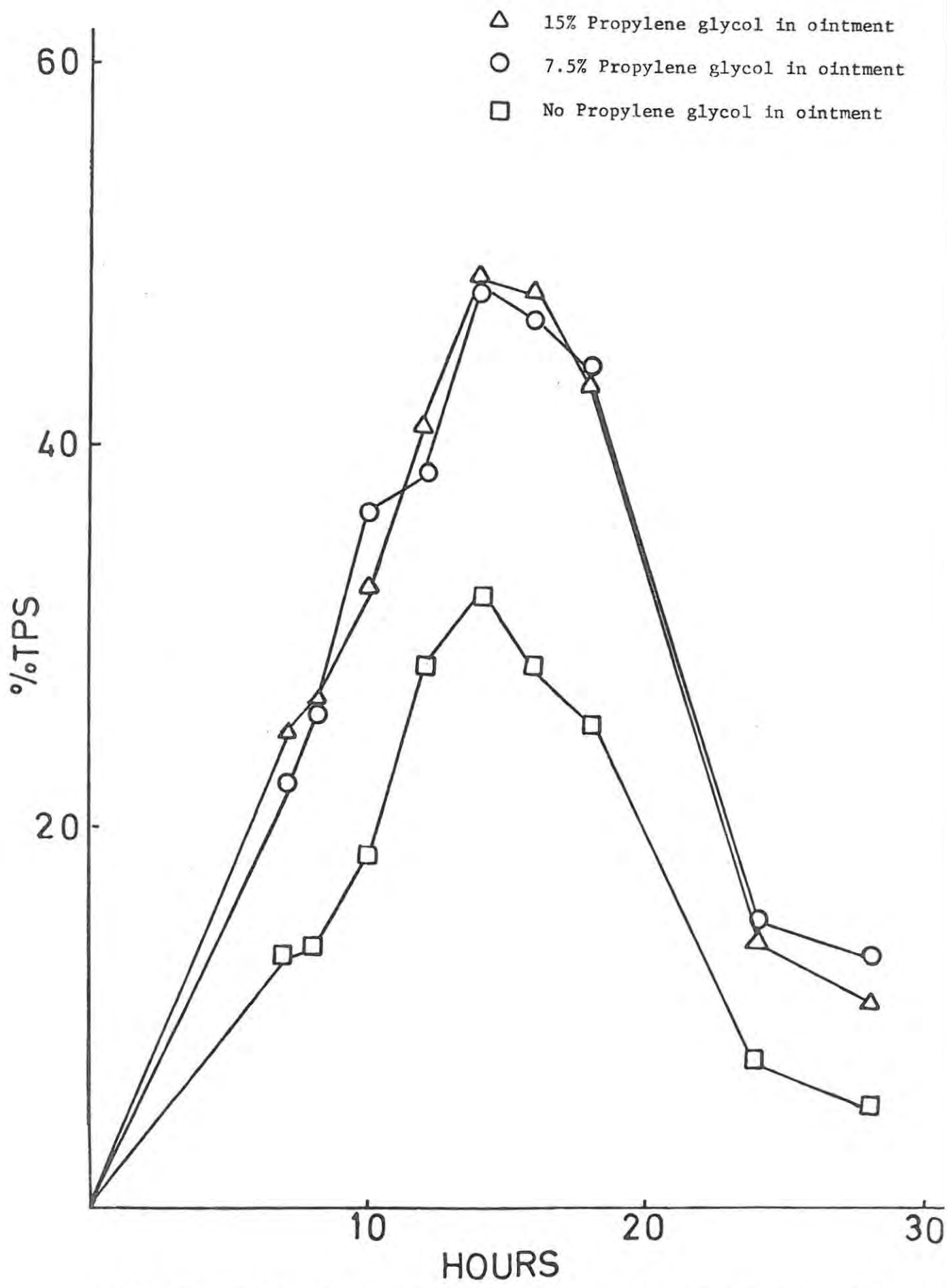


fig 3. Blanchings profiles obtained from Trial A. (unoccluded ointments)

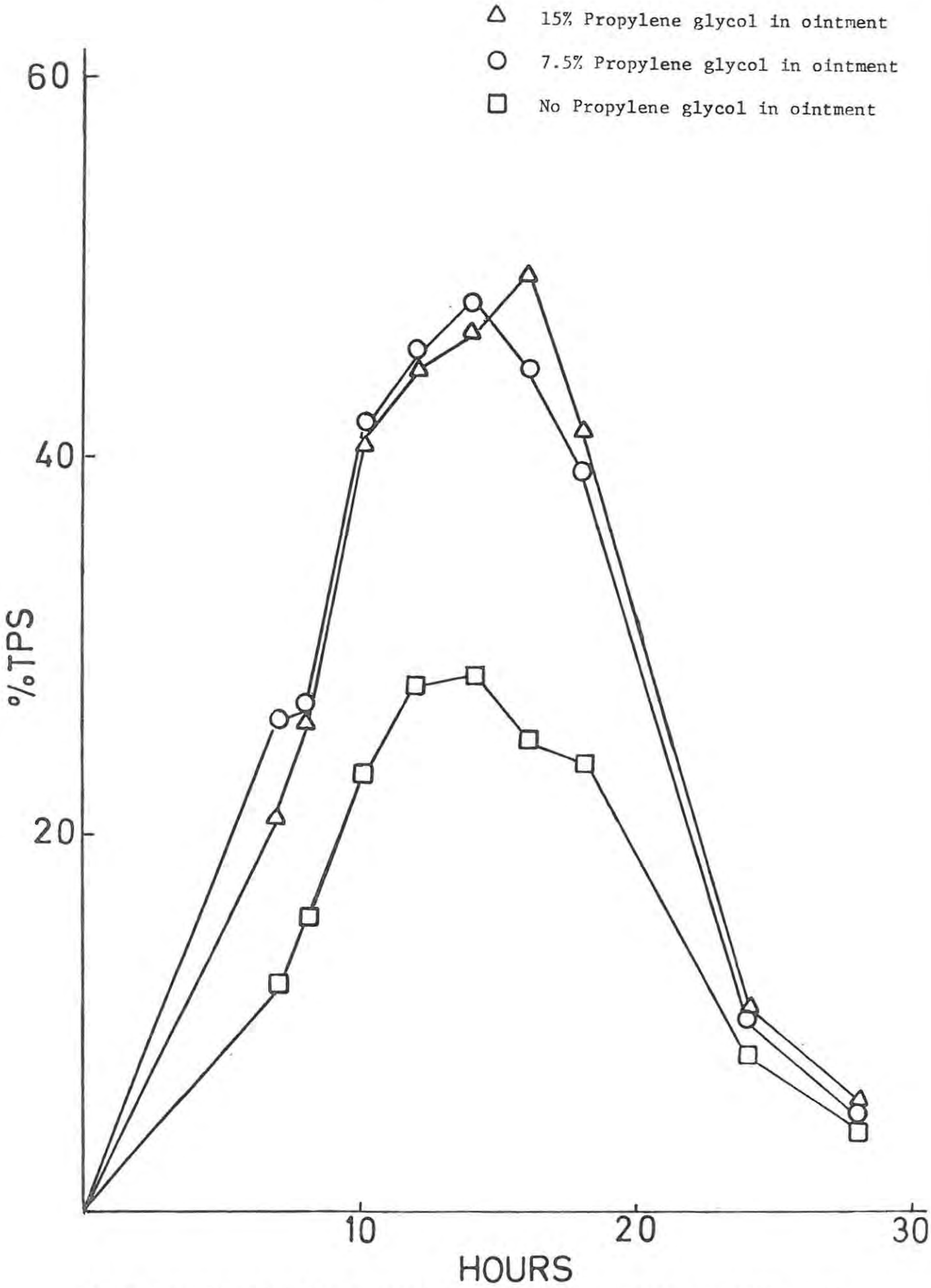


fig 4. Blanching profiles obtained from Trial A. (occluded ointments)

Similar results were obtained in the occluded study on the ointments, except that the differences for the 24-28 hour period were not significant when the ointments containing no propylene glycol and $7\frac{1}{2}$% propylene glycol were compared (table 7).

Creams and Ointments

The design of the trial also permitted the comparison of the cream containing $7\frac{1}{2}$% propylene glycol with the ointment which contained $7\frac{1}{2}$% propylene glycol. Very few statistical differences were observed between the cream and ointment , in either application mode (tables 6 and 7).

In the unoccluded application mode the ointment containing $7\frac{1}{2}$% propylene glycol yielded a larger area under the curve (A.U.C.) value than the corresponding cream. In the occluded application mode this order was reversed (table 8). The occlusive nature of the ointment base accounts for the higher A.U.C. value obtained for the ointment in the unoccluded application mode. When an occlusive dressing is applied the stratum corneum becomes hydrated, so hydration due to base effects are minimized, and hence the better delivery system would result in a better rate of release and therefore generate a larger A.U.C. value, as observed in the case of the cream.

The time taken for the creams to reach a maximum response for the unoccluded application mode in this trial was approximately $13\frac{1}{2}$ hours. Under occlusion the peak time was reduced to approximately $12\frac{1}{2}$ hours. This was not found to be the case for the ointments. The peak appeared $13\frac{1}{2}$ hours after application, irrespective of mode of application. Due to the high viscosity of an ointment diffusion of the active

ingredient to the skin surface will be hindered. The viscosity of the cream base will not effect the diffusion of the active ingredient from the base to the skin as greatly as ointments and hence the rate of release from creams of the active ingredient into the skin will be enhanced by occlusion.

The effect of propylene glycol on cream formulations does not appear to be as critical as it does on ointment formulations. This is evident both from the statistical treatment of the data and from A.U.C. measurements.

Seven and a half percent propylene glycol gave a marginally better rate of release of fluocinolone acetonide from ointment formulations, whereas 15% propylene glycol gave a better release rate of this corticosteroid from creams. It was decided to include $7\frac{1}{2}\%$ propylene glycol into both cream and ointment formulations of fluocinolone acetonide on the basis of this in vivo investigation.

In a trial conducted by Ostrenga, Steinmetz and Poulsen[46] on 0,025% fluocinolone acetonide gels it was found that a gel containing 15% propylene glycol resulted in a poor release of this corticosteroid compared to a gel containing $7\frac{1}{2}\%$ propylene glycol using both in vivo and in vitro techniques. However in this trial on propylene glycol containing creams and ointments, a good blanching response compared to $7\frac{1}{2}\%$ propylene glycol was obtained for the cream and ointment which contained 15% propylene glycol. A gel is a relatively simple delivery system compared to creams and ointments and hence differences in the optimum propylene glycol concentrations may vary from one type of formulation to another depending upon the components of the vehicle.

TRIALS B and C. Comparison of extemporaneous fluocinolone acetonide formulations with Synalar formulations.

These two trials permitted the direct comparison of Synalar cream and ointment with extemporaneously prepared creams and ointments containing 0,025% fluocinolone acetonide. Cream A and Ointment A were prepared according to Formula 1 and 2 (Trial A) respectively. Fluocinolone acetonide was solubilized in $7\frac{1}{2}$% propylene glycol. In order for these comparisons to be valid quantitative analyses (see p88) were performed on all preparations to ensure that they were all of standard strength.

In Trial B five volunteers were independently evaluated by two observers. There were 12 sites per arm. One forearm was occluded, the other being left unoccluded. The design of the trial did not permit all three types of statistical treatment. Paired comparison of adjacent sites was not possible. Placebo preparations, Cream A base and Ointment A base were also applied to the forearms in order to determine whether the vehicles alone were capable of producing blanching.

Trial C was performed on six volunteers, each of whom was evaluated by three observers. Four preparations were applied to three sites per arm. Both forearms were used, one set of applications being occluded, the other being left unoccluded. In Trial B the bases alone did not not produce any marked blanching and thus placebo preparations were not included in this trial. All forms of statistical data treatment were performed.

Creams

Figures 5 and 6 graphically represent the unoccluded and occluded results respectively of Trial B.

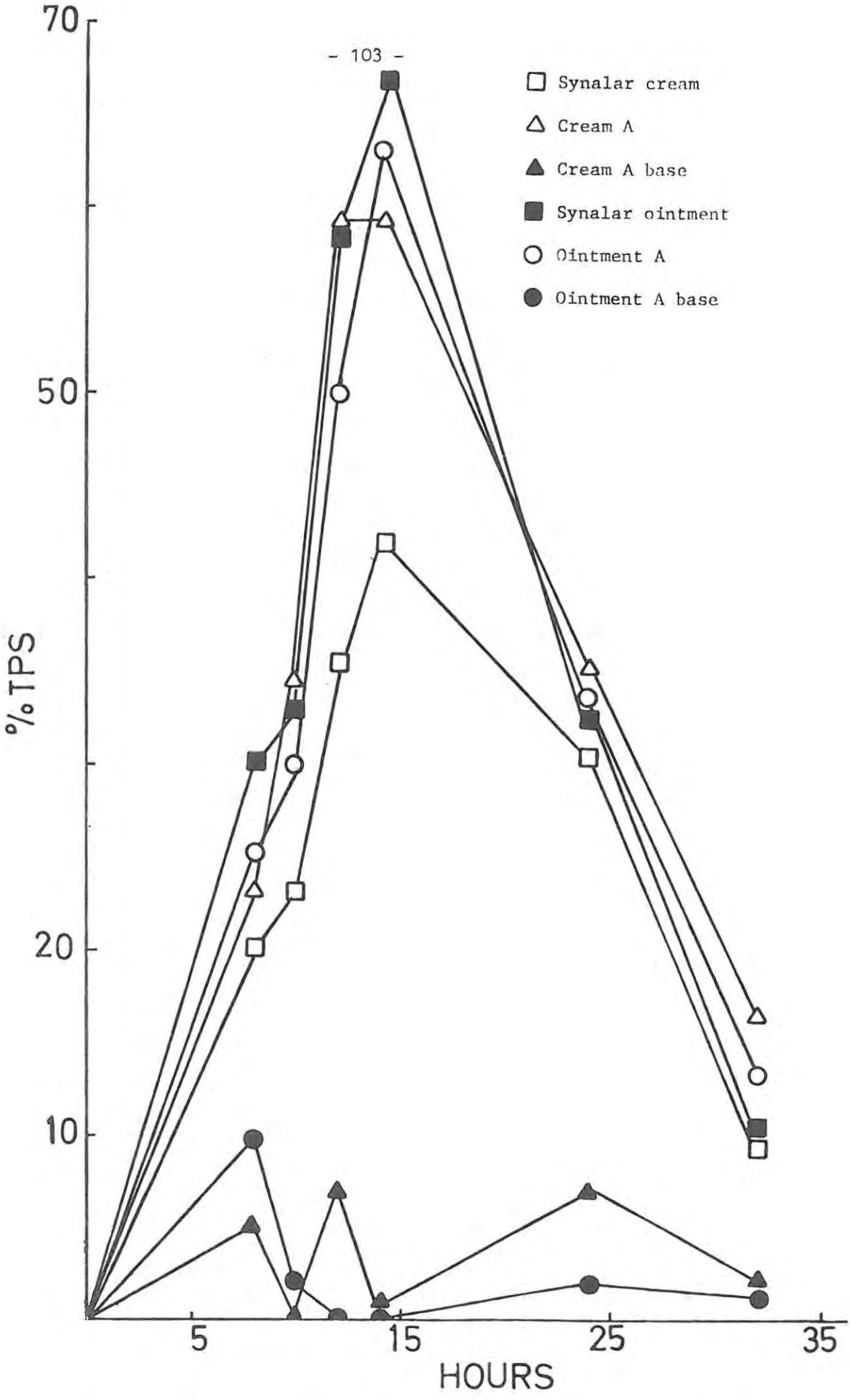


fig 5. Blanching profiles obtained from Trial B. (unoccluded)

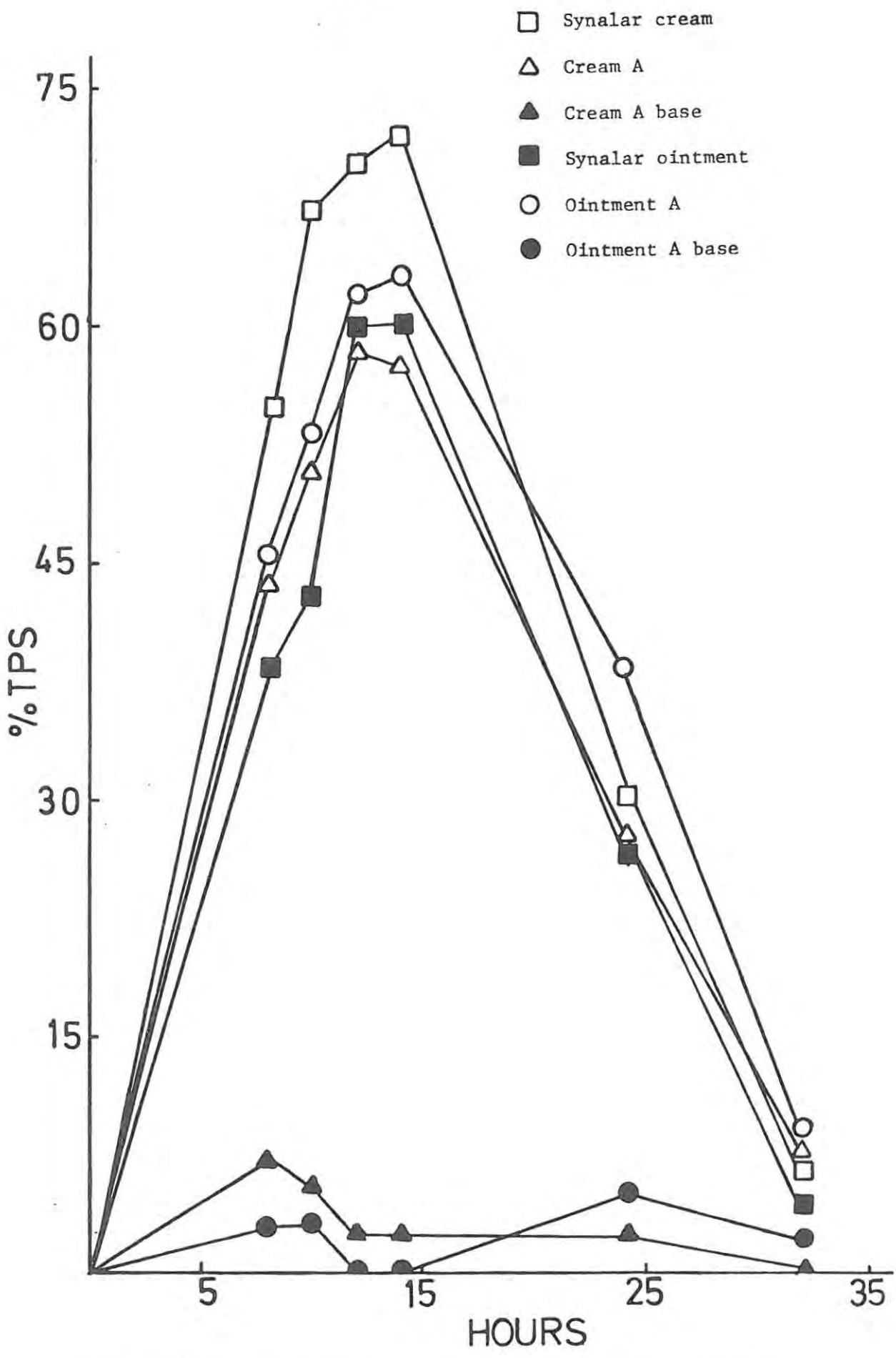


fig 6. Blanching profiles obtained from Trial B. (occluded)

Generally fluocinolone acetonide was not as well released from Synalar cream base when compared with the release of the same corticosteroid from Cream A, both in the unoccluded and occluded application mode as evidenced by the blanching profiles (figures 5 and 6). There are few statistical differences when analysed by χ^2 techniques (tables 10 and 13). An exception was observed in trial B where Synalar cream in the occluded mode gave a higher blanching response than Cream A (table 11). When this trial was repeated (Trial C) this trend was reversed and the extemporaneously prepared cream gave a higher blanching response than its proprietary counterpart, Synalar cream in both application modes (table 14).

Ointments

In trial B (figures 5 and 6) Synalar ointment was superior, although not statistically, to Ointment A in the unoccluded application mode (table 10). This situation was reversed under occlusion (table 10). As in the cream study of Trial C, Ointment A was superior to Synalar ointment in both the unoccluded and occluded application modes (table 13).

The anomalies in the results of Trial B may be due to the fact that each preparation was only applied to a total of 20 sites, and evaluated by only two observers, whereas in Trial C 36 applications were made per preparation tested. These 36 sites were then evaluated by three observers to give a total possible score (T.P.S.) per preparation of 162, compared with the T.P.S. of 60 for Trail B in each application mode.

Placebo Effect

Cream A and Ointment A bases were assessed in Trial B. As can be seen

from figures 5 and 6 placebo effects are minimal in both application modes, being high at the initial reading times and tending to be very low when the active preparations were displaying their maximum responses.

Considering the two trials together fluocinolone acetonide was released from Cream A and Ointment A at a rate equal to, if not better than the commercially available preparations, Synalar cream and ointment.

In addition to optimizing the release of the corticosteroid from the base, the overall formulation should be cosmetically acceptable and all the ingredients compatible. Unfortunately Cream A and Ointment A tended to separate out, after 6-8 months. Hence it was necessary to reformulate these preparations to permit good release characteristics and have a longer shelf life.

TRIAL D. Comparison of reformulated fluocinolone acetonide preparations with Synalar preparations.

This trial investigated the rate of release of fluocinolone acetonide from reformulated bases and compared this release with that of fluocinolone acetonide from Synalar bases. The new formulae for the cream and ointment formulations are listed below.

CREAM B		OINTMENT B	
Liquid paraffin	90,00 g	Cholesterol	30,00 g
Cetyl alcohol	90,00 g	Stearyl alcohol	32,00 g
Lanette Wax SX	30,00 g	Bleached white beeswax	85,00 g
Fluocinolone acetonide	0,25 g	Fluocinolone acetonide	0,25 g
Propylene glycol	75,00 g	Propylene glycol	75,00 g
Benzyl alcohol	15,00 g	White soft paraffin to	1000,00 g
Purified water to	1000,00 g		
FORMULA 3		FORMULA 4	

Cream B was prepared in a manner essentially similar to the manufacture of Cream A. Liquid paraffin was added to a melted mixture of cetyl alcohol and lanette wax. Fluocinolone acetonide was dissolved in propylene glycol, heated to approximately 65 ^{o}C and added to the liquid paraffin, cetyl alcohol and lanette wax mixture, while the latter mixture was still molten. Benzyl alcohol was dissolved in water and gently heated to approximately 65 ^{o}C and added to the mixture obtained from the previous step. The cream was homogenized until it set.

In the manufacture of Ointment B, stearyl alcohol, bleached white beeswax and white soft paraffin were gently heated until all the components had melted. Cholesterol was dissolved in this mixture with constant stirring. This mixture of base components was stirred until cool. Fluocinolone acetonide was dissolved in proylene glycol and incorporated into the mixture of base components, which had been heated to a temperature of 35-40 ^{o}C. The ointment was mixed until it set.

In Trial D nine subjects were evaluated by three observers. Both arms were employed, one forearm being occluded, the other being left unoccluded. There were 14 sites per forearm. The four steroid containing preparations, Synalar cream and ointment, Cream B and Ointment B, were applied to three sites per forearm. The remaining two sites per forearm were filled with Cream B base and Ointment B base. The total possible score per active preparation was 243.

Creams

The results are depicted graphically in figures 7 and 8.

In the unoccluded mode Cream B was significantly superior from 12-28 hours after application to Synalar cream, considering all statistical methods of analysis (table 16). Under occlusion these differences

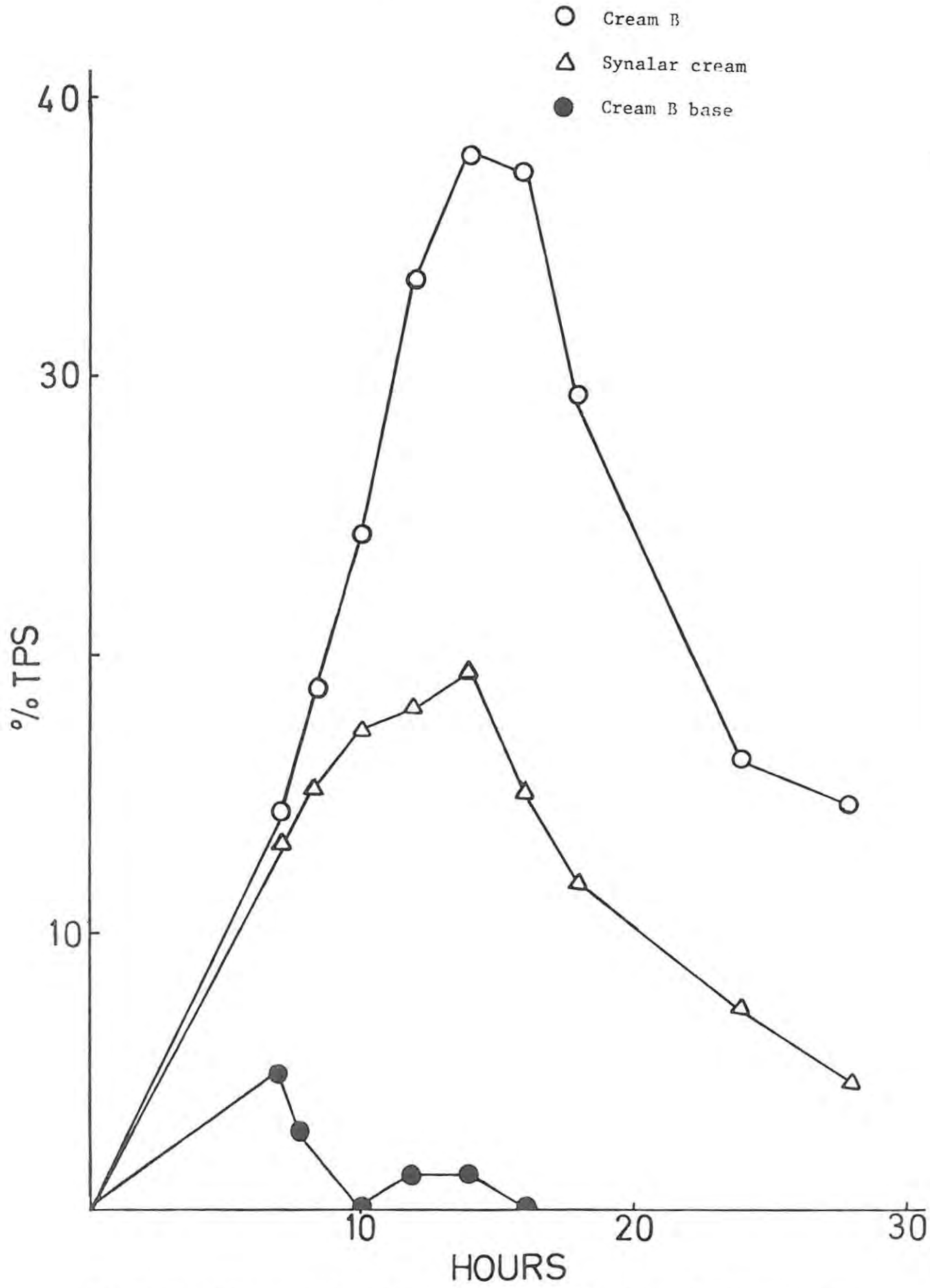


fig 7. Blanching profiles obtained from Trial D. (unoccluded creams)

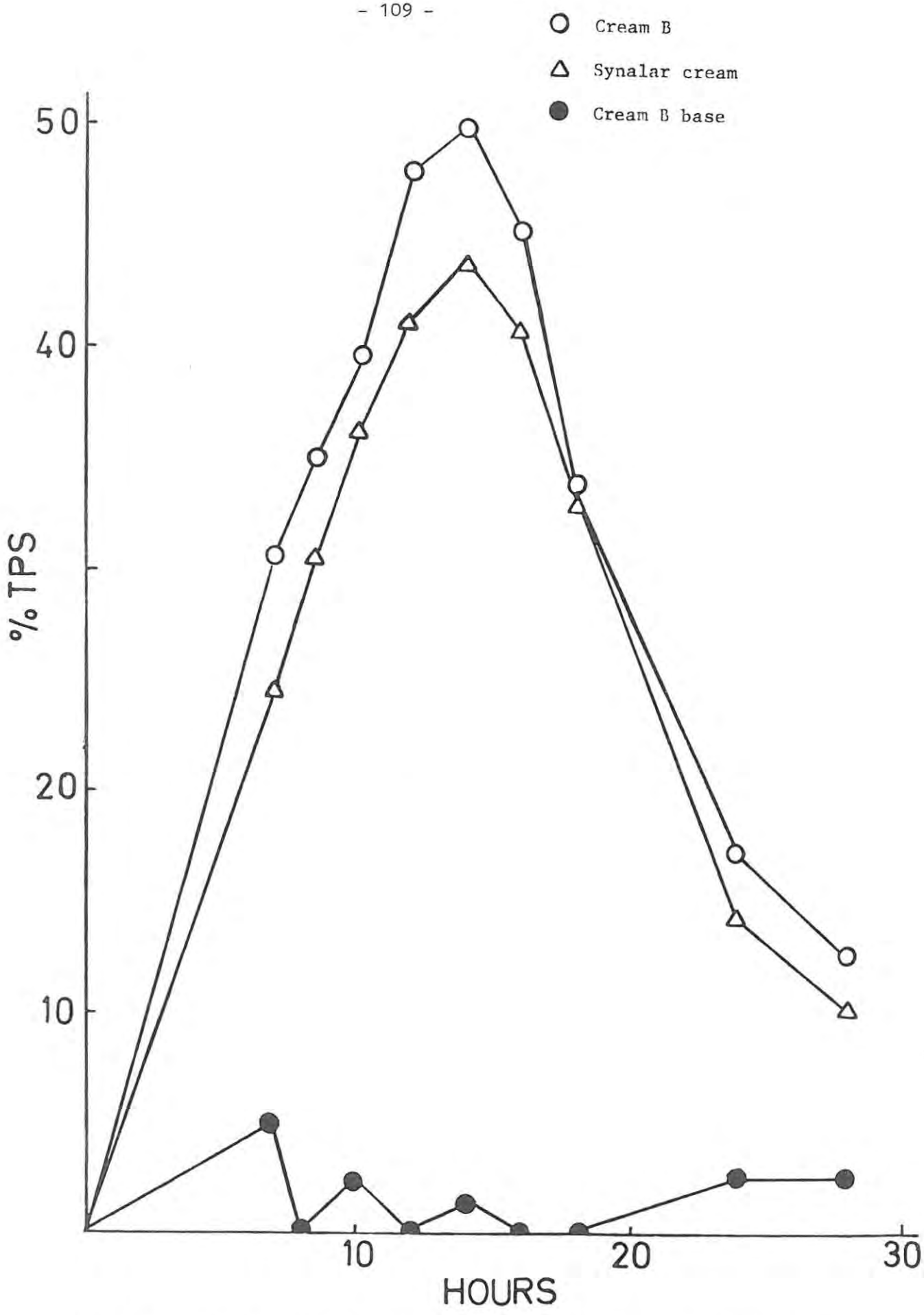


fig 8. Blanching profiles obtained from Trial D. (occluded creams)

became less marked with the result that only isolated statistically significant differences were observed (table 17). Occlusion results in the maximum response occuring after a slightly shorter period of time, a higher maximum response and a more rapid removal from the site of action.

The A.U.C. value for Synalar cream in the unoccluded application mode is 350,4. In the occluded mode this value is 766,1 (table 18). Hence the area increases by a factor of 2,19 under occlusion. The increase in area under occlusion, relative to the unoccluded application mode, for Cream B is only 1,15. The A.U.C. value for Base B also increases under occlusion by a factor of 1,55. This indicates that placebo effects are also enhanced under occlusion. Care should be exercised when comparing the unoccluded application mode with the occluded, especially when all the bases have not been evaluated.

It was not possible to obtain Synalar cream base for inclusion into this trial, but it would appear as though Cream B releases fluocinolone acetonide to a greater extent than does Synalar cream as seen by the very large differences between the two application modes. Occlusion tends to favour the poorly designed base. Synalar cream is favoured more by occlusion than Cream B.

Ointments

The results are depicted graphically in figures 9 and 10.

Ointment B produces a better blanching response than Synalar ointment, both in the unoccluded and occluded application modes. Statistically the differences are not significant except in the unoccluded application mode for the paired comparison method (tables 16 and 17). Here Ointment

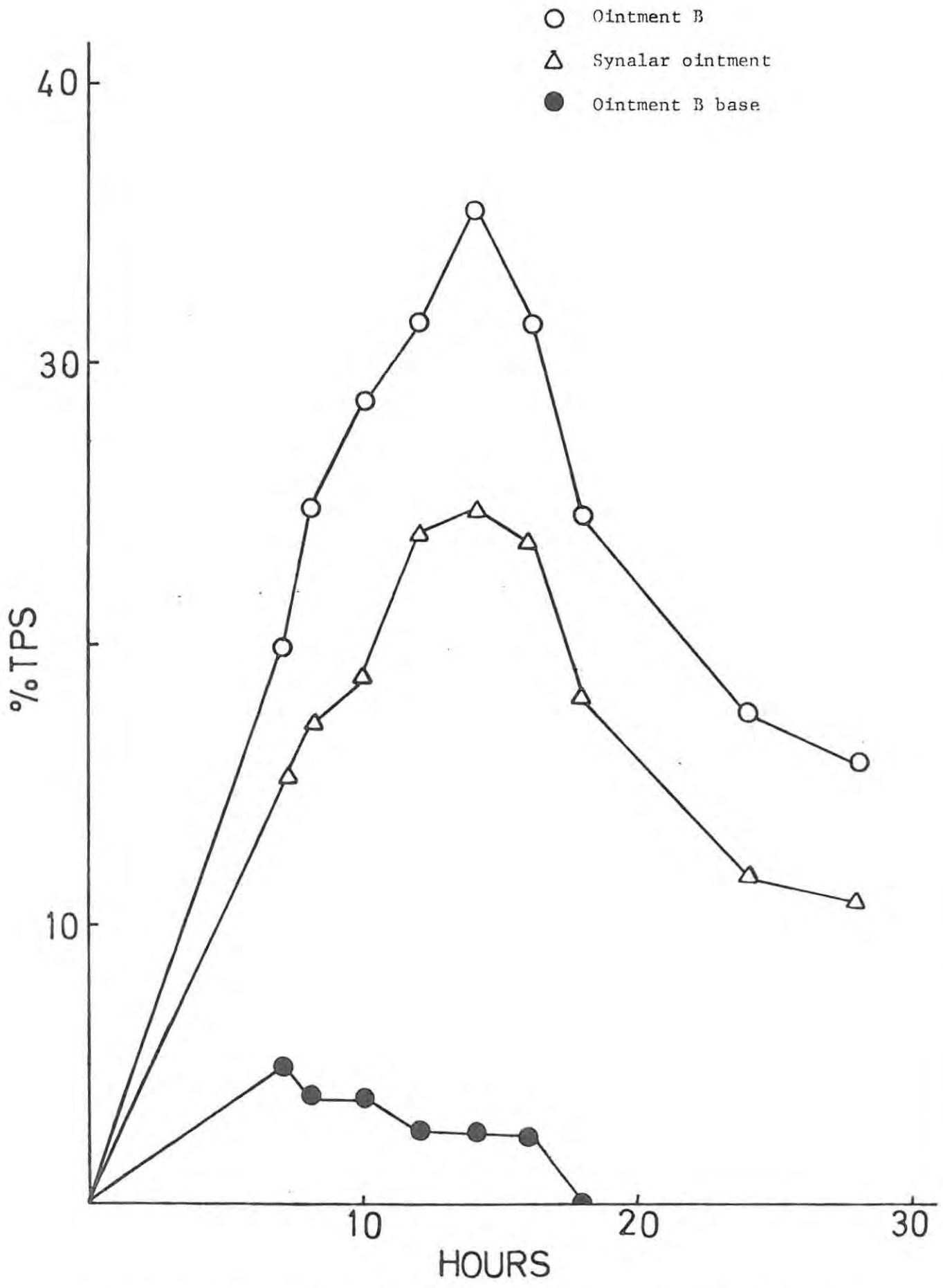


fig 9. Blanching profiles obtained from Trial D. (unoccluded ointments)

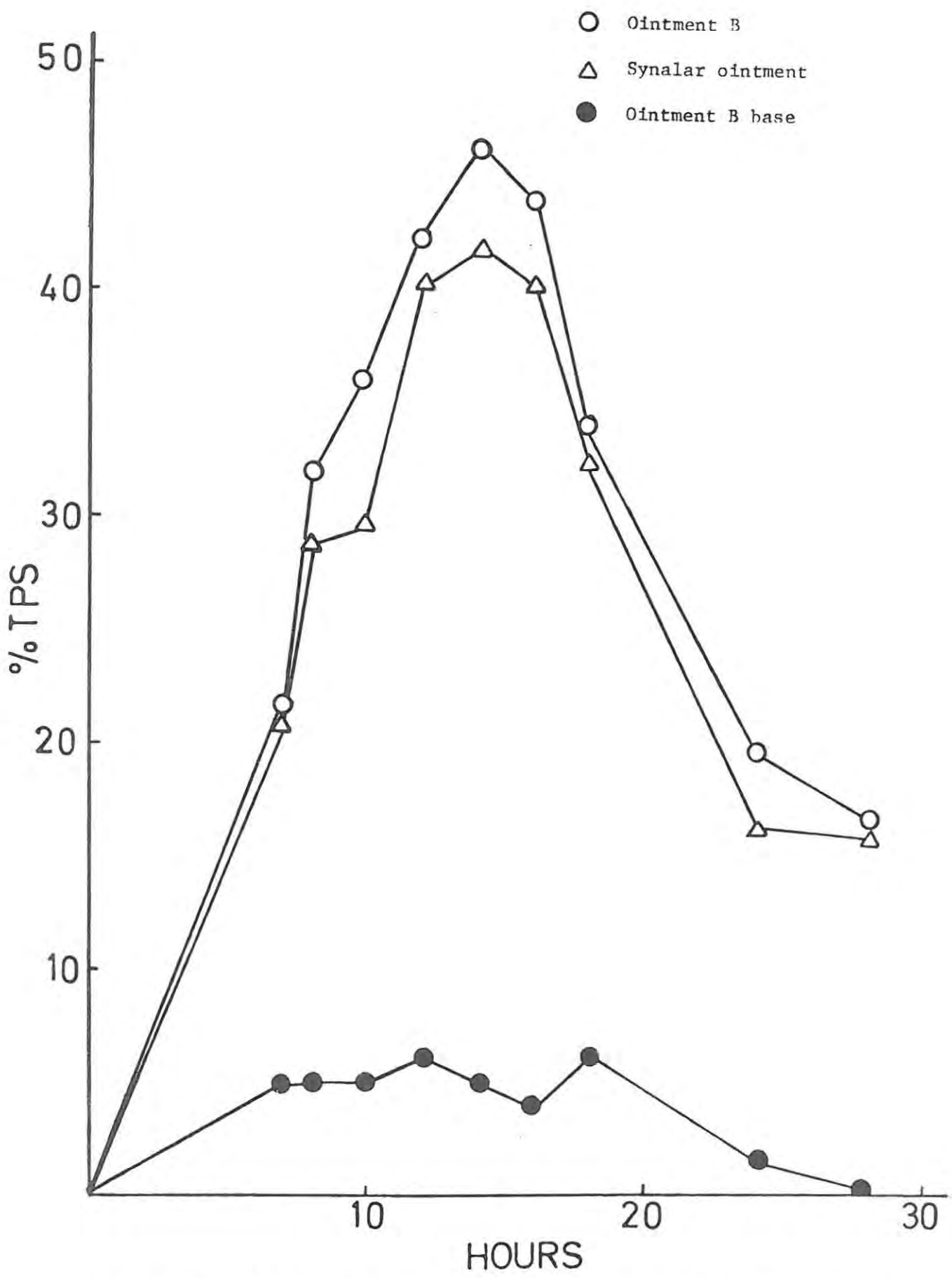


fig 10. Blanching profiles obtained from Trial D. (occluded ointments)

B has better release characteristics than Synalar ointment throughout the whole timespan of the trial. This trend is not observed when the other statistical methods are considered. As with the creams, occlusion of the ointments results in increased A.U.C. values. However, this increase is not as marked as for the creams. The A.U.C. value for Synalar ointment is 626,2 for the unoccluded application mode and 905,2 for the occluded mode. Occlusion results in an A.U.C. value which is 1,45 times greater than that of the unoccluded application mode. The increase in area attributable to occlusion for Synalar cream was 2,19. Likewise the increase in area attributable to occlusion for Ointment B was 1,04, compared to the increase observed for Cream B of 1,15 (table 18). Hence occlusion results in a better rate of release of fluocinolone acetonide from cream bases than it does from ointment bases, as may be expected.

Placebo Effect

The high placebo response of petroleum bases has previously been shown.[52,53] In the unoccluded application mode the ointment base produced an area of 5% relative to the steroid containing preparation. This ratio doubled under occlusion to yield a placebo response of 10%. The placebo response attributable to the cream base under occlusion is 5%, and 4% in the unoccluded application mode. The ointment base produced a higher amount of placebo response than the cream base. This may be due to the ointments tendency to hydrate the skin and thus cause a whitening of the skin. This was more evident in the occluded mode than in the unoccluded application mode.

Cream B and Ointment B produce a better blanching response than Synalar cream and ointment, respectively. The differences are not statistically

significant, with the exception of Cream B over Synalar cream in the unoccluded application mode. Base effects account for a maximum of 10%.

TRIAL E. Comparison of commercially available dilutions of triamcinolone acetonide.

Commercially available dilutions of triamcinolone acetonide creams were assessed using the McKenzie-Stoughton bioassay. The preparations studied were Aristocort H.P. (0,5% triamcinolone acetonide), Aristocort R.P. (0,1% triamcinolone acetonice), Aristocort L.P. (0,025% triamcinolone acetonide) and Ledercort-D (0,01% triamcinolone acetonide). Also included in this trial was Synalar cream (0,025% fluocinolone acetonide). Synalar formulations were used throughout this work as the standard preparations.

Five volunteers were assessed in both the unoccluded and occluded application modes by three observers. There were 12 application sites per arm. Figures 11 and 12 represent the blanching profiles obtained from this trial.

It follows that the ranking order obtained from A.U.C. measurements for the same corticosteroid should be the same as the order which is based on concentration. The order should be the same for both application modes. The cream which contains the greatest amount of triamcinolone acetonide is Aristocort H.P. This cream contains more corticosteroid than Aristocort R.P., which in turn is more concentrated than Aristocort L.P. It would be expected that Ledercort-D should give the lowest A.U.C. value since this cream contains the lowest concentration of triamcinolone acetonide tested.

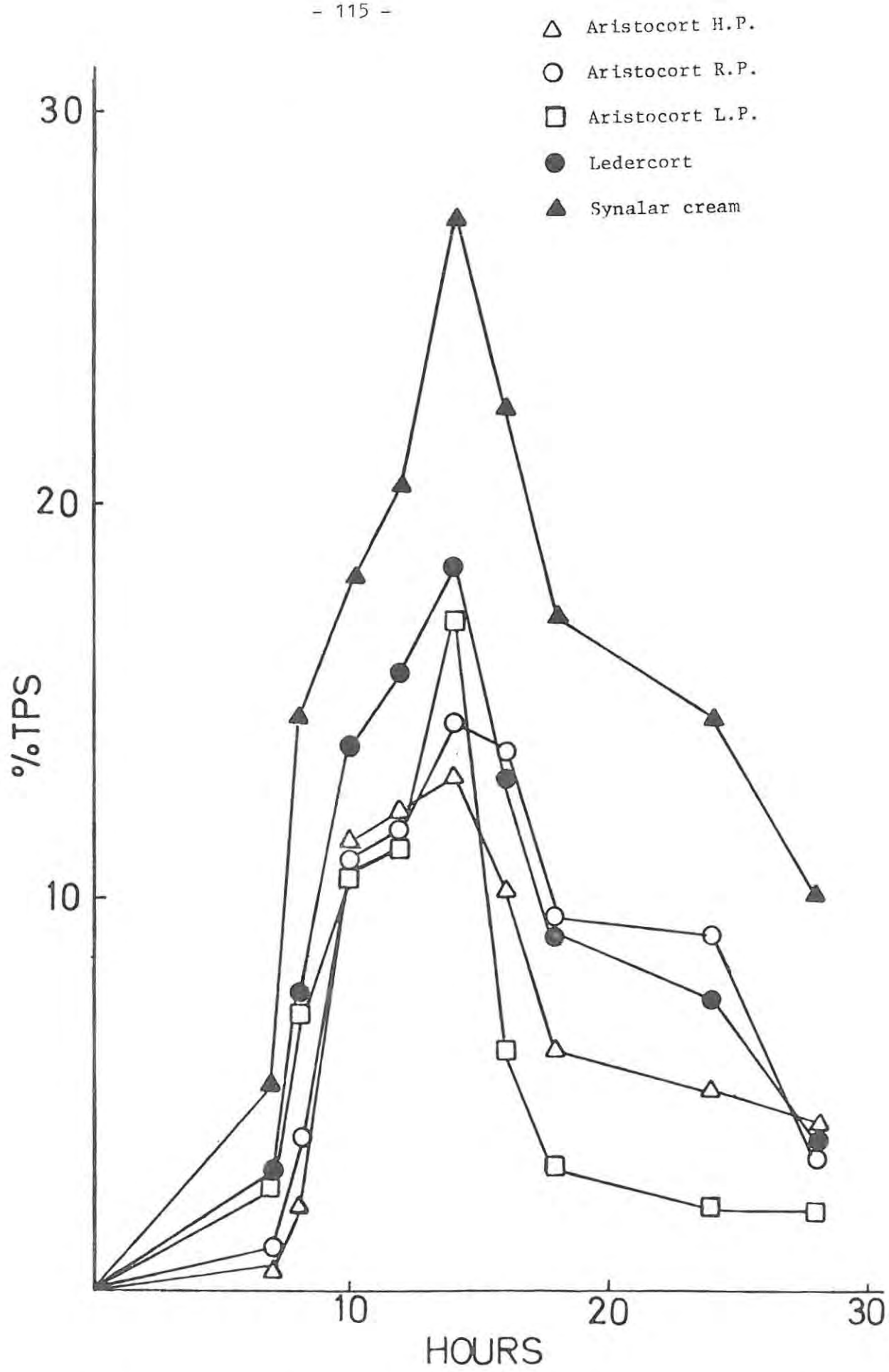


fig 11. Blanching profiles obtained from Trial E. (unoccluded)

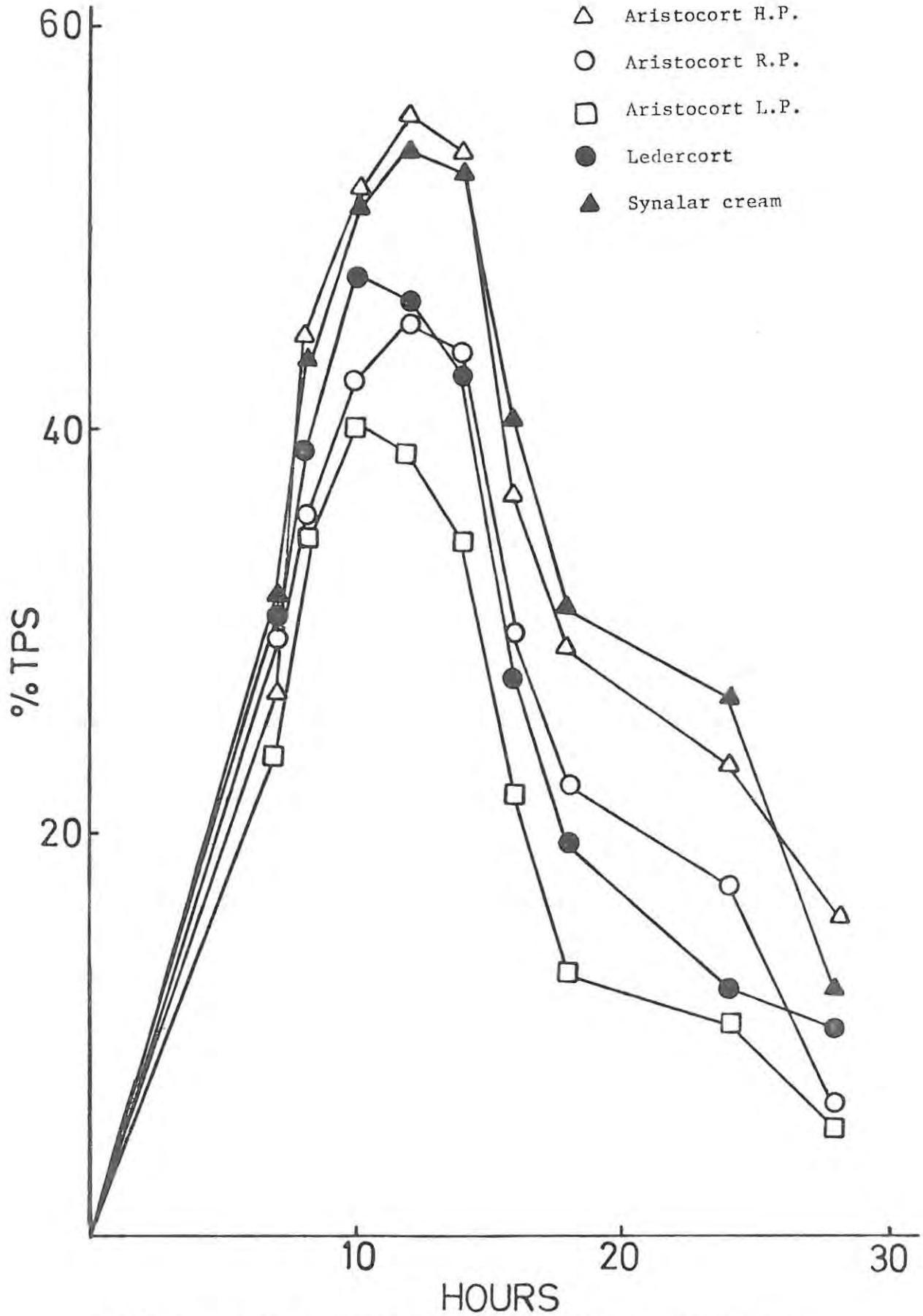


fig 12. Blanching profiles obtained from Trial E. (occluded)

The A.U.C. values (table 22) of the Aristocort creams for both the unoccluded and occluded application modes follow the anticipated ranking order i.e. Aristocort H.P. >R.P. > L.P. However Ledercort-D produces a larger A.U.C. value than the most concentrated preparation, Aristocort H.P. in the unoccluded application mode, while in the occluded mode Ledercort-D is ranked between Aristocort H.P. and Aristocort R.P.

The Aristocort range, which contains the same corticosteroid in the same base, produces predictable trends. When the vehicle composition varies, Aristocort and Ledercort-D bases are different, the ranking order predicted by concentration differences is not observed in that the cream which contains the lowest concentration of triamcinolone acetonide (Ledercort-D) produces an A.U.C. value comparable with the most concentrated preparation, Aristocort H.P. Hence vehicle composition affects the rate of release of triamcinolone acetonide. It would appear as though the corticosteroid is released better from Ledercort-D base than it is from Aristocort base.

Under occlusion the availability of triamcinolone acetonide from the Aristocort base is increased, when compared to Ledercort-D. Since occlusion tends to favour the poorly designed base,[105] this too indicates that Ledercort-D base is a better delivery system than Aristocort base.

The same constituents are used in both bases, but the proportions of these constituents are unknown. However different preservatives are employed. Ledercort-D is preserved with 0,16% methylparaben, 0,04% propylparaben and 0,2% potassium sorbate while the Aristocort base uses 0,1% sorbic acid and 0,1% potassium sorbate. The difference in the release rates from Aristocort and Ledercort-D bases may be due to

different proportions of the same constituents or it may be due to the different preservatives which are used.

Comparison of formulated triamcinolone acetonide with fluocinolone acetonide cream.

Triamcinolone acetonide and fluocinolone acetonide are closely related corticosteroids. The effect of the second fluorine atom in the 6 α position in fluocinolone acetonide is to increase topical activity. Using alcoholic solutions in the blanching test fluocinolone acetonide has an activity of 100 compared to the 75 of triamcinolone acetonide.[17] Coldman, Lockerbie and Laws[106] tested the blanching activities of a proprietary formulation of fluocinolone acetonide and two proprietary triamcinolone acetonide creams. Using an unoccluded technique the fluocinolone acetonide (0,025%) cream gave an uncorrected A.U.C. value of 1316, compared to the 1024 and 700 obtained from the two triamcinolone acetonide (0,1%) creams, giving a ratio of 0,78 and 0,53, respectively, relative to fluocinolone acetonide (0,025%) cream. From this study, Trial E, a ratio of 0,45 was obtained for Aristocort R.P. cream in the unoccluded application mode (table 22).

Barry and Woodford[27] have also assessed fluocinolone acetonide and triamcinolone acetonide cream formulations using an occluded application mode. Two triamcinolone acetonide (0,1%) creams available in the United Kingdom were tested. Vastly different blanching profiles were obtained for the two creams containing the same amount of corticosteroid. Ratios of 1,23 and 0,83 relative to the A.U.C. of fluocinolone acetonide (0,025%) cream were obtained by those workers. A ratio of 0,76 (table 22) was obtained for Aristocort R.P. (0,1% triamcinolone acetonide)

relative to Synalar cream (0,025% fluocinolone acetonide) in the occluded application mode in Trial E.

These two trials and the present study show that the release of triamcinolone acetonide is greatly affected by formulation.

Fluocinolone acetonide cream produces a more intense blanching response and is longer acting than triamcinolone acetonide cream. Increasing the concentration of triamcinolone acetonide in the same base increases the duration of action.

Statistically few differences were noted between Synalar cream and Aristocort H.P. cream in either application mode (tables 20 and 21). Synalar cream was significantly superior to Aristocort L.P. cream from 14 hours onwards, considering both application modes. Only isolated statistically significant differences were observed between Synalar cream and Ledercort-D cream. Generally few statistically significant differences were observed between the Aristocort preparations. As would be expected more differences were seen when Aristocort H.P. was compared with Aristocort L.P.

This trial illustrates the effect of formulation on the release rate of the same corticosteroid derivative from different bases i.e. Aristocort and Ledercort-D. The anticipated ranking order for the Aristocort preparations was observed in both application modes and so it is possible to detect concentration differences using the blanching bio-assay.

TRIALS F and G. Comparative bioavailability of proprietary formulations.

TRIAL F. Comparative bioavailability of betamethasone 17-valerate, fluocinolone acetonide and fluclorolone acetonide creams and ointments.

The blanching activity of four proprietary commercially available topical corticosteroid creams and ointments were assessed. The preparations tested were Betnovate and Celestoderm-V creams and ointments, both of which contain 0,1% betamethasone as the 17-valerate, Topilar cream and ointment, which contains 0,025% fluclorolone acetonide and Synalar cream and ointment which contain 0,025% fluocinolone acetonide. Topilar cream and ointment are prepared in the United Kingdom, while the other three preparations are manufactured in South Africa.

Two trials were performed, one on the creams, the other evaluating the ointments. Six volunteers took part in both trials. The degree of blanching was evaluated by three observers. Each formulation was applied to three sites per arm. Both arms were used, one set of application sites were occluded, the other being left unoccluded.

Creams

The results are depicted graphically in figures 13 and 14.

Betnovate and Celestoderm-V creams, formulations which contain the same corticosteroid in the same concentration, gave almost identical blanching profiles, in both the unoccluded and occluded application modes (figures 13 and 14). These profiles show no significant differences when analysed statistically (tables 24 and 25). This is not entirely unexpected since both these preparations contain the same corticosteroid in the same concentration. As the formulae of their bases are unknown, it may be assumed, that even in the event of different bases having been used, or different manufacturing processes employed, these factors do not appear to influence the rate of release of the active ingredient.

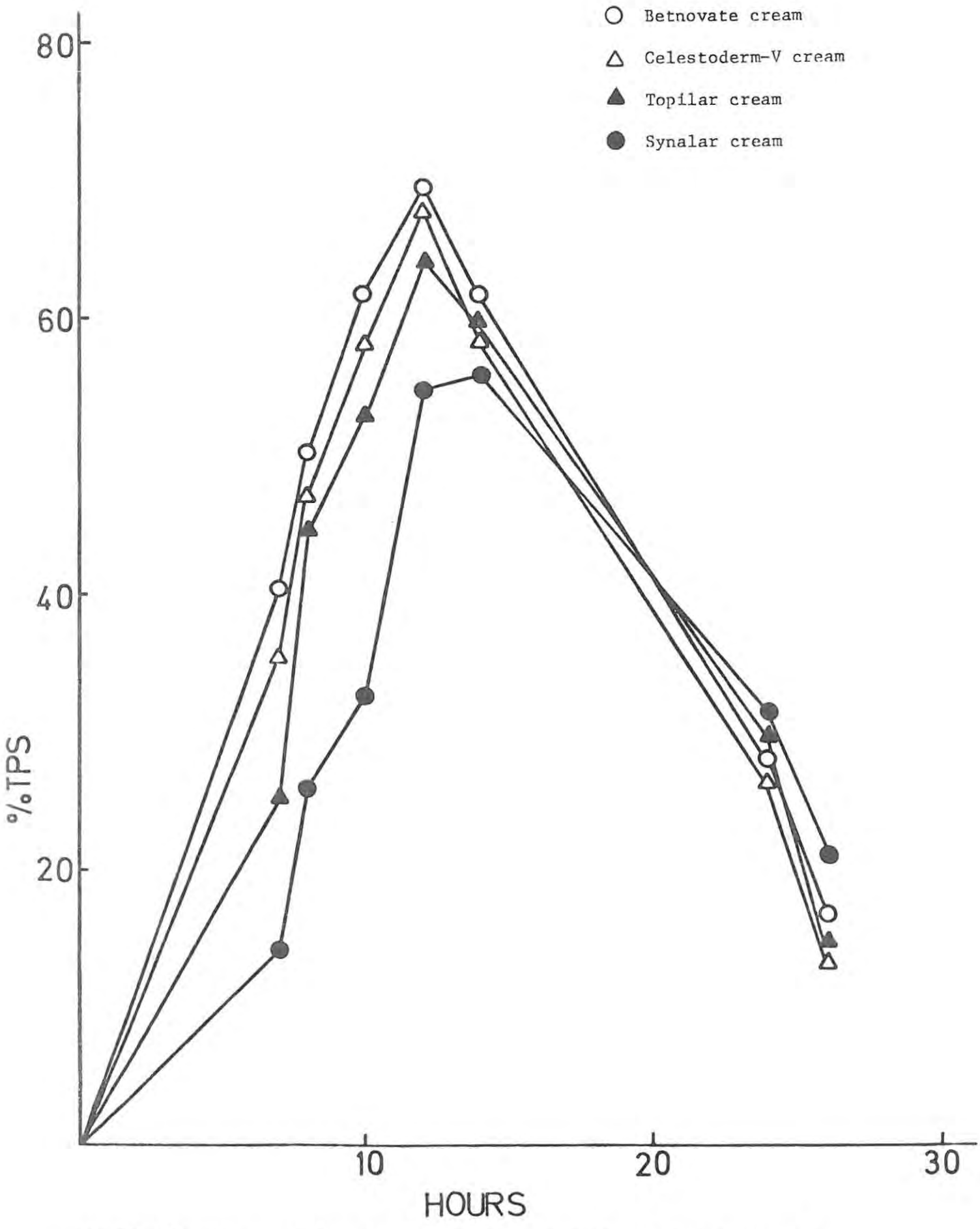


fig 13. Blanching profiles obtained from Trial F. (unoccluded creams)

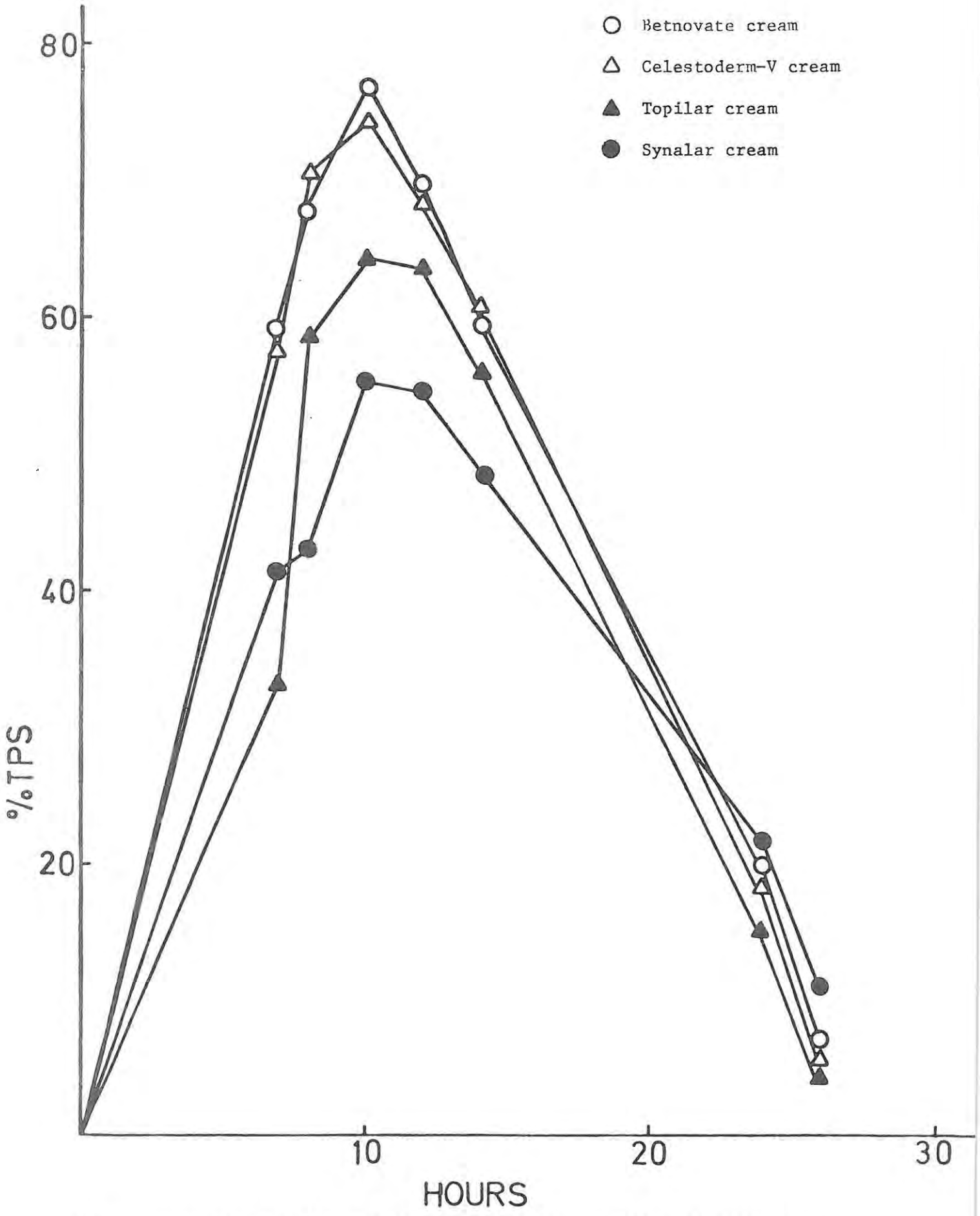


fig 14. Blanching profiles obtained from Trial F. (occluded creams)

Differences in blanching response are seen when the profiles produced by the three different corticosteroids are compared. Betamethasone 17-valerate gave the highest response followed by fluclorolone acetonide and then fluocinolone acetonide. During trials[20] involving the use of alcoholic solutions of fluclorolone acetonide and betamethasone 17-valerate the superiority of the former over the latter was clearly demonstrated. The results found in Trial F show the opposite trend i.e. Betnovate and Celestoderm-V produce a greater degree of blanching than Topilar cream. Similar trials[27] conducted in Britain indicate that Topilar cream shows much greater vasoconstriction than Betnovate cream, as would be expected. The Topilar cream tested here was imported from the United Kingdom and shows a considerably reduced blanching response. This is possibly due to the fact that the cream is formulated for use in a temperate climate and the base may not be a good releasing medium in a subtropical climate. Another possibility is that during the transportation process, extremely wide temperature conditions may be encountered which may cause changes to the vehicle which in turn may account for the change in release rate.

In another study[19] comparing alcoholic solutions of corticosteroids it was found that betamethasone 17-valerate produced a greater degree of blanching than fluocinolone acetonide. Both the present study (Trial F) on the locally produced creams and the study[27] on the same creams prepared in the United Kingdom show that Synalar produces a lower degree of blanching than Betnovate and Celestoderm-V, as would be expected. It must however be borne in mind that Synalar contains 0,025% active ingredient whereas Betnovate and Celestoderm-V contain 0,1%.

The alcoholic vasoconstrictor studies[19,20] rank fluclorolone acetonide above fluocinolone acetonide. Topilar and Synalar creams contain the same concentration of active ingredient, hence the differences in the blanching response can be attributed to the different corticosteroid derivatives used, assuming that the bases have been optimally formulated. Topilar cream produces a better blanching response than Synalar cream, as would be expected from the alcoholic vasoconstrictor studies.

On statistical examination of the unoccluded data for the creams (table 24) significant differences were observed 7-12 hours after application in favour of Betnovate and Celestoderm-V compared to Synalar. These differences became less statistically significant under occlusion (table 25), so once again occlusion masks any differences apparant in the unoccluded application mode.

There are isolated points of statistical significance between Betnovate and Celestoderm-V creams, in both application modes (tables 24 and 25). Betnovate cream produces a marginally larger A.U.C. value than Celestoderm-V cream i.e. the occluded A.U.C. values are 1139,8 and 1126,0, respectively (table 28). This close similarity in response is not unexpected.

Topilar cream is significantly superior to Synalar cream in the period 8-12 hours after application, considering the unoccluded application mode, but with occlusion these differences became non significant.

The results for the cream study indicate that Topilar and Synalar creams produce a less intense blanching response than Betnovate and Celestoderm-V creams. However, if the whole time span of the trial is considered it can be seen that Synalar cream and Topilar cream peak later and have a longer duration of action. The A.U.C. value is a measure of the blanching

of a preparation from zero time to infinite time and hence this parameter can be used to gain an overall measure of the cumulative amount of active ingredient released from any one preparation in the same trial. The A.U.C. values calculated for this trial show that there is little difference in the area produced by the various creams. The ratio of the A.U.C. values relative to Synalar cream in the unoccluded mode are 1,18; 1,07 and 1,04 for Betnovate, Celestoderm-V and Topilar creams, respectively (table 28).

Ointments

The results are depicted graphically in figures 15 and 16.

The blanching profiles of Betnovate and Celestoderm-V ointments should theoretically be approximately the same, as was found with the cream formulations, as they contain the same corticosteroid in the same concentration. Throughout the whole timespan of the trial Celestoderm-V ointment gave a higher blanching response than Betnovate ointment. The better response indicates that the betamethasone 17-valerate is more efficiently released at a greater rate from Celestoderm-V ointment base than from Betnovate ointment base. There were more statistically significant differences in the unoccluded mode than in the occluded study (tables 26 and 27). This is also borne out by their A.U.C. values of 493,1 and 703,4 for Betnovate ointment and Celestoderm-V ointment, respectively, in the unoccluded mode compared to the values of 738,8 and 862,7, respectively in the occluded mode (table 28).

As has been previously noted, the three corticosteroids have been ranked in the order fluclorolone acetonide > betamethasone 17-valerate > fluocinolone acetonide. In both the unoccluded and occluded application modes the ointments follow this expected order, i.e. Topilar ointment

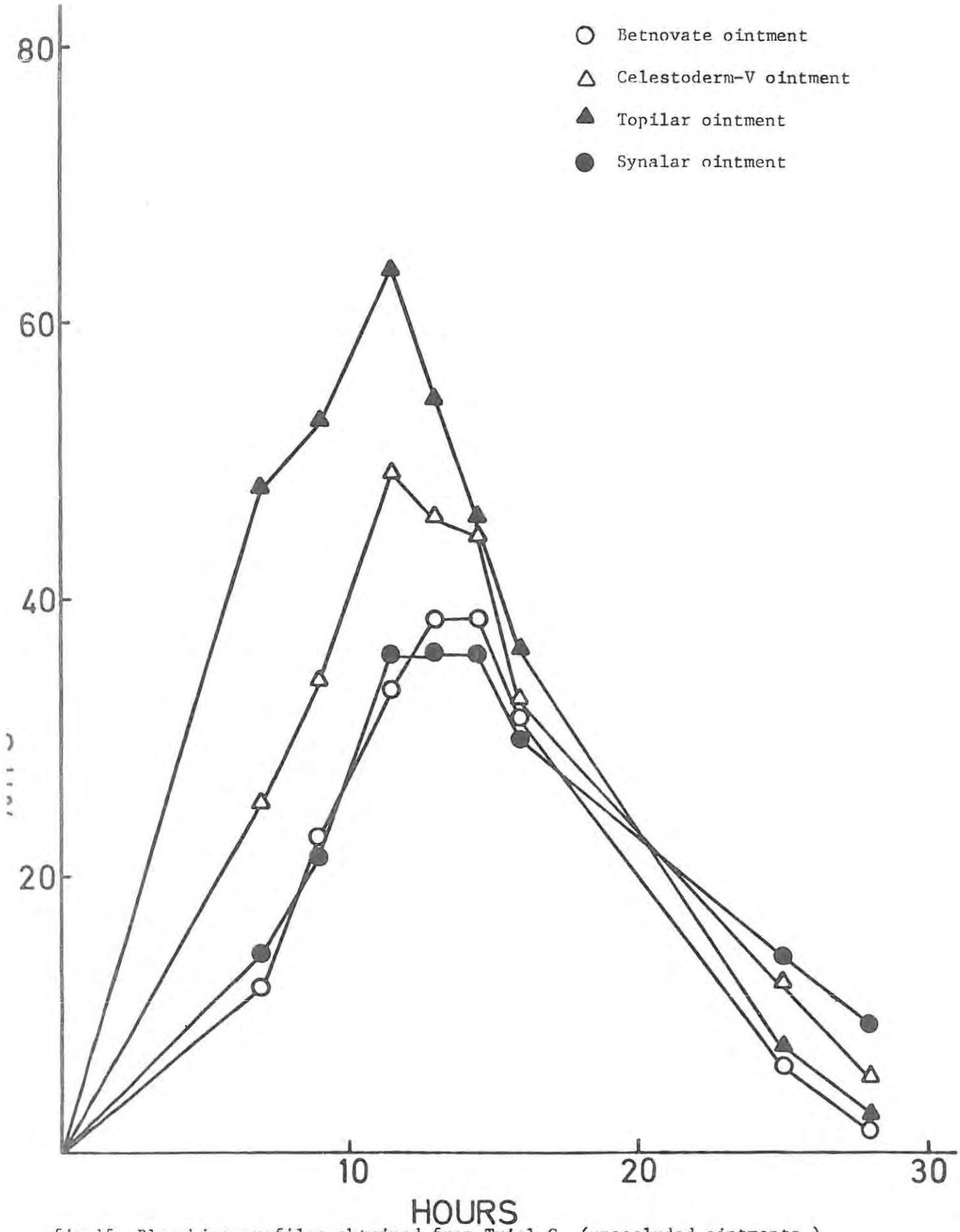


fig 15. Blanching profiles obtained from Trial G. (unoccluded ointments)

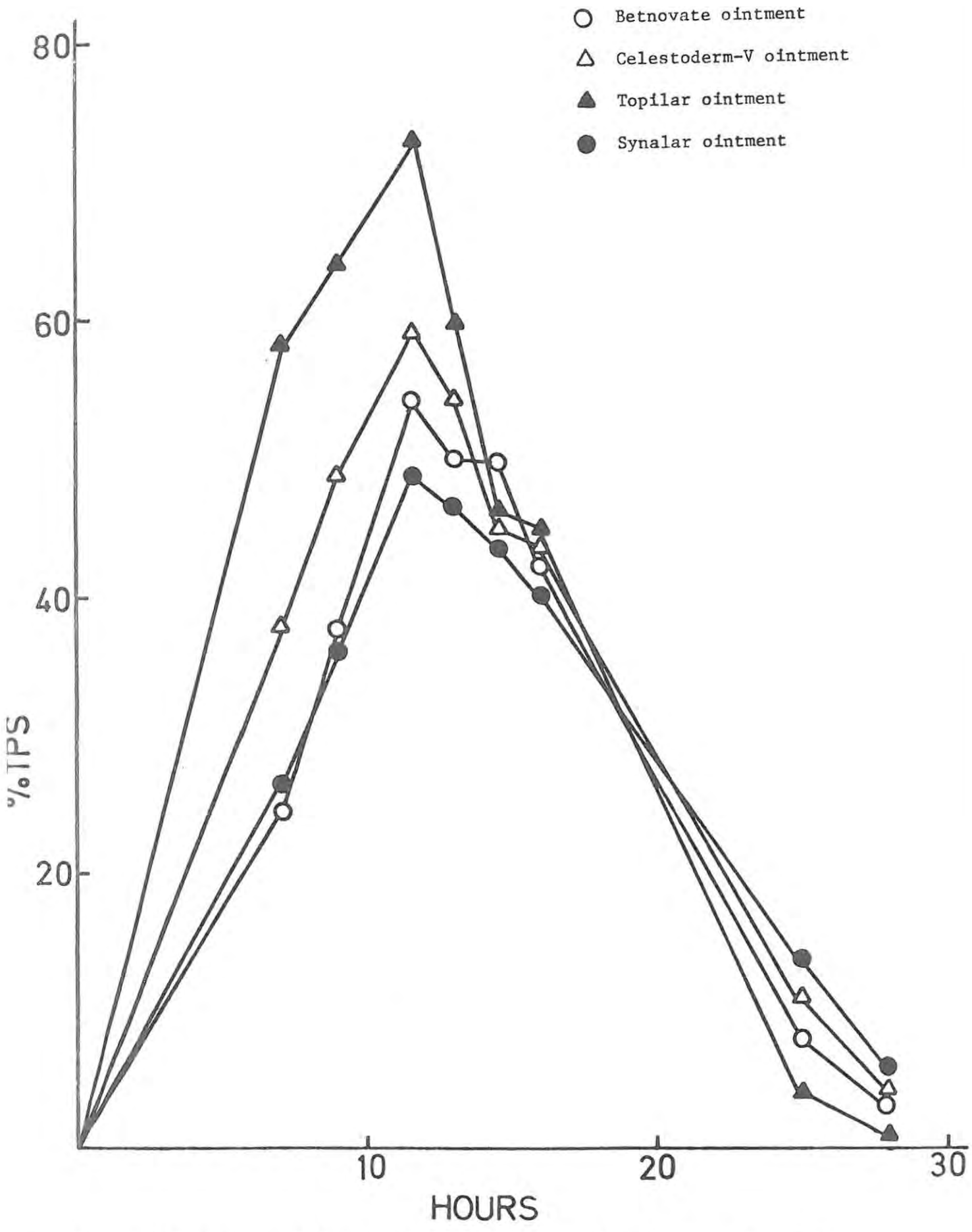


fig 16. Blanching profiles obtained from Trial G. (occluded ointments)

shows a higher degree of blanching than Betnovate and Celestoderm-V ointments, which in turn are higher than Synalar ointment. It is worth noting that the ranking order is followed even though Betnovate and Celestoderm-V contain four times more active ingredient than Synalar and Topilar.

When comparing Synalar ointment with Topilar ointment, a similar situation prevails as did for the cream preparations, i.e. Topilar displays a better initial release rate, but Synalar is longer acting. Hence, by substituting the fluorine atom in the 6 α position in fluocinolone acetonide with a chlorine atom to yield fluclorolone acetonide a more potent compound with a quicker onset of action but with a shorter duration of action is produced. This mirrors the alcoholic vasoconstrictor studies.

It is interesting to note that Topilar ointment gives a very similar blanching profile to the one determined for the same ointment in another study.[28] This would appear to suggest that the ointemnt base is not as susceptible to the various factors which may affect the cream base. Differences in manufacturing procedure could affect release rates. It would appear that the products prepared in South Africa resemble those prepared in the United Kingdom. In general the United Kingdom preparations seem to display a longer duration of action, but the initial release rate and intensity of blanching are similar and always, with the exception of Topilar cream, in the same order.

Of the creams studied there seems to be little difference except that Synalar cream is longer acting than the rest. The Topilar cream base is apparently affected by various factors causing the release of fluclorolone acetonide to be considerably less than previously shown.[27]

In the case of the ointments, Topilar displays a considerably greater response in both occluded and unoccluded modes. In the unoccluded application mode Celestoderm-V ointment displays a better release rate than Betnovate ointment, whereas in the occluded mode there is little difference between Betnovate, Celestoderm-V and Synalar ointments. Synalar ointment produces a larger A.U.C. measurement than Betnovate ointment in both application modes and also shows a more prolonged release in both the occluded and unoccluded modes compared to the other ointments.

TRIAL G. Comparative bioavailability of diflucortolone valerate preparations.

The preparations tested were Nerisone fatty ointment, ointment and cream, prepared in Germany, and Temetex fatty ointment, ointment and cream, prepared in Switzerland. The Nerisone and Temetex formulations contain 0,1% diflucortolone valerate. Synalar cream and ointment, 0,025% fluocinolone acetonide, prepared in South Africa, were also assessed.

Two trials were performed. In the first trial the blanching activities of Nerisone ointment and fatty ointment were compared with those produced by Temetex ointment and fatty ointment. The degree of blanching produced by Synalar ointment was also monitored. In the second trial Nerisone cream was compared with Temetex cream. Synalar cream and Nerisone ointment were also evaluated. Inclusion of the latter formulation permitted intercomparison between the two trials.

Ten volunteers were used per trial. For each volunteer both arms were masked to produce 12 application sites per arm. One set of applications were occluded, the other being left unoccluded. The degree of blanching was assessed by three observers.

Creams

Figures 17 and 18 depict the blanching profiles obtained for the creams.

In the case of the creams no statistically significant differences were noted between Nerisone and Temetex in either application mode (tables 30 and 31). Nerisone cream had a slightly larger A.U.C. value than Temetex in both application modes (table 32). The difference was more marked in the unoccluded application mode, as may be expected from previous trials. Both the diflucortolone valerate cream preparations and Synalar cream show statistical equivalence until about 13 hours after application in both the unoccluded and occluded modes. From 13-28 hours after application Synalar cream shows significant differences over Nerisone and Temetex creams.

Ointments and Fatty Ointments

Figures 19 and 20 depict the results graphically.

Chi-square analysis showed statistically significant differences in favour of both Nerisone and Temetex ointments relative to Synalar ointment from the 7-18 hour period post application in both application modes (tables 34 and 35). From 18-28 hours after application fluocinolone acetonide displays its longer acting effect, and accordingly Synalar cream produces a blanching profile depicting this effect.

There are few statistically significant differences between the ointment and fatty ointment formulations in either the unoccluded or occluded application modes. The ointments always produced a higher degree of blanching than the fatty ointments (table 36).

There are no statistically significant differences between Nerisone and Temetex ointments, or between Nerisone and Temetex fatty ointments in

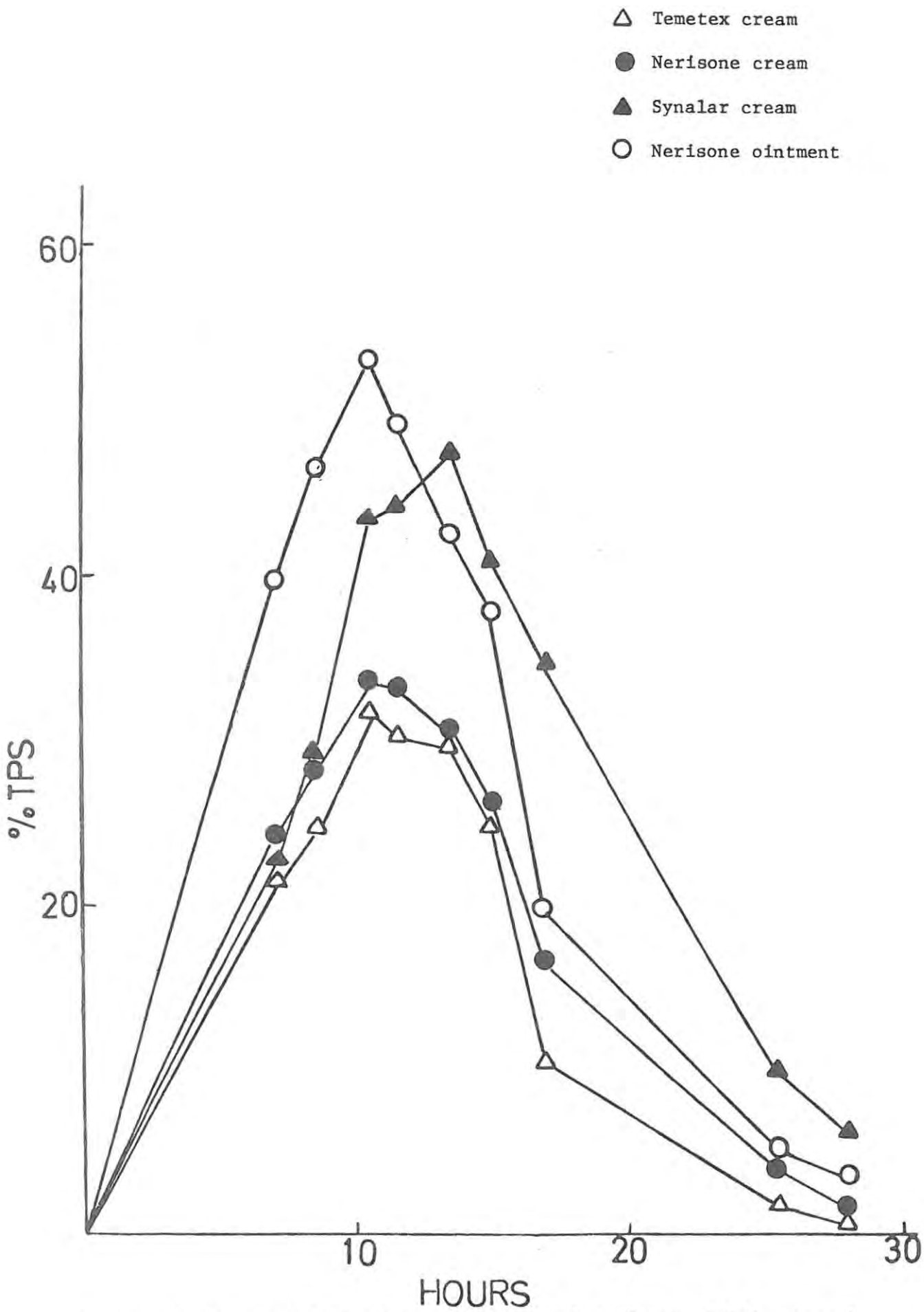


fig 17. Blanching profiles obtained from Trial G. (unoccluded creams)

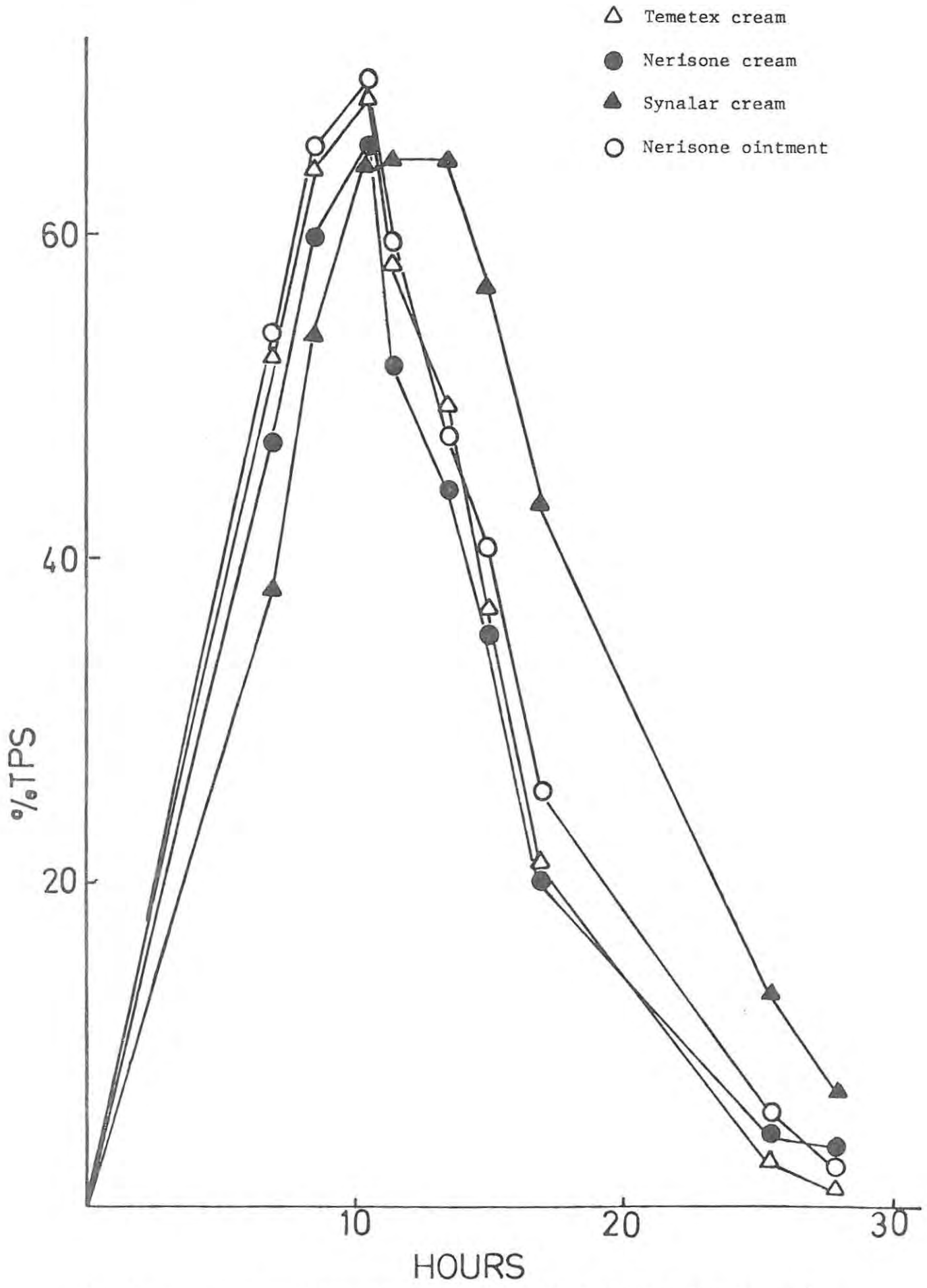


fig 18 Blanching profiles obtained from Trial C (occluded creams)

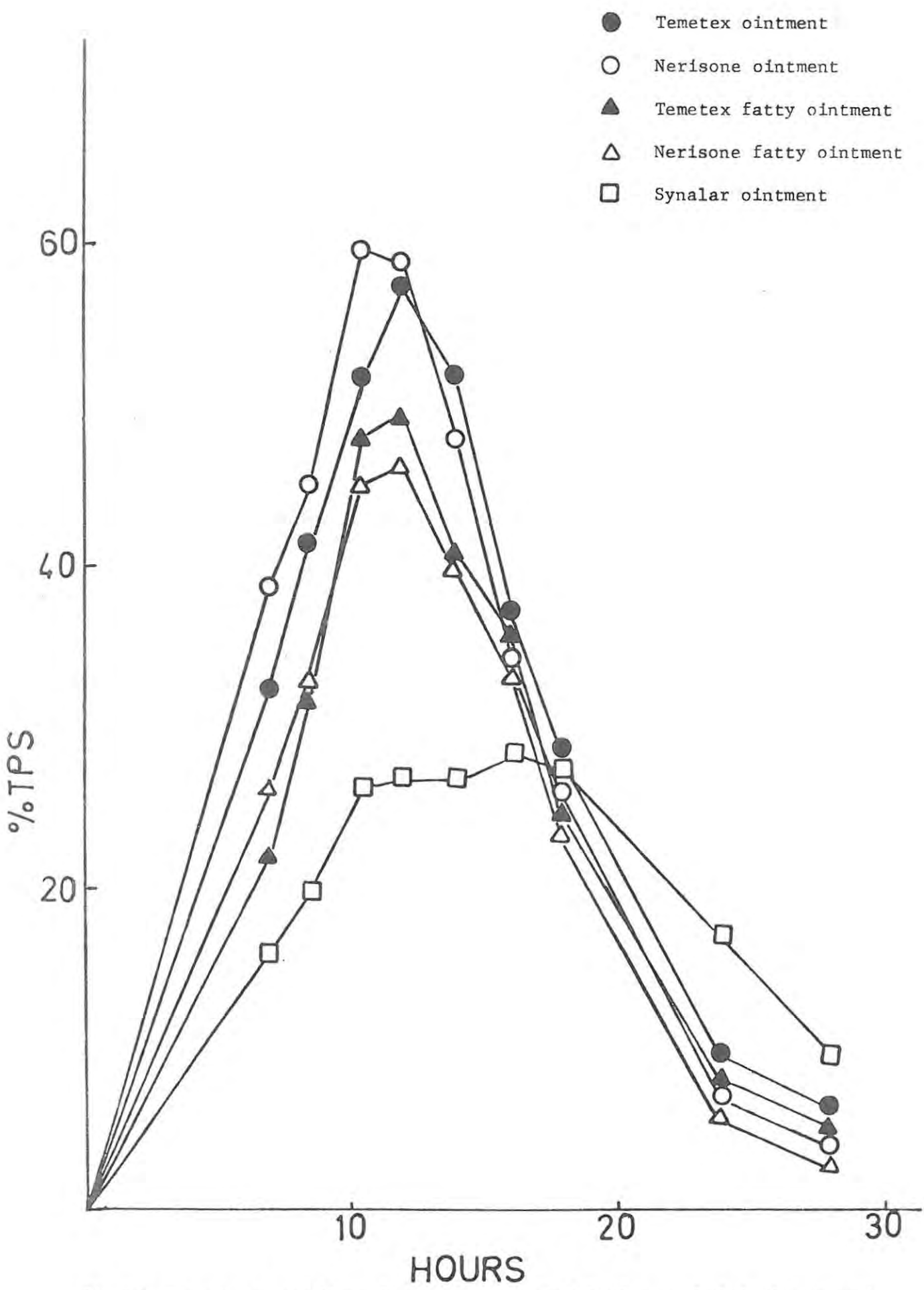


fig 19. Blanching profiles obtained from Trial G (unoccluded ointments)

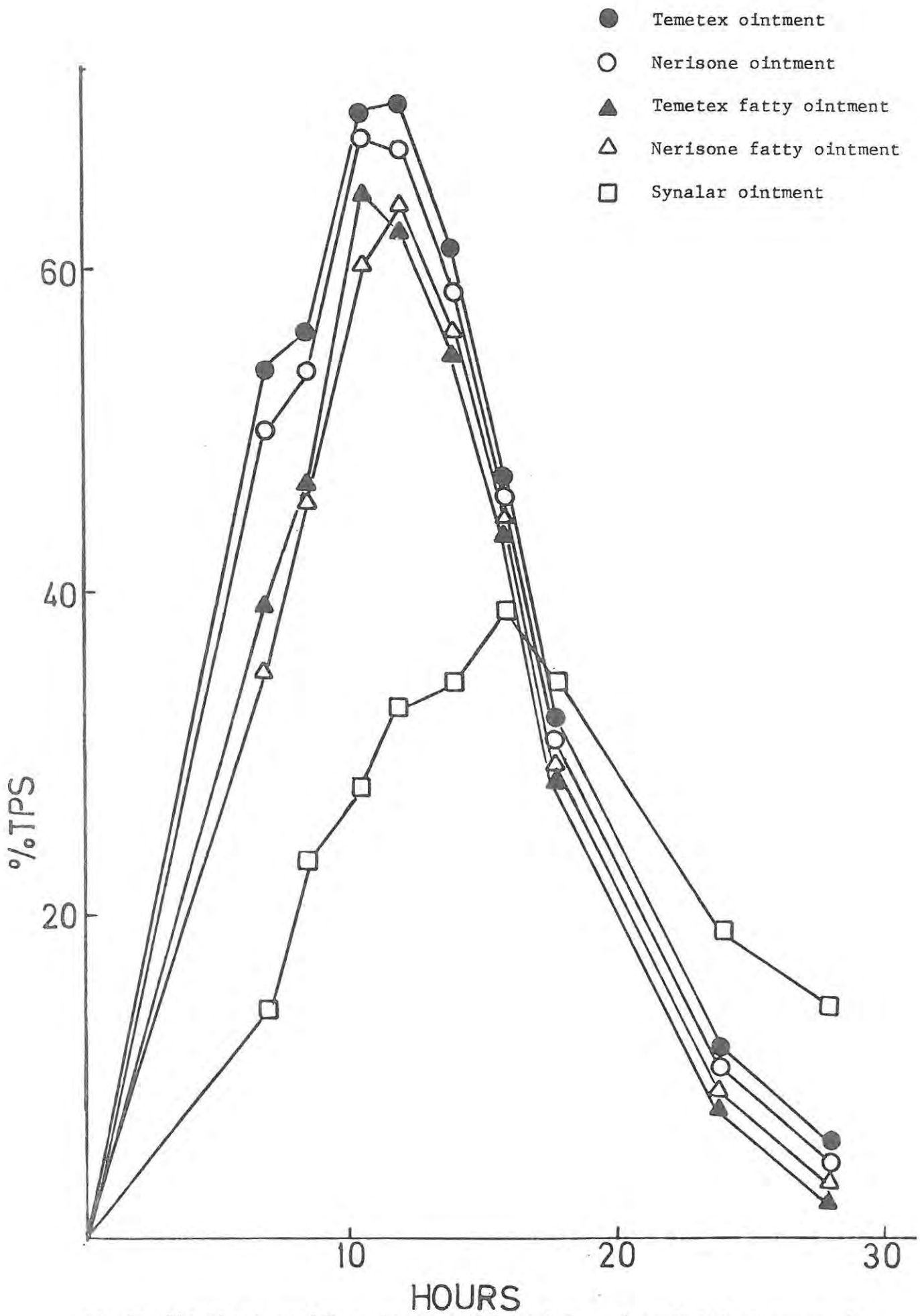


fig 20. Blanching profiles obtained from Trial C (occluded ointments)

either of the application modes. It is interesting to note that Temetex ointment produces a higher A.U.C. value than Nerisone ointment in both application modes as does Temetex fatty ointment compared to Nerisone fatty ointment (table 36). Conversely, in the cream study, Nerisone cream produces a larger A.U.C. value than Temetex cream.

Creams and Ointments

No statistically significant differences were noted between Nerisone ointment and cream in the occluded mode (table 31). In the unoccluded application mode Nerisone ointment demonstrated a higher degree of blanching than Nerisone cream (table 32). This is expected due to the occlusive nature of the base. In this application mode scattered differences were observed. In the unoccluded application mode the A.U.C. values for Nerisone ointment and cream were 494,99 and 462,40, respectively. The order is reversed under occlusion, and areas of 775,91 and 833,40, respectively were obtained (table 32). Occlusion enhances the rate of release of corticosteroids from cream bases more so than from ointment bases.

The blanching profiles for both the cream and ointment study indicate that diflucortolone valerate shows a more rapid rate of release from the vehicle, associated with a higher degree of blanching and a more rapid removal from the site of action when compared to fluocinolone acetonide. The rapidity of onset of blanching has previously been demonstrated.[107]

In this study generic equivalence is shown between the various diflucortolone preparations. There were few statistically significant

differences between the ointments, fatty ointments and creams. Hence the formulation most suited to the skin condition can be employed i.e. cream bases which have a low fat and high water content are suitable for application to acute and weeping conditions.

2. ANALYTICAL METHODS

A number of quantitative assays, colorimetry, ultraviolet spectrophotometry and high performance liquid chromatography, were performed on formulated fluocinolone acetonide preparations.

The colorimetric method is not a direct analysis. It depends upon the addition of colour forming agents to the corticosteroid derivative. Colour development proceeds at a slow rate. Development is halted after one hour by the addition of glacial acetic acid. Since colour development is dependent upon a stoichiometric relationship, any variation in the amount of the two colour reagents added will affect the intensity of the colour reaction. Likewise, variations in development time may affect colour intensity.

Since colour development is stopped after a specific time the total concentration of corticosteroid is not determined. However, this is justifiable as the standard preparation is treated in an identical manner, and the analysis is based on the comparison of the absorbances of the test and standard solutions.

The crude extract is treated with the colour reagents, accordingly it may be possible that the base components may interfere with colour formation. This apparently did not occur with the preparations studied here.

In the ultraviolet assay it is necessary to ensure that the only species absorbing in the ultraviolet region is the corticosteroid if a quantitative analysis is to be performed on formulated products. Accordingly it is necessary to pass the extract through a chromatographic column in order to separate out the base components from the active ingredient.

The mobile phase used to elute the extract through the column is comprised of solvents which exhibit strong absorbances in the ultra-violet region, hence it is essential to use spectroscopically pure solvents and to ensure that they are absent when the corticosteroid is analysed.

The U.S.P. XIX imposes a restriction on the purity of the mobile phase by giving an absorbance value which cannot be exceeded after redissolving in alcohol an aliquot of the mobile phase which has been evaporated to dryness. This acts both as a check on the mobile phase purity and on the efficiency of evaporation. In order not to exceed the specified absorbance value it is necessary to evaporate under reduced pressure, with the aid of heat ($60^{o}C$). This must be carefully performed as the chloroform portion of the mobile phase tends to bump.

This assay, like the colorimetric analysis, is a comparative technique, with the standard being subjected to the same procedure as the test extract.

Both of the foregoing analyses involve numerous manipulative procedures and are lengthy, taking 4-6 hours per analysis.

Variations in the absorbances of aliquots of the same sample may be due to different amounts of colour reagents being added in the case of the colorimetric assay, or, in the case of the ultraviolet assay, varying amounts of the mobile phase being left behind after evaporation. In spite of these inherent variables close correlation was obtained for the various formulations when analysed by these two techniques.

High performance liquid chromatography is both a quantitative and separatory technique, hence it is possible to eliminate repetitive

isolation steps. Extraction need only remove insoluble materials. Reverse phase chromatography is useful for the analysis of any sample which is sparingly soluble.

The average analysis time is short. A complete assay takes 1-2 hours. The time taken for the injected sample to be analysed is in the order of 15 minutes.

Use was made of the internal standard method of quantitation. This compensates for varying injection volumes and day to day instrumental changes. In this method the sample peak of interest is quantitated relative to the internal standard. The area ratio between the sample peak and internal standard will always remain constant, and is independent of injected volume. This is a great advantage over the external standard method, where injection volume must remain constant.

In the case of Cream B a flow rate of 100 ml/hr was used. Due to the inclusion of different preservatives in Synalar cream, the flow rate was reduced to 60 ml/hr to achieve complete separation. At the latter flow rate the area ratio between the sample peak and internal standard is different. This is due to the broadening of the sample peak relative to the internal standard peak. Here difficulty is encountered by the electronic integrator in determining when the slope changes sufficiently for the integrator to start and stop calculating the area under the peak.

High performance liquid chromatography is not without its drawbacks. The cost of the instrument and solvents are high. Columns, which are also costly, are subject to deterioration and hence operating conditions may vary. Solvent concentrations too, should be accurately determined, as often small changes in solvent polarity will greatly affect separation.

The main advantage of H.P.L.C. lies in its ability to perform rapid, precise and reliable assays. Simple extraction procedures can be employed.

The content of fluocinolone acetonide in the formulation tested fell within the U.S.P. limits of 90-110% of the stated strength irrespective of analytical technique used. H.P.L.C. has the advantage of reduced analysis time and high reproducibility. In the absence of this costly instrumentation colorimetry would appear to be the next best analytical method of choice as interaliquot variation is less than with the ultraviolet method. In addition the ultraviolet method calls for more manipulations and is therefore more time consuming than the colorimetric method.

E. SUMMARY

Therapeutic equivalence or equal biological activity cannot necessarily be inferred from equivalence in the chemical constitution of different formulations of the same drug i.e. generic equivalence cannot always be assumed. Equivalence in blanching activity was shown for diflucortolone valerate preparations in that Temetex formulations and Nerisone formulations produced almost identical blanching profiles even though manufactured in Switzerland and Germany, respectively. The various triamcinolone acetonide preparations exhibited inequivalence. The most dilute cream, Ledercort-D, produced a blanching response equivalent to a preparation containing 50 times more corticosteroid, Aristocort H.P., in the unoccluded application mode. Accordingly formulation can affect the rate of release of a corticosteroid from a topical delivery system.

The same proprietary formulation manufactured in different countries may show different release characteristics due to different methods of manufacture, variations in raw materials, and in active ingredients. Similar trends were generally observed between preparations manufactured in South Africa and those prepared elsewhere, mainly in the United Kingdom. Topilar cream, manufactured in the United Kingdom performed poorly in the blanching test, while the corresponding ointment produced a similar blanching response to that reported for Topilar ointment in the United Kingdom. The performance of Topilar cream in the blanching assay may be due to deterioration during transport, or climatic variation.

The effect of propylene glycol on the release rate of fluocinolone acetonide from various bases was monitored. Undersolubilization of the corticosteroid affects its release from ointments to a greater extent than from creams.

Occlusion hydrates the stratum corneum thereby allowing for better percutaneous absorption. The occlusive nature of the ointment base enables the ointment to perform better in the blanching test than the cream in the unoccluded, therapeutically employed application mode. However, occlusion with an impermeable dressing results in creams displaying better release characteristics than ointments. Hence, in most cases occlusion of creams results in the best release rate of the steroid from the base.

Formulated fluocinolone acetonide preparations were quantitatively analysed by colorimetry, ultra-violet spectrophotometry and high performance liquid chromatography. High performance liquid chromotography would appear to be the most efficient technique. Analysis time is short, while precision and reproducibility are good.

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