

PLETHYSMOGRAPHIC RECORDINGS OF SKIN PULSES

IV. *The Vasoconstrictive Effect of Steroids in Normal and Stripped Skin*

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Abstract. Plethysmographic measurements of the vasoconstrictive activity of hydrocortisone acetate, betamethasone-17-valerate, fluocinolone acetonide and fluoclorolone acetone on normal and stripped skin, are described. The vascular action is considered to be evidence that the steroids have reached the dermal vessels and the results are thus related to percutaneous absorption. In normal skin, significant constriction of the pulsating vessels was not consistently obtained by the present method. The results indicate that the clinical blanch produced in normal skin during the vasoconstriction test, is, for the most part, due to decongestion of the capillaries only. On stripped skin the results were more consistent and hydrocortisone acetate produced a marked vasoconstrictive effect which reached its maximum within 30 min, versus 10 hours for the fluorinated steroids. The data are compatible with an increase in steroid concentration adjacent to the dermal vessels. In the present study the steroids, in particular betamethasone-17-valerate, seemed to induce special changes in the form of the pulse wave.

In previous papers the results of photoelectric and piezoelectric pulse recordings from psoriatic skin were reported (22, 23). It was shown that corticosteroids produced a reduction in pulse height indicating vasoconstriction of the dermal vessels and a marked difference in vascular action between betamethasone-17-valerate and hydrocortisone acetate was demonstrated. The vasoconstriction is induced after penetration of the epidermal barrier. Consequently the results may be related to percutaneous absorption.

Preliminary investigations performed by the present author on normal skin of the forehead, indicated that similar measurements were possible by the piezoelectric method (21). In the course of studies, however, it appeared that the results were inconsistent. This may be related to the particular mode of blood supply in this region as described by Illig (10). It was therefore decided

to examine other and more suitable skin sites and to use a photoelectric plethysmograph with a particular sensitive photocell. In the present investigation the vascular effect of hydrocortisone acetate, betamethasone-17-valerate, fluoclorolone acetone and fluocinolone acetone has been measured plethysmographically on (a) normal skin, and (b) following removal of the skin barrier by stripping.

MATERIAL AND METHODS

Sixty-four skin sites in 8 healthy subjects aged 22-50 years were examined after application of the following steroids: betamethasone-17-valerate 0.1% in ointment base (Betnovate®—Nyco, Nyegaard & Co. A/S, Oslo, Norway), fluoclorolone acetone 0.05% (in stearyl alcohol, polyethylene glycol 4 000, glycerin and propylene glycol),¹ fluocinolone acetone 0.2% in cream base (Synalar forte®—Imperial Chemical Industries Ltd., Macclesfield, Cheshire, England) and hydrocortisone acetate 1% in petrolatum. The flexor aspect of the forearms was selected as test area, 4 sites measuring 2 sq. cm. being used on each arm. The penetration of each steroid was studied in different persons at 8 normal and 8 stripped areas. Stripping was done with cellophane tape until the skin surface appeared red and glistening. The test sites were delineated with a violet pen and covered with polythene sheets secured by adhesive occlusive strapping. Following application of ointment on the first day only and during continued occlusion, measurements on normal skin were performed every day for periods up to 6 days at 32 sites in total. On stripped skin, during continued application of ointment and occlusion, measurements were performed at 32 other skin sites. The vascular effect produced by hydrocortisone acetate was examined every 20 min during the first hours. Examinations were made every second hour following application of the other steroids. All measurements were made on seated pa-

¹ Kindly supplied by Syntex Pharm. Ltd., Maidenhead, Berks. England.



Fig. 1. Pulse curves recorded from normal skin of the forearm. Dichrotism and rebound phenomenon are observed.

tients with the forearm resting comfortably in a horizontal position at heart level. The room temperature was kept constant at 25°C ($\pm 1^\circ$). Further precautions were taken as mentioned in previous reports (22, 23).

The cutaneous blood flow was measured by photoelectric plethysmography. The principles of this method have been outlined previously (22). The emitted light penetrates the skin and is reflected back to the photodetector. The reflected light fluctuates with the pulsating blood flow of the skin and is sensed by the photocell as a pulse wave. The resistance of the semiconductor varies inversely with the light intensity. Vasodilatation is indicated by an increase in pulsation and vasoconstriction by a decrease. As the pulse height varies interindividually and also from one skin site to another, only recordings from the same areas were compared.

In the present device which was secured by a constant power supply, cadmium sulphide was used as photoconductive material (16). To avoid a possible influence on the skin blood flow by heat generation from the light source, the 0.5 W lamp was placed behind the photoconductive material. No measurable effect on skin temperature was produced by the photocell as controlled by skin thermometry. The photocell described is very light-sensitive and makes the apparatus even more suitable for measurements than the photo-field-effect transistor employed in studies on diseased skin (21, 22).

RESULTS

Following application to normal skin, distinct pallor was produced by all fluorinated steroids during the whole period of investigation. The pallor produced by hydrocortisone acetate was less pronounced and of short duration only. It was absent at 4 skin sites. By pulse plethysmography

large pulsations could be recorded at all areas of intact skin (Fig. 1). The curves were distinctly outlined illustrating the great sensitivity of the photocell. In fact, the pulsations had to be dampened. No significant reduction in pulse height could be measured during the treatment. Dichrotism and accentuated rebound phenomenon were observed at some areas.

On stripped skin the fluorinated steroids induced pronounced blanching extending beyond the stripped areas. It was less pronounced by hydrocortisone acetate but no further attempts were made to quantitate the degree of pallor. Plethysmographically, great variations in periods of latency and duration of maximal response was observed from one site to another. As opposed to the results obtained on normal skin, hydrocortisone acetate induced a marked reduction in pulse height on stripped skin during the first 30 min (Figs. 2 and 3). This effect was still detectable after 20 hours. At this moment the occlusive bandage was removed and control measurements taken 4 hours later showed a significant increase in pulse height. Following application of the

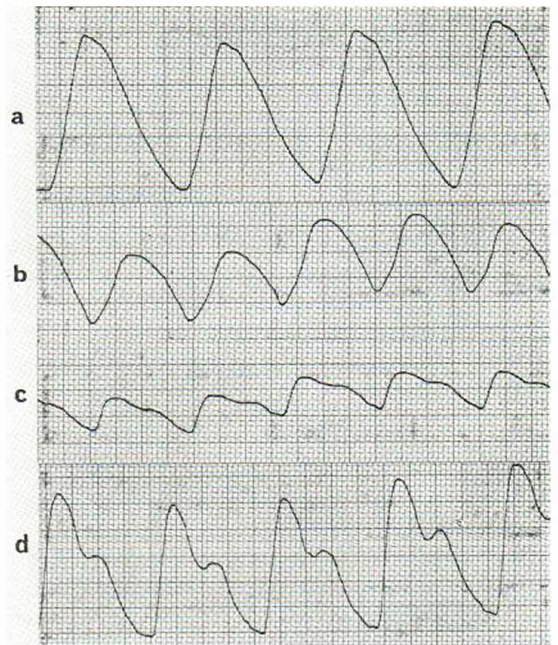


Fig. 2. Effect of hydrocortisone acetate 1% on the cutaneous blood flow. (a) After stripping. (b, c) At 20 min and 20 hours' occlusion. (d) Four hours after removal of occlusion and 24 hours after application of ointment.

fluorinated steroids a latent period was observed before vascular action could be recorded (Fig. 4). The maximum effect was obtained within 10 to 14 hours. Following betamethasone-17-valerate the pulse wave changed into 3 small waves distinctly separated from each other (Fig. 5). This did not occur after the other steroids which generally produced a small, double-wave or merely a flattening of the pulse curve (Figs. 6 and 7).

Recordings taken at 20 hours' occlusion were uniform for all steroids and showed that the same degree of vasoconstriction was still detectable. Four hours after removal of the occlusive bandage the pulsations increased, indicating vasodilatation. No significant difference in the vasoconstrictive activity between the various fluorinated steroids could be observed.

DISCUSSION

The blanching produced in normal skin by the vasoconstriction test has proved to be a very sensitive physiological marker for evaluation of new steroids (15). However, as mentioned by Stoughton et al. (20), the opinions concerning the mode of action in inducing skin pallor are controversial. It has been suggested that steroids may act partly by decongestion the capillaries of the dermal papillae (1). The piezoelectric and photoelectric measurements previously performed by

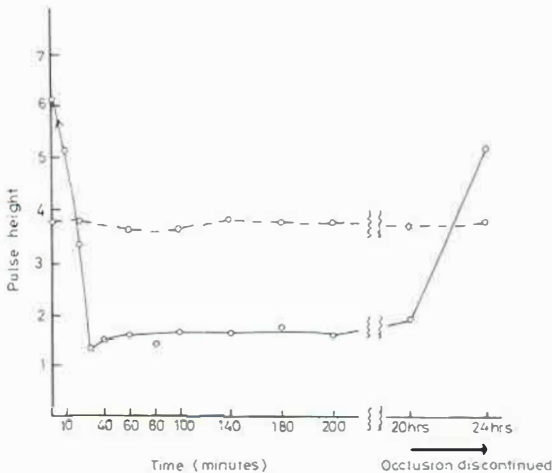


Fig. 3. Results of plethysmographic measurements on normal and stripped skin showing the vascular effect produced by hydrocortisone acetate 1% shortly after application. ○—○, Normal skin; □—□, stripped skin.

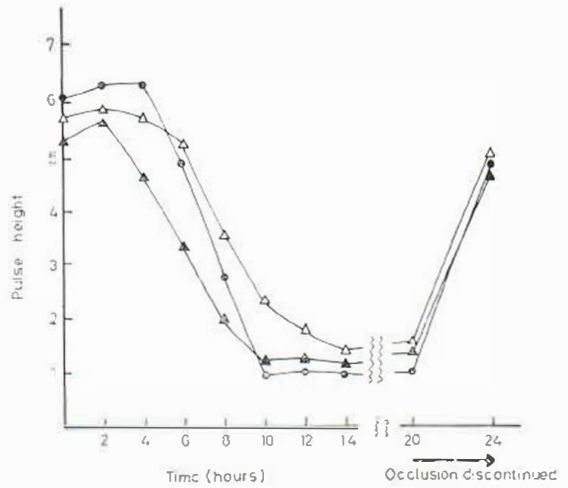


Fig. 4. Results of plethysmographic measurements on stripped skin showing the vascular effect of the fluorinated steroids. The pronounced vasoconstriction is indicated by a decrease in pulse height. ●, Betamethasone-17-valerate 0.1%; △, flucinolone acetonide 0.2%; ▲, flucorolone acetonide 0.05%.

the author on patients with psoriasis (23) are consistent with this theory. These measurements disclosed only a low degree of vasoconstriction after 24 hours' occlusion with betamethasone-17-

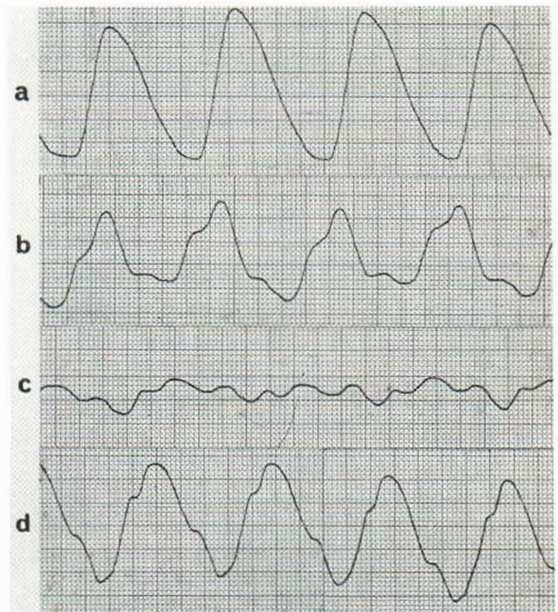


Fig. 5. Effect of betamethasone-17-valerate 0.1% on the cutaneous blood flow. (a) After stripping. (b, c) At 6 and 20 hours' occlusion. (d) Four hours after removal of occlusion and 24 hours after application of ointment.

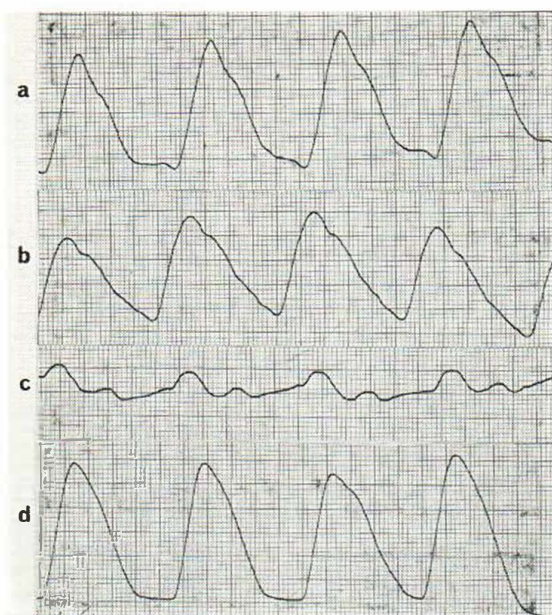


Fig. 6. Effect of fluoclorolone acetone 0.05% (a) After stripping. (b, c) At 4 and 20 hours' occlusion. (d) Four hours after removal of occlusion and 24 hours after application of ointment.

valerate although the treated skin area appeared distinctly pale. During the next 3 days a marked reduction in pulse height was observed, suggesting an increase in concentration of steroid or metabolites adjacent to the dermal vessels. Photoelectric measurements performed after 12 hours' occlusion with betamethasone-17-valerate further confirmed this theory and substantial evidence was provided for the view that the blanching observed at this moment in psoriatic skin was due to decongestion of the capillaries. Vasoconstriction of the pulsating dermal vessels was consequently produced at a later moment.

However, many questions related to this matter are still unsolved. Stoughton et al. (20) have suggested a predominant role of the deeper vessels for the blanching phenomenon produced by catecholamines. Demis et al. (5) using capillary microscopy, stressed the importance of venoconstriction in addition to contraction of precapillary sphincters and arterioles. The results previously obtained by means of pulse plethysmography are compatible with the view that the deeper vessels participate in adrenaline-induced pallor (23). Cummings (4) utilized this method in measure-

ments of penetration of a histamine-releasing vasodilator through normal skin. The end-point of skin-traverse was indicated by the vasodilatory effect produced by the substance. He suggested that erythema and wheal formation were not directly related to the pulsatile arteriolar flow but rather to non-pulsatile components of the circulation. Furthermore, one must assume that the amount of oedema in epidermis and dermis and the filling state of the veins have some influence.

Percutaneous absorption involves the passage of materials from the outside through the entire thickness of the skin and into the circulation. The main barrier is situated in the stratum corneum and the molecules move across this barrier by passive diffusion (2). The steroid molecules possess large molecular volumes and usually several polar groups which lower the diffusion rates (3). As shown by Scheuplein et al. (18) the amount of steroid diffusing through skin appendages, i.e. shunt diffusion, is of major importance particularly during the initial period, but may control percutaneous absorption in later phases also. The pallor induced by occlusion in the vasoconstrict-

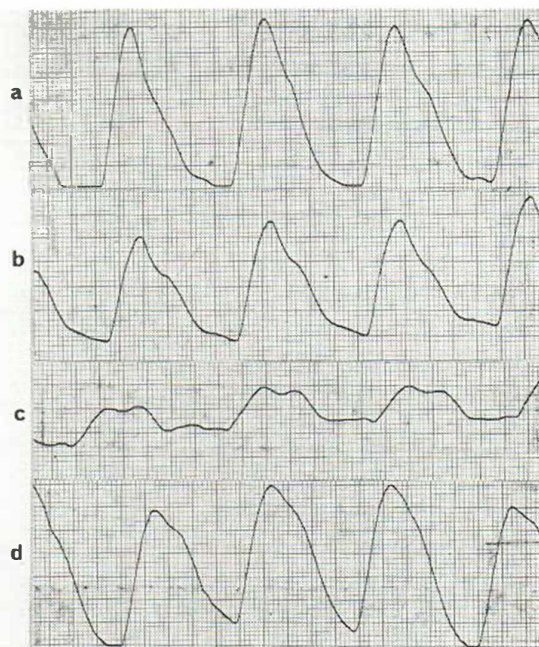


Fig. 7. Effect of fluocinolone acetone 0.2% cream on the cutaneous blood flow. (a) After stripping. (b, c) At 6 and 20 hours' occlusion. (d) Four hours after removal of occlusion and 24 hours after application of cream.

tion test is probably a result of the shunt diffusion (18).

The vascular action of the steroids is produced after penetration of the epidermal barrier and probably within a distance of 250 μ from the skin surface (2). Normally the molecules are rapidly removed by the blood stream which maintain a large concentration drop across the barrier facilitating diffusion. But this process is in part counteracted by the steroids, since they induce vasoconstriction and consequently reduce the blood flow. This may in turn lead to prolonged tissue retention and an increase in concentration adjacent to the dermal vessels. Furthermore, this effect will be enhanced by an increase in diffusion distance, brought about vasoconstriction in the upper part of the dermis. Plethysmographic measurements performed by the author on psoriatic skin (23) and the data obtained in the present study support this interpretation.

In the present investigation no significant vasoconstriction could be obtained on normal skin. This may indicate that (a) the vasoconstriction induced is too small to be measured by the present method, or (b) the pallor produced by the steroids is due to constriction of non-pulsatile vessels. Considering the great sensitivity of the photoplethysmograph employed (16), however, it seems reasonable to conclude that the pallor induced in the vasoconstriction test is due mainly to decongestion of the capillaries and small veins beneath the epidermis. Normally this effect produces an increase in pulsations (16) allowing more light to be reflected from the pulsating and deeper situated vessels. Accordingly a slight increase was observed on stripped skin during the first hours after application. In normal skin the pulsations are much weaker than in psoriasis and the non-pulsating vessels are not congested. Consequently the vascular effect will be less pronounced. Presumably the pulse curve would have been influenced if distinct constriction of the pulsating vessels had taken place. It is possible that the dichotism observed and the accentuated rebound phenomenon are related to the pallor induced. A decongestion of the veins will alter the curve in a direction corresponding with the arterial pulsations.

The results obtained have to be evaluated on the basis of the different concentrations and vehicles applied although the molecular charac-

teristics are probably more important (8). The influence of formulation and selection of vehicles have been discussed by Sarkany & Hadgraft (17) and they have shown that improved absorption may result from a correct prescription. Furthermore, variations in vascular action may be connected not only with biological variations of the various skin areas but also with different degrees of spreading of the substances applied. The physical spread of the preparation on the skin is difficult to control and may interfere with the area and degree of vasoconstriction produced. In the present study this seems to be of minor importance since large amounts of preparation remained on the skin during the investigation.

The penetration through normal skin is small compared with the amount penetrating in eczema or psoriasis. This is also evident from data obtained by plethysmographic measurements as no effect was recorded on normal skin in contrast to the pronounced vasoconstriction observed in psoriasis or stripped skin. The difference in penetration is clearly illustrated by the data obtained by hydrocortisone: in normal skin the effect may be neglected, in psoriatic skin the vascular action occurred after 12 hours (23) and following stripping after 10 min. This may be explained by the absence or poor functioning of the epidermal barrier and the largely increased blood flow observed in the latter conditions favoring penetration.

According to Malkinson & Kirschenbaum (13) the amount absorbed in normal skin is in the range of 2% or less and as shown by Feldmann & Maibach (7) it may be spread out as long as 10 days. Furthermore, the two latter authors observed that stripping only doubles penetration whereas a ten-fold increase is caused by occlusion.

The rapid and pronounced vascular action produced by hydrocortisone acetate on stripped skin accords with the data obtained by Malkinson (12) on penetration of radioactive hydrocortisone from the skin surface. He observed a rapid drop of surface ^{14}C radioactivity through stripped skin followed by a slowing of absorption. As much as 60–80% was absorbed during the first 3–4 hours. His findings may be interpreted as an initial rapid penetration while the tissue (corium) levels were rising. On the other hand Feldmann & Maibach (7) have reported urinary recovery of ^{14}C after occlusive application of radioactive

hydrocortisone. Significant amounts were excreted over a period of 10 days. There was a lag period of several hours followed by a peak lasting several days. The excretion after intravenous or intradermal application was very rapid with little delay. Consequently, these authors suggested two skin barriers, one in stratum corneum and one in the Malpighian and basal layers. A second barrier situated at the base of the stratum corneum has also been discussed by Dugard & Embery (6). According to Blank & Scheuplein (2), however, the stratum germinativum offers very little resistance to movement of molecules and the transport across this layer is probably similar to transport through the dermis. The plethysmographic findings confirm the data given by Malkinson (12) and clearly illustrate the rapid penetration through stripped skin producing an increase in concentration adjacent to the vessels. Additional support for this interpretation is obtained by the observations of the development of the clinical blanch (12, 23).

Malkinson & Kirschenbaum (13) in their work on absorption of triamcinolone, mention two factors which remain to be evaluated: (i) decreased rate of local metabolic inactivation of the steroid, and (ii) prolonged and decreased rate of circulatory removal dependent on local tissue storage in corium. The pronounced and prolonged vascular action of the fluorinated steroids may be explained in part by these two postulations. Although the fluorinated steroids penetrate intact skin in approximately the same quantities as does hydrocortisone (13) their vascular action is different. This is also clearly demonstrated by the plethysmographically recorded data on stripped skin: Hydrocortisone acetate induces vasoconstriction within the first 30 min, versus 10 hours or more for the fluorinated steroids.

An infinite reservoir of ointment was formed on stripped skin by repeated applications, thus explaining the prolonged vasoconstriction observed during continued occlusion. The prolonged vascular effect of the fluorinated steroids could have been studied after removal of all steroid from the skin surface and a possible reservoir effect measured during continued occlusion. Such studies have previously been performed on psoriatic skin (23) and substantial evidence for differences in vascular action between hydrocortisone acetate and betamethasone-17-valerate have been pro-

vided. Difficulties would arise from similar measurements on stripped skin as the influence of regeneration of the epidermal barrier and regional and individual variations might have disturbed the results.

As deduced from the measurements on skin denuded of its epidermal barrier the high concentration of fluocinolone acetonide did not increase its vascular effect compared with the other steroids. There is, however, general agreement that this concentration increases the therapeutic effect and beneficial results have been reported (14). This illustrates the difference which exists between clinical application and experimental trials.

Many factors are related to the vascular effect of the steroids amongst which must be considered the significance of the penetration through the vessel walls, the question of steroid metabolites, and the influence of steroids on catecholamines. In his studies on the blanching produced by fluocinolone, Juhlin (11) assumed this to be due to a vasoconstrictor effect of the steroid *per se*. Solomon et al. (19) suggested that steroid vasoconstriction is mediated by norepinephrine release in normotensive subjects. Fulton et al. (9) using motion picture records, observed that responses were not obtained consistently by catecholamines applied topically to the cheek pouch of hamsters. They related the difficulty in obtaining constriction to the fact that the blood vessels are embedded in a gelatinous, mucoid matrix of connective tissue which may interfere with penetration of vasoactive substances. All the factors mentioned above are important in percutaneous absorption but the role they play in the mechanism of action of topical corticosteroids remains unknown.

The pronounced vasoconstrictive action on the pulsating vessels has been clearly illustrated by plethysmographic measurements on stripped and psoriatic skin. The results have been related to percutaneous penetration and great variations in absorption have been demonstrated. Such measurements concern physiological responses and are of practical value since they have the advantage of being applied to living skin. Possibly, comparable data cannot be obtained from the concentrations used in the present study, but it is certainly of importance that the results have been obtained by topical therapeutics which are

in clinical use. Yet our knowledge of the exact mechanism involved in vasoconstriction of steroids leaves much to be desired. In the present investigation the various steroids apparently induced special characteristic changes in the form of the pulse curve. This was most evident following betamethasone-17-valerate. The present data, however, do not permit any definite conclusion and further studies are required to elucidate the connection between these changes and variations in vasoconstrictive activity.

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