Acta Dermatovener (Stockholm) 52: 303–307, 1972

PLETHYSMOGRAPHIC RECORDINGS OF SKIN PULSES

VI. *Further Measurements of the Vasoconstriction Produced by Corticosteroids*

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Abstract. Plethysmographic measurements of the vascular effect produced by betamethasone-17-valerate 0.1% ointment and 1% solution on normal and stripped skin, are described. Evidence is presented that the vasoconstriction induced by fluorinated corticosteroid ointment on stripped skin is plethysmographically measurable and accordingly also concerns pulsatile vessels. Despite distinct blanching, no measurable effect was produced by the solution on normal or stripped skin. Hydrocortisone acetate ointment had a weak and less constant effect. The study indicates that fluorinated steroid ointment induces a more pronounced vasoconstriction than solution of the same steroid. The results are related to percutaneous absorption and the amount of steroid remaining on the skin surface.

Plethysmographic measurements of the vasoconstrictive action of corticosteroids on skin denuded of the epidermal barrier were reported in a previous paper (7). The results were obtained with plastic occlusion of ointments and the vasoconstriction was indicated by a decrease in pulse height. Several areas in a few individuals were investigated. Intra- and interindividual variations in vascular response were observed. Similar investigations performed on psoriasis made it possible to measure objectively the vascular action of various therapies (8). The superior effect of the fluorinated steroids compared with that of hydrocortisone acetate was illustrated.

In the present work the previous investigations have been continued in order to study more closely the vasoconstrictive action of the fluorinated steroids and hydrocortisone acetate on intact and stripped skin, and to compare the influence of ointment base with that of solution.

MATERIAL AND METHODS

The present work comprised 15 healthy persons aged 22–50 years. The flexor aspect of both forearms was selected as test area. Two or 4 skin sites (1 × 1 cm) were studied at each side. The substances were investigated as follows:

1. Betamethasone-17-valerate 0.1% in ointment base (Betnovat®-Nyco, Nyegaard & Co. A/S, Oslo, Norway) was applied to 8 skin sites denuded of the epidermal barrier by stripping (2 subjects). About 3 mg of ointment was used at each site. The effect was measured at 14 hours of occlusion since it was assumed that the vascular action had occurred within that period of time (7).

2. Betamethasone-17-valerate dissolved in ethyl alcohol at a concentration of 1%, was applied to 10 stripped and 10 normal skin sites (5 subjects). Aliquots of 0.02 ml were pipetted at each site of examination and allowed to evaporate before occlusion. On stripped skin, measurements were performed every second hour for the first 14 hours and later on at 20 hours' occlusion. On intact skin, measurements were performed each day for 3 days.

3. Hydrocortisone acetate 1% in petrolatum was applied to 6 stripped skin sites (3 subjects). About 3 mg of ointment was used at each site. Measurements were performed after 3 and 20 hours' occlusion.

4. Placebo ointment, i.e. the ointment base only of Betnovat®, was applied to 10 stripped skin sites (5 subjects). About 3 mg of ointment was used at each site. Measurements were performed after 2, 14 and 20 hours' occlusion.

Recordings of skin pulses were also taken before application and 4 hours or more after removal of the occlusion. The skin sites were marked with a gentian violet pen and covered with polythene sheets secured by adhesive occlusive strapping during the occlusive period. The ointments and the solution were reapplied after each examination during this period. Stripping of the epidermal barrier was done with Scotch Tape using about 50 strippings at each area. The surface then appeared red and glistening.

The pulse meter used in the present study has been described earlier (7). Cadmium sulphide was used as photoconductive material. A power supply unit providing constant current for the photoconductive cell and an interface circuit were connected between the photocell and the electrocardiograph. In order to obtain representative curves the sensitivity was adjusted for each area. The deflection by 1 mV was noted and on each area the same sensitivity was used at each examination.

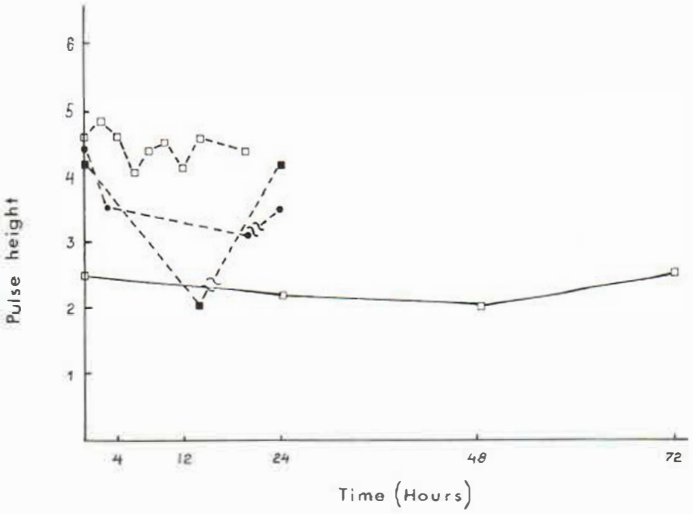

Fig. 1. Results of pulse measurements on stripped (- - -) and intact (—) skin showing the effect of 1% betamethasone-17-valerate solution (□), 0.1% betamethasone-17-valerate ointment (■) and 1% hydrocortisone acetate ointment (●).

RESULTS

The vascular response varied from one area to another. Regional differences in skin vascularization were probably responsible for some of the variations. It is also possible that stripping of the stratum corneum does not produce an even removal of the epidermal barrier.

At some areas of normal or stripped skin repeated measurements were necessary in order to obtain representative curves. The configuration of the pulse curves varied from one site to another on both normal and stripped skin. On some sites of stripped skin variation in pulse shape from one moment to another could be observed, while the pulse height remained more constant. This was probably related to fluctuations in vasomotor tone.

Fig. 1 shows the mean values of skin pulses obtained in the present study. Betamethasone-17-valerate ointment produced a distinct decrease in pulse height at all skin sites after 14 hours of occlusion. After removal of the occlusion the pulse height increased, indicating that the vasoconstriction observed was due to the occlusive treatment with the steroid.

The vascular response obtained by hydrocortisone acetate ointment on stripped skin was much weaker. At some areas a distinct vascular effect could be recorded, whilst at other areas little or no effect was observed. At a few areas there was a distinct reduction in pulse height but no increase was observed after removal of the occlusion. The reduction in pulse height may accordingly be related to causes other than the vascular effect of ointment. In one individual no vasoconstriction could be observed after 4 and 20 hours of occlusion.

Spontaneous variations in pulse height within a short period of time could be observed on stripped skin during occlusion with ointment base only. Usually the variations were within the range of 1 unit of measurement when large amplitudes were recorded. There was no permanent reduction in pulse height during the occlusion. A decrease occurred at some areas after removal of the occlusive bandage. This was probably related to the increase in temperature and vascularization induced by the occlusion.

Betamethasone-17-valerate solution produced distinct blanching on normal skin. On stripped skin it was less pronounced and of a shorter duration than that produced by the fluorinated steroid ointment. With this the induced blanch persisted as long as the application remained on the skin. The solution produced no significant reduction in pulse height during the occlusive period on normal or stripped skin. In fact the same variations occurred as observed with the placebo ointment. The average values of the skin pulses obtained are shown in Fig. 1.

DISCUSSION

The data obtained in the present and previous study (7) clearly indicate that the vascular effect produced by the fluorinated steroid ointments on

stripped skin is plethysmographically measurable. Table I shows the statistical analysis of the mean values for the pulse height measured before occlusion, at 14 hours of occlusion and after removal of the occlusive bandage. The induced reduction in pulse height during occlusion and the increase after removal of it, are statistically significant with only 4 degrees of freedom ($0.005 > P > 0.001$ and $P < 0.001$, respectively).

Normally some decrease in pulse height might be expected when the hyperemia produced by the stripping method subsides. The decrease noted at some areas during treatment with hydrocortisone acetate ointment might be related to this factor. However, control measurements with placebo ointment produced no distinct reduction in pulse height. The increase observed after removal of the occlusion of the fluorinated steroid ointments also favours the assumption that the named factor is of less importance. Presumably, the increase in temperature and vascular flow induced by the occlusion sustains the hyperemia produced by the stripping. A decrease in pulse height after removal of the occlusion might be expected if the vasoconstrictive effect of the steroids was too weak. Actually this was also observed on some areas following applications other than the fluorinated steroid ointments.

According to the principles of pulse plethysmography, the decrease in pulse height observed during treatment with the fluorinated ointments on stripped skin is caused by constriction of the pulsating vessels. No such effect has been obtained on intact skin (7) or with a solution of the same steroid on stripped or intact skin. The results thus provide additional evidence for the assumption that the induced blanch in normal skin is due to decongestion of non-pulsating vessels.

Table I. *Statistical analysis of all data obtained with the various fluorinated steroid ointments on stripped skin and after 14 hours of occlusion and after removal*

Subject number	Occlusive period 0	Occlusive period 14 hrs	Mean values: Difference 0–14 hrs	Mean values: Difference Removed	Difference Removed–14 hrs
1[a]	4.375	3.000	1.375	4.750[c]	1.750
2[a]	4.875	2.000	2.875	4.625[c]	2.625
3[b]	4.5625	2.125	2.438	4.000[d]	1.875
4[b]	3.9375	2.3125	1.625	4.9375[d]	2.625
5[b]	3.1875	1.250	1.938	2.8125[d]	1.562
X			2.050		2.087
S.E.$_X$			0.2720		0.2251
t (4 degrees of freedom)			7.54		9.27
			$0.005 > P > 0.001$		$P < 0.001$

[a] Four observations per person at each moment.
[b] Eight observations per person at each moment.
[c] Occlusive bandage removed at 14 hours' occlusion.
[d] Occlusive bandage removed at 20 hours' occlusion.

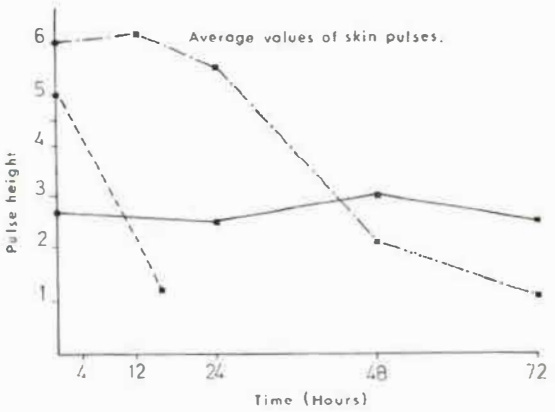

Fig. 2. Vascular effect of 0.1% betamethasone-17-valerate ointment (■) on psoriatic skin (–·–), intact skin (—) and after removal of the epidermal barrier by stripping (- - -).

The present data indicate that fluorinated steroid ointment or cream induce a stronger vascular effect than the solution. This is in keeping with the observations of Tronnier (9) and Munro (4). The betamethasone-17-valerate solution was far in excess of the minimal dose required to demonstrate the blanching phenomenon. Why it failed to induce measurable vasoconstriction is unclear, but it may be related to the depth of penetration and rate of clearance. With the application of ointment, a continuous source of steroid is formed, whereas the steroid molecules left after evaporation of the ethyl alcohol may be rapidly absorbed and brought into circulation during the occlusion. This interpretation accords with the investigation of Vickers (10) who demonstrated that stripped skin has no depot effect when solutions are applied, and the induced blanch disappears within 8 or 16 hours. On the other hand, as shown in the present study and by Malkinson & Kirschenbaum (3) fluorinated steroid cream or ointment induces a pronounced

blanch which persists as long as the application remains on the skin.

The data obtained illustrate the superior effect of the fluorinated steroids compared with that of hydrocortisone acetate. The vasoconstrictive action of the former substance is measurably stronger and much more constant. Measurable vasoconstriction may be produced by the latter within a short period of time in some individuals. However, the effect is more influenced by local and individual variations and is not always of a significant degree. This may of course also be related not only to the steroid molecules but to the base applied. The influence on the penetration of various vehicles has been shown by many investigators (e.g. 2, 5, 6) and petrolatum is probably less suitable than the other bases.

Besides the cellular and vascular action of each steroid, among other factors the following are concerned: the releasing capacity of the vehicle, the rate of diffusion through the epidermal barrier and other molecular relationships. Also each class of corticosteroid may have a pattern in terms of the time relationships to vasoconstriction. Thus with an appropriate base and by continuous measurements the lag time, i.e. the time until maximal vascular action, may be determined. Differences in lag time have been observed by the Mackenzie-Stoughton assay (1) but not by means of pulse plethysmography.

When compared with previous studies (7, 8) the results obtained may be related to percutaneous absorption and the effect of the epidermal barrier may to a certain extent be reflected in the lag time. The data obtained with betamethasone-17-valerate ointment on 32 stripped skin sites in 5 subjects and on 10 psoriatic lesions in 6 subjects, are illustrated in Fig. 2. It is shown that the measurable vascular action appears much sooner on stripped than on psoriatic skin. On intact skin there is no measurable effect (24 skin sites in 3 subjects). These 3 conditions, normal, stripped, and psoriatic skin, have a different vascularization and a different rate of steroid diffusion from the skin surface. Also, the amount of steroid molecules accumulating in the upper part of the dermis differs. Accordingly, a different degree of vasoconstriction may be produced.

Pulse measurements on normal or stripped skin require a very sensitive plethysmograph such as used in the present study. This implies that the pulse curves are more easily disturbed by external stimuli or movements of the subject. Pulsations are more readily recorded on fully developed psoriatic lesions. This may be related to the rich vascularization of the latter. Furthermore, the measurable vascular effect produced by the fluorinated steroid ointments is greater in such lesions than on stripped or normal skin.

On psoriatic skin the values shown in Fig. 2 are recorded with a less sensitive pulse meter than the one used on stripped or normal skin. Accordingly, the values are not directly comparable and the difference between psoriatic and stripped skin may be even greater. Presumably, the onset of measurable vasoconstriction is not influenced by the application of two different methods.

Although the results obtained by pulse plethysmography on skin denuded of the epidermal barrier are significant, the method seems too time-consuming for testing of new steroid compounds when compared with the Stoughton-McKenzie (1) assay. Furthermore, stripped skin is subjected to greater vascular variation than is a fully developed psoriatic lesion. It therefore seems reasonable to assume that psoriatic skin is preferable when comparative measurements of the vascular effect of various steroids have to be performed by pulse plethysmography.

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Received November 16, 1971

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