

## PENETRATION KINETICS OF FOUR DRUGS IN THE HUMAN SKIN

### *Influences of Various Pharmaceutical Vehicles on Penetration in Vitro*

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**Abstract.** A substance applied topically to the skin should penetrate to the intended site of action in sufficient quantities. The galenical preparation should facilitate the passage of correspondingly large quantities of the substance to the target site of action. The investigations were carried out with tritium-labelled 1% hydrocortisone ointment, and a 0.1% fluocinonide ointment, a 0.1%  $^{35}\text{S}$ -labelled heparin ointment and a 0.1% 4-chlortestosterone-acetate ointment. Four ointment bases of the German Pharmacopoea DAB 7 were applied to excised human skin. The penetration properties were investigated during the subsequent 300 minutes. Each substance exhibited penetration kinetics which were related to the ointment base employed. A water-soluble substance penetrated more rapidly from a 50% aqueous water-oil emulsion than from a fatty hydrocarbon (vaseline). Hydrocortisone and 4-chlortestosterone-acetate exhibited penetration properties which were unrelated to the water content of the ointment base. A graph of the absolute values, measured in  $\text{dpm} \cdot \text{cm}^{-2} \cdot 10 \mu\text{m}^{-1}$  plotted against the penetration time, was linear. The depth of the layers was expressed in an average depth of  $85 \mu\text{m}$  ( $70\text{--}100 \mu\text{m}$ ) for the epidermis and an average depth of  $520 \mu\text{m}$  ( $480\text{--}560 \mu\text{m}$ ) for the cutis. These types of kinetic measurements make possible an assessment of the steady state of the substance under investigation in the skin. The rate of the individual substance expressed as moles per minute can be calculated. The flow rate of hydrocortisone applied to the skin in 1% vaseline is therefore  $0.018 \cdot 10^{-11}$  moles per minute.

Local therapy of skin diseases must take into consideration the individual skin layers. A prerequisite is that a substance applied topically to the skin should penetrate to the intended site of action in sufficient quantities. The penetration of drugs into the skin depends on:

1. The release of an active substance from a galenical preparation.
2. The uptake of the substance by the horny layer.
3. The transfer of the substance from the horny layer to the epidermis and cutis.

The carrier itself may be able to penetrate up to three-quarters of the thickness of the horny layer (10). The galenical preparation should facilitate the migra-

tion of large quantities of the substance from here to the target-site.

If the substance is to achieve any effect epidermally then as high as possible a concentration should be aimed for in the horny layer (stratum corneum) (6, 7). Of decisive importance for the further permeation in this case is the difference in affinities of the substance to the corneal keratin in comparison with epidermal protein. A higher affinity of a substance to the epidermal protein means that a continuous diffusion into the epidermis is guaranteed.

A cutaneously active substance should be distinguished by a rapid penetration through the horny layer and the epidermis as well as its high affinity to cutaneous proteins only, as far as this is possible.

The suitability of a drug for application to skin can be judged by its clinical effectiveness. Further penetration and excretion studies need to be undertaken, so that the use of a particular drug may be recommended.

## METHODS

In each case, these investigations were carried out with tritium-labelled 1% hydrocortisone ointment, a 0.1% fluocinonide-acetonide-acetate (fluocinonide) ointment, and a 4-chlortestosterone-acetate ointment 0.1%. A 0.1%  $^{35}\text{S}$ -labelled heparin ointment was also employed.

### *1. Ointment bases*

The following ointment bases of the German Pharmacopoea DAB 7 (2) were employed according to Sarkany (5).

1. A fatty hydrocarbon mixture (white vaseline).
2. A water-oil emulsion (aqueous wool wax alcohol ointment).
3. An oil-water emulsion (aqueous hydrophilic cream).
4. A polyethyleneglycol ointment.

### *2. Labelling of the ointment*

The tritium-labelled substances were diluted with acetone as required. Volumes containing about  $5 \mu\text{Ci}$  were applied to a slide by means of a micropipette.

Table I. The relationship of percentage yield activity recovered (horny layer = 100%) to the ointment base (penetration time 300 minutes)

E = epidermis, C = cutis

| Substance                      | Skin layer | Vaseline | Aqueous wool wax alcohol ointment | Aqueous hydrophilic ointment | Polyethylene-glycol ointment |
|--------------------------------|------------|----------|-----------------------------------|------------------------------|------------------------------|
| Hydrocortisone                 | E          | 4.9      | 3.0                               | 2.6                          | 8.5                          |
|                                | C          | 10.4     | 1.9                               | 6.7                          | 45.0                         |
| Fluocinolone-acetonide-acetate | E          | 0.0      | 0.0                               | 21.6                         | 1.8                          |
|                                | C          | 0.0      | 0.0                               | 14.9                         | 3.9                          |
| 4-Chlorotestosterone-acetate   | E          | 8.2      | 3.2                               | 5.7                          | 0.6                          |
|                                | C          | 10.9     | 5.1                               | 11.1                         | 3.9                          |
| Heparin                        | E          | 5.0      | 12.4                              | 0.7                          | Not measured                 |
|                                | C          | 7.6      | 14.2                              | 0.02                         | Not measured                 |

On evaporation of the solvent, the labelled substance was mixed to homogeneity in 30 mg of the corresponding ointment base. The ointment was then transferred to a glass spatula and its exact weight ascertained.

### 3. Preparation of the excised skin

Skin, generally from mammary tissue, was taken directly following operations and separated immediately at the subcutis level. These specimens were stored for short periods at  $-35^{\circ}\text{C}$ .

A  $7\text{ cm}^2$  area of skin was marked out for investigations and known amounts of ointment were rubbed into this surface for 30 sec.

Then this piece of skin was fixed in a draught-free "penetration chamber". The lower surface (subcutis) of this treated skin was in direct contact with physiological saline, which was agitated continuously by means of a magnet stirrer. The glass chamber was maintained at  $32^{\circ}\text{C}$ .

### 4. Investigation of skin

The skin was removed and cleaned four times with cotton wool, after a 300 min penetration time. Then the skin was mounted on a vulcanized rubber base and the horny layer was removed layer by layer using cello tape. After some  $15 \pm 5$  strips have been taken the shiny tissue and areas of moisture seepage are usually reached. This subjective evaluation of the

completeness of the horny layer stripping was tested for its reproducibility in preliminary experiments by means of resistance measurements (11). An extremely intense radioactivity in the initial horizontal epidermal sections relative to the later ones indicated that remaining horny layer lamella were situated on the epidermis. These values were then assigned to the horny layer.

Although the amount of tissue adhering to the cello tape decreased with each successive strip, it could be shown that this process did not have any significant effect on the results of substance distribution (9, 12, 13). This is explained by the fact that the amount of adhering tissue decreases by a factor of 10, whereas the radioactivity decreases by a factor of 100.

Each strip was then placed in a test tube. After removal of the horny layer,  $28\text{ mm}^2$  pieces were punched out and sectioned horizontally with a freeze-microtome. 15 sections of  $10\text{ }\mu\text{m}$  thickness and the  $20 \pm 10$  sections of  $40\text{ }\mu\text{m}$  thickness were prepared from the remaining tissue. All tissue adhering to the microtome, and thus not sectionable, as well as the individual sections, were placed in separate test tubes.

The samples thus obtained were appropriately processed and their radioactivity measured by means of a Philips Liquid Scintillation Counter.

## RESULTS AND DISCUSSION

The individual substances were applied to the skin in the four different ointment bases and their penetration properties were investigated during the subsequent 300 minutes. The data are summarized below:

1. Each substance exhibited penetration kinetics which were related to the ointment base employed, and on the physicochemical properties of the substance itself.

A polar water-soluble substance such as heparin, for example, penetrated more rapidly from a 50%

Table II. Percentage yield of total activity recovered in the skin (penetration time 300 minutes; Vaseline)

|             | Hydrocortisone | Fluocinolone-acetonide-acetate | 4-Chlorotestosterone-acetate | Heparin |
|-------------|----------------|--------------------------------|------------------------------|---------|
| Horny layer | 85.9           | 99.9                           | 82.8                         | 87.9    |
| Epidermis   | 4.2            | 0.0                            | 6.7                          | 4.4     |
| Cutis       | 9.0            | 0.0                            | 9.0                          | 6.7     |

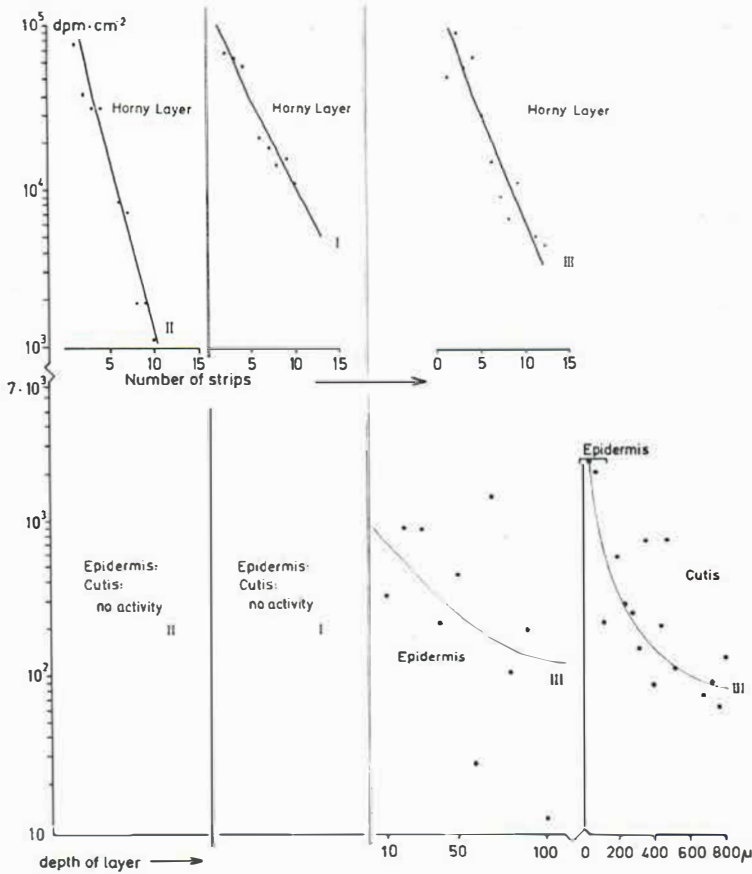


Fig. 1. Penetration kinetics of fluocinolone-acetonide-acetate (0.1%) in vaseline (I), aqueous wool wax alcohol ointment (II), and in polyethyleneglycole ointment (III).

aqueous water-oil emulsion than from vaseline (Table I).

Less polar substances such as hydrocortisone and 4-chlortestosterone exhibited penetration properties which were unrelated to the water content of the ointment base.

Both substances penetrated equally well from the vaseline base (Table II).

2. These kinetics can vary if different substances are used with the same base (Tables I and II).

Penetration into the epidermis and cutis is twice as high for 4-chlortestosteroneacetate as for hydrocortisone from a 70% aqueous oil-water emulsion (aqueous hydrophilic cream). 4-chlortestosteroneacetate therefore does not behave like testosterone and ethinylestradiol, both of which can penetrate more easily from a fatty ointment base than from an oil-water emulsion, according to Kolbe (3).

Anabolic effective 4-chlortestosterone-acetate seems to have intermediate penetration properties, being approximately equally capable of epidermal or

dermal penetration from fatty ointment and from oil-water emulsion.

Very clear differences in the penetration properties of different substances in the same base have been noticed when using a polyethyleneglycole ointment (Table I). 4-Chlortestosterone-acetate, which is less polar than hydrocortisone, does not penetrate into the epidermis and cutis. On the other hand, the non-polar substance fluocinolone-acetonide-acetate, whose free chemical groups are protected is only capable of penetrating the epidermis and cutis when in this polyethyleneglycole base (Figs 1, 2).

3. Non-polar substances apparently penetrate only from ointment bases in which they are completely soluble. Their penetration properties are therefore particularly dependent on the ointment base chosen. Thus fluocinolone-acetonide-acetate is incapable of skin penetration from vaseline or water-oil emulsions. On the other hand, the free form fluocinolone-acetonide was found to penetrate through to the blood capillaries of the skin layer, as tested by the

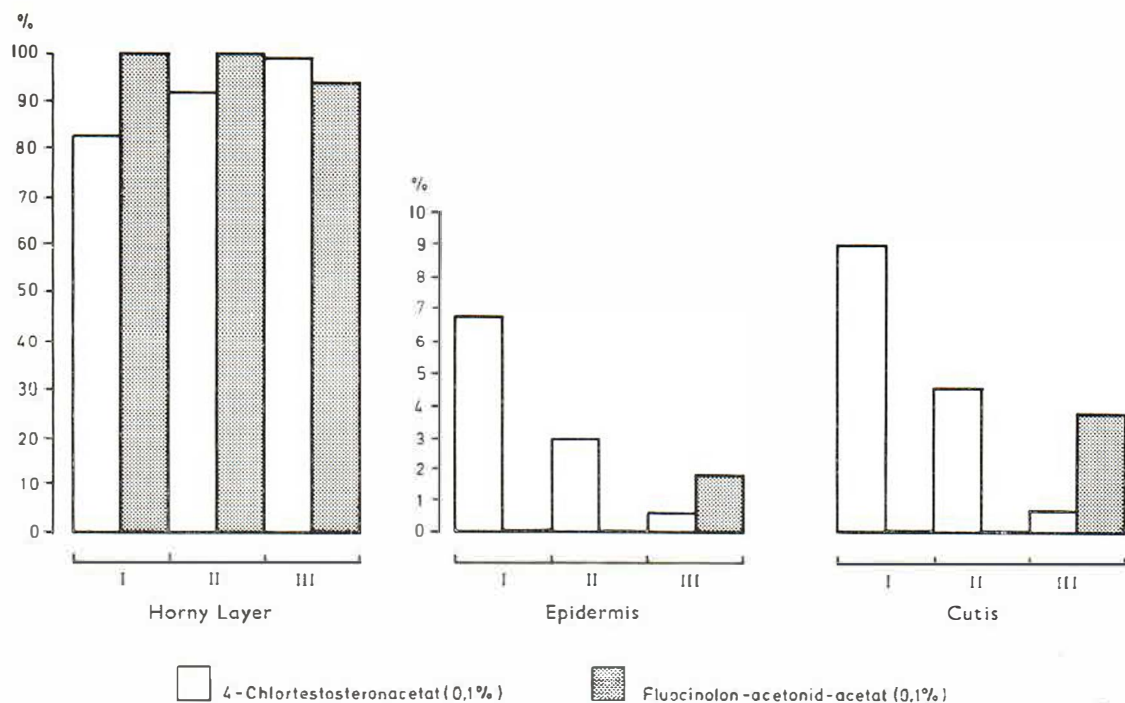


Fig. 2. Distribution of the total activity recovered in the skin in percent. 4-chlortestosterone-acetate (0.1%) and fluo-

cinolone-acetonide-acetate 0.1% in different ointments (Bases I, II, III see Fig. 1).

McKenzie vasoconstriction test (4) as carried out by Brode et al. (1) and Sarkany and colleagues (5).

The extent of penetration, they found, was dependent on the base employed. Thus good penetration values were obtained from a water-oil- or oil-water emulsion and moderate penetration from a vaseline-base ointment, although no such penetration was to be found from a polyethylene-glycole base. Dissolving the substance first in propyleneglycole and then preparing in a vaseline-base improved the penetration properties considerably.

In contrast to this (according to the Sarkany group (5)) betamethasone caused identical vasoconstriction in polyethyleneglycole or vaseline or oil-water- and water-oil emulsions. It therefore showed penetration properties most similar to hydrocortisone.

4. A considerable reservoir of the substances investigated was evident in the horny layer (Fig. 2 and Table II).

Between 82 and 99.9% of the substance administered could be recovered there. Stüttgen (8) suggested that each substance can now diffuse out of this reservoir according to its physicochemical properties and functionally independently of the carrier used to transport it there (Table II).

*Acta Dermatovenere (Stockholm) 54*

The shallower the slope of the graph of layer depth against logarithmic values of activity, the greater is the depot available for its further absorption.

Fig. 1 shows very surprisingly that these slopes are practically identical for vaseline and polyethyleneglycole.

Both, it is noted, are ointment bases in which further penetration of this substance is continuous. Therefore the substances must be present in both ointments in excess. The steep slope seen with the water-oil emulsion indicates a good absorption of the substances in the horny layer. The whole horny layer therefore functions as a barrier, allowing only small amounts to penetrate deeper. This is the reason for the weak concentration in the epidermis and cutis.

Figs. 3, 4 and 5 show that the graph of the absolute values measured in  $\text{dpm} \cdot \text{cm}^{-2} \cdot 10 \mu\text{m}^{-1}$ , plotted against the penetration time, was linear. The depth of the layers was, on average, 85  $\mu\text{m}$  for the epidermis (70–100  $\mu\text{m}$ ) and 520  $\mu\text{m}$  for the cutis (480–560  $\mu\text{m}$ ). The scatter observed was a reflection of the differences in biological material taken (i.e. skin variations).

The various types of kinetic measurements make

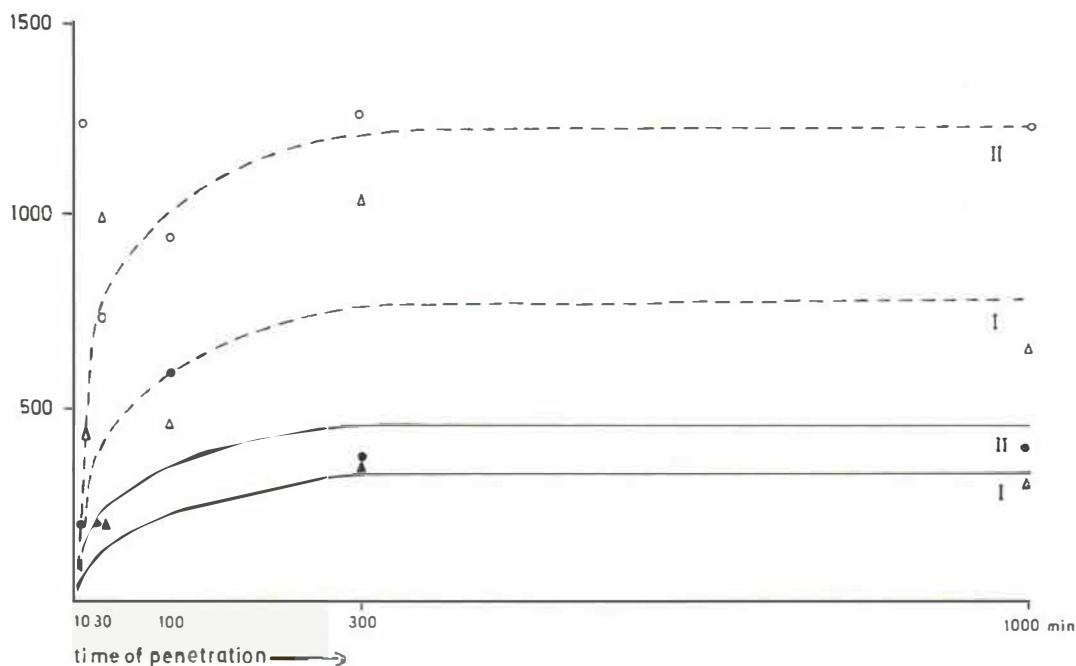
dpm · cm<sup>-2</sup> · 10<sup>-1</sup> μ<sup>-1</sup>

Fig. 3. Average of activity in a skin depth of 85  $\mu\text{m}$  (---) and 520  $\mu\text{m}$  (—) (10  $\mu\text{m}$  thick layers each) during a penetra-

tion time of 10–1 000 minutes (hydrocortisone: vaseline (I) and aqueous wool wax alcohol ointment (II)).

possible an assessment of the steady state of the substance under investigation in the skin. The steady state represents the equilibrium between the influx of a substance in the epidermis from above and its efflux to the underlying cutis and vascular layer.

The graphic representation of the kinetics permits an evaluation of the time taken to achieve a steady state as well as its course and possible physiological or unphysiological anomalies.

In the case of hydrocortisone-vaseline (I) and hydrocortisone-water-oil emulsion (II) (Fig. 3), the steady state is reached at about 300 minutes. For 4-chlortestosterone-acetate in the water-oil emulsion the steady state is arrived at after a period of 800 minutes (Fig. 4). If the penetration kinetics of a substance are as described above then the rate of migration of the individual substance expressed as moles per minute can be calculated for the period up to the attainment of the steady state.

Fig. 3 indicates that until reaching the steady state (attained at 300 minutes), an average of  $5.4 \times 10^{-11}$  moles hydrocortisone have migrated through a

layer 435  $\mu\text{m}$  thick, from the epidermis at 85  $\mu\text{m}$  to the cutis at 520  $\mu\text{m}$ . The flow rate of hydrocortisone applied to the skin in 1% vaseline during the steady state is therefore approximately  $0.018 \times 10^{-11}$  mole per minute.

In the case of hydrocortisone in oil-water emulsion (IV) the steady state is attained after 30 minutes but is then interrupted at 300 minutes (see Fig. 5).

For the hydrocortisone-polyethyleneglycol ointment (III) the rate of uptake in the epidermis and cutis becomes equal within 30 to 300 minutes (region of proportionality). A steady state was not measurable up to 1 000 minutes.

With 4-chlortestosterone-acetate-vaseline, on the other hand, neither a region of proportionality, nor a steady state existed during the first 1 000 minutes.

Details of the rate of flow and steady state allow the establishment of the pharmacological and biochemical dimensions, so that one can estimate which known pharmacological effects were feasible, i.e. biochemical actions of steroids or inhibition effect, etc.

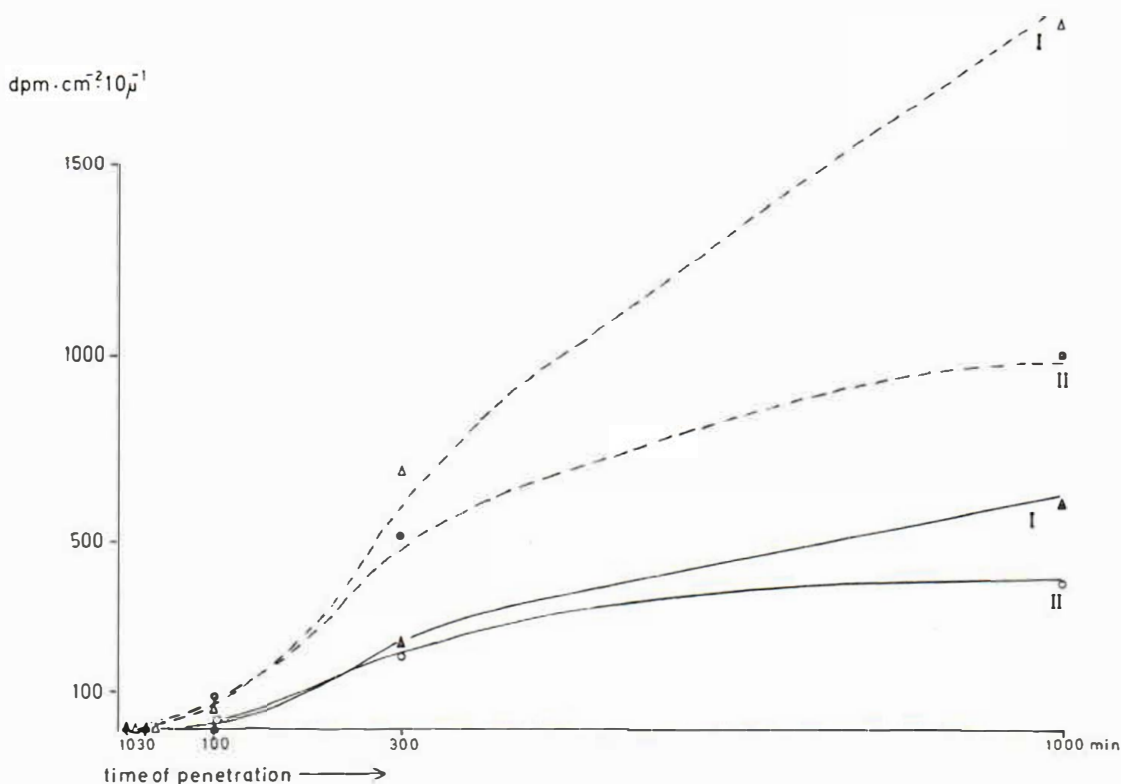


Fig. 4. Average of activity in a skin depth of 85  $\mu\text{m}$  (---) and 520  $\mu\text{m}$  (—) (10  $\mu\text{m}$  thick layers each) during a penetra-

tion time of 10–1 000 minutes (4-chlortestosterone-acetate: vaseline (I) and aqueous wool wax alcohol ointment (II)).

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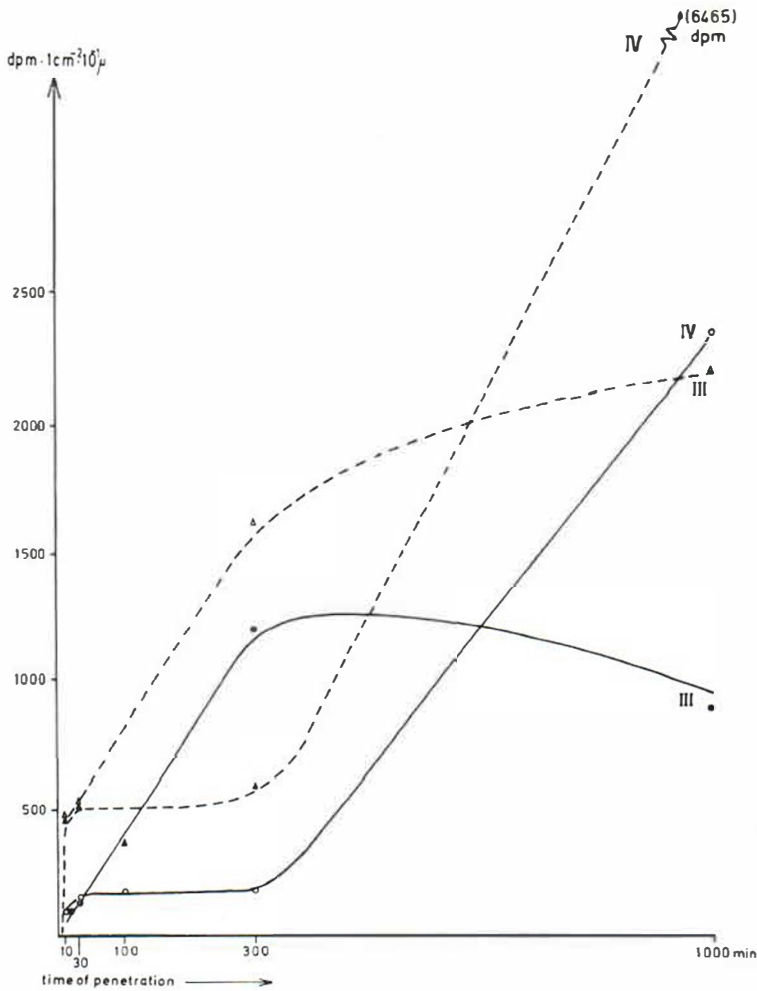


Fig. 5. Average of activity in a skin depth of 85  $\mu\text{m}$  (---) and 520  $\mu\text{m}$  (—) (10  $\mu\text{m}$  thick layer each) during a penetration time of 10–1 000 minutes (hydrocortisone; aqueous hydrophilic ointment (IV) and polyethyleneglycole (III)).