

Bioavailability of Topically Administered Steroids: A "Mass Balance" Technique

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The percutaneous absorption of four steroids (hydrocortisone, estradiol, testosterone, and progesterone) has been measured in vivo in man under occluded and "protected" (i.e., covered, but non-occlusive) conditions. The experimental approach, involving simple modifications of standard radiochemical methodology, has enabled excellent "mass balance" and dose accountability to be achieved. Consequently, the utility of the procedure for the measurement of in vivo topical bioavailability can be inferred. In addition, because of the precision and accountability of the results, the technique offers a potential means to establish quantitative structure-penetration relationships for skin absorption in man. It was found that steroid absorption increased with increasing lipophilicity up to a point, but that penetration of progesterone (the most hydrophobic analog studied) did not continue the trend and was at least partly rate-limited by slow

interfacial transport at the stratum corneum-viable epidermis boundary. Comparison of data obtained from the occluded and "protected" experiments permitted the effect of occlusion (defined as the complete impairment of passive transepidermal water loss at the application site) to be assessed. Occlusion significantly increased percutaneous absorption of estradiol, testosterone, and progesterone but did not effect the penetration of hydrocortisone. A mechanism is proposed to explain why the absorption of the more lipophilic steroids is enhanced by occlusion but that of the most water-soluble (i.e., hydrocortisone) is not. It is suggested that the rate-determining role of the sequential steps involved in percutaneous absorption can be revealed by experiments of the type described using related series of homologous or analogous chemicals. *J Invest Dermatol* 90:29-33, 1988

In the development of a dosage form intended for topical administration on the skin, an essential step is to determine the percutaneous absorption of the drug. Of the various alternatives available for the assessment of skin penetration, there is little doubt that an in vivo measurement in man is most appropriate and desirable [1]. However, in vivo percutaneous absorption experiments in man are much more difficult to perform than either animal model or in vitro penetration studies. Furthermore, most of the in vivo investigations which have been carried out have not allowed accountability of the applied dose and, hence, have not produced results which can be interpreted unequivocally.

The majority of human in vivo percutaneous absorption measurements have used indirect radiochemical methods [2-6]. Typically, a ^{14}C labeled chemical is applied topically from a volatile solvent vehicle and penetration is evaluated from the excretion of the ^{14}C radiolabel over the next 5-10 d. Correction for incomplete elimination is made by performing an identical protocol after intravenous or intramuscular administration of the same ^{14}C labeled material. The approach has some clear limitations: any conclusions are based on radiolabel data, not specific information about the parent compound and its metabolites; the elimination profile after topical and parenteral dosing must be assumed identical; the fate of that fraction of the topical dose which is not absorbed immediately into the skin post-application is not controlled so that the meaning of "dose" in this situation is usually poorly defined.

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In this paper, simple modifications of the conventional in vivo experiment are described and the improvement in resulting data quality is illustrated for four steroids: progesterone, testosterone, estradiol, and hydrocortisone. The procedures involve i) covering the application site for the entire duration of the study, ii) washing the dosed skin surface at the end of the dosing period, and iii) on occasion, when monitoring of urinary excretion is terminated, tape-stripping the upper layer of stratum corneum. The key improvement afforded by these changes is that the radiolabeled dose can be totally accounted for, i.e., mass balance is possible. The approach has been applied to both single and multiple-dosing regimens and measurements have been made under both occlusive and non-occlusive ("protected") covering conditions. The results obtained demonstrate that the technique may have significant potential for establishing quantitative structure-penetration relationships for skin absorption in man, and revealing quantitatively the effects of occlusion on the transport of compounds across the cutaneous barrier.

MATERIALS AND METHODS

The penetrants considered were four steroids: progesterone, testosterone, estradiol, and hydrocortisone. The ^{14}C -labeled chemicals (RPI Corp., Mount Prospect, IL) were applied in acetone to the ventral forearm of healthy male volunteers ($n \geq 5$), from whom informed consent, approved by the UCSF Committee on Human Research, had been previously obtained. Chemical and radioactivity doses were $4 \mu\text{g}/\text{cm}^2$ and $1 \mu\text{Ci}/\text{cm}^2$, respectively; the area of application was 2.5 cm^2 and the dose was administered in $20 \mu\text{l}$ of acetone.

After evaporation of the vehicle ($<0.5 \text{ min}$), the application site was covered with a semirigid, polypropylene Hilltop[®] (Hilltop Research, Inc., Cincinnati, OH) chamber (HTC), which was affixed to

the skin with hypoallergenic adhesive tape. The cotton pads, with which the chambers are supplied, were removed prior to application on the subjects' forearms. In the occluded studies, intact chambers were employed; for the penetration experiments under "protected" conditions, the chambers were "ventilated" by boring several small holes through the plastic (such that about 50% of the surface area was exposed). To prevent loss of surface material (squames, undissolved penetrant, etc.), the roof of the chamber was covered with a piece of Gore-Tex® (W.L. Gore & Associates, Inc., Elkton, MD) membrane (0.2 µm pore size). It was found that Gore-Tex® did not impede transepidermal water loss to any significant extent and hence the objective of dosing site protection without occlusivity was achieved (Bucks et al, in press).

The subjects collected their urine for 7 d, post-steroid application, according to the schedule: 0–4, 4–8, 8–12, and 12–24-h; day 2, 3, 4, 5, 6, and 7. Urine volumes were determined gravimetrically for each time period, and duplicate 3-ml samples were analyzed for radioactivity. ¹⁴C-Toluene was added, as an internal standard, to a third 3-ml sample to determine quenching. The percent "dose" (as total radioactivity) excreted was determined for each time interval. At 24 h after dosing, the chamber (or chamber + Gore-Tex®) was removed, placed in scintillation fluid, and sequestered ¹⁴C was counted. An appropriate quench correction was again made. The application site was washed with a standardized procedure [7] using 5 cotton balls consecutively soaked in soap solution (Ivory Liquid Soap, Proctor and Gamble Co., Cincinnati, OH; diluted 1:1 with water), water, soap solution, water, and water. All washings were collected and were processed for liquid scintillation counting to assay for residual surface chemical. For the remaining 6 d of the urine collection period, the administration site was again covered with a (new) chamber. Finally, this chamber was also assayed for ¹⁴C-chemical; also, at this time, in the "protected" experiments, stratum corneum at the site of application was stripped 10 times with adhesive tape (Scotch Cellophane Tape®, 3M, St. Paul, MN) and the skin strips were analyzed for residual radioactivity (once more, corrected accordingly for scintillation quenching).

A parallel protocol was also performed following a multiple-dosing regimen [8] for testosterone, estradiol, and hydrocortisone under occluded conditions. The compounds were applied every 24 h for 14 d at a dose of 4 µg/cm² to the same skin site. The first and eighth applications utilized ¹⁴C-labeled drug and urinary excretion for 7 d (using the collection schedule described above) after each of these doses was followed. In these studies, the 24-h washing procedure was performed daily (prior to that day's dosing) and a new chamber was provided on each occasion.

Partition coefficients of the penetrants between isopropyl myristate and water, and tetradecane and water, were determined using a standard technique [9]. Octanol-water partition coefficients were obtained from the literature [10].

RESULTS

Data from the single dose experiments performed under occlusive conditions are presented in Table I and should be compared to the corresponding results from the "protected" studies given in Table II. Total recoveries are, in general, high and were greater for the "protected" measurements. These experiments were performed after the occluded investigation and incorporated obligatory evaluations of i) ¹⁴C-radiolabeled sequestered on the second HTC, ii) chemical in the second set of washings, and iii) material remaining in the upper layers of the stratum corneum at the end of 7 d. This more thorough determination of penetrant disposition probably accounts for the improved mass balance in the "protected" studies. The percentage dose absorbed columns in Tables I and II show the effect of occlusion on the topical bioavailability of the four steroids. With the exception of hydrocortisone, unpaired t-tests show that occlusion significantly increases the percutaneous absorption ($p < 0.01$) of these compounds in man. This finding is further emphasized in Fig 1, which shows, for each of the four steroids, the rate of excretion of radiolabel following their topical application under both occluded and "protected" conditions. To optimize clarity, data for estradiol, testosterone, and progesterone are plotted semi-logarithmically because of the difference in absorption between oc-

Table I. Disposition of Topically Applied ¹⁴C-labeled Steroids Following a Single Dose under Occluded Conditions

Steroid	Absorbed ^b	Percentage of Applied Dose ^a				Total
		1st HTC ^c	1st Wash ^d	2nd HTC ^e	2nd Wash ^f	
Hydrocortisone	4.0 ± 2.4	28 ± 5.6	36 ± 3.0	n.d. ^g	n.d. ^g	68 ± 3.9
Estradiol	27 ± 6.4	41 ± 10	18 ± 7.2	0.5 ± 0.3	n.d. ^g	87 ± 13
Testosterone	46 ± 15	41 ± 8.4	3.0 ± 4.1	0.3 ± 0.2	n.d. ^g	90 ± 8.4
Progesterone	33 ± 8.9	46 ± 10	1.2 ± 0.8	.07 ± .02	.01 ± 0.0	80 ± 5.5

^a Mean ± standard deviation (n = 5, except for progesterone, for which n = 6).

^b Values corrected for incomplete renal elimination [3].

^c Material sequestered on Hilltop chamber (HTC) removed at 24 h post-dosing.

^d Chemical found in combined washings performed 24 h post-dosing.

^e Material sequestered on HTC removed at end of measurement period.

^f Chemical found in combined washings performed at end of experiment.

^g n.d.: not determined.

Table II. Disposition of Topically Applied ¹⁴C-labeled Steroids Following a Single Dose Under "Protected" Conditions

Steroid	Absorbed ^b	1st HTC ^c	Percentage of Applied Dose ^a			SC "strips" ^g	Total
			1st Wash ^d	2nd HTC ^e	2nd Wash ^f		
Hydrocortisone	4.4 ± 1.7	27 ± 11	51 ± 18	3.2 ± 1.7	2.7 ± 1.3	2.5 ± 1.1	89 ± 5.6
Estradiol	3.4 ± 1.2	38 ± 13	58 ± 12	0.7 ± 0.4	0.3 ± 0.4	0.1 ± 0.1	100 ± 0.9
Testosterone	18 ± 8.6	46 ± 7.5	30 ± 15	1.4 ± 0.4	0.1 ± .08	n.d. ^h	96 ± 2.0
Progesterone	13 ± 6.3	54 ± 7.7	27 ± 8.7	1.2 ± 0.6	0.3 ± 0.4	n.d. ^h	96 ± 3.4

^a Mean ± standard deviation (n = 6).

^b Values corrected for incomplete renal elimination [3].

^c Material sequestered on HTC + Gore-Tex® removed at 24 h post-dosing.

^d Chemical found in combined washings performed 24 h post-dosing.

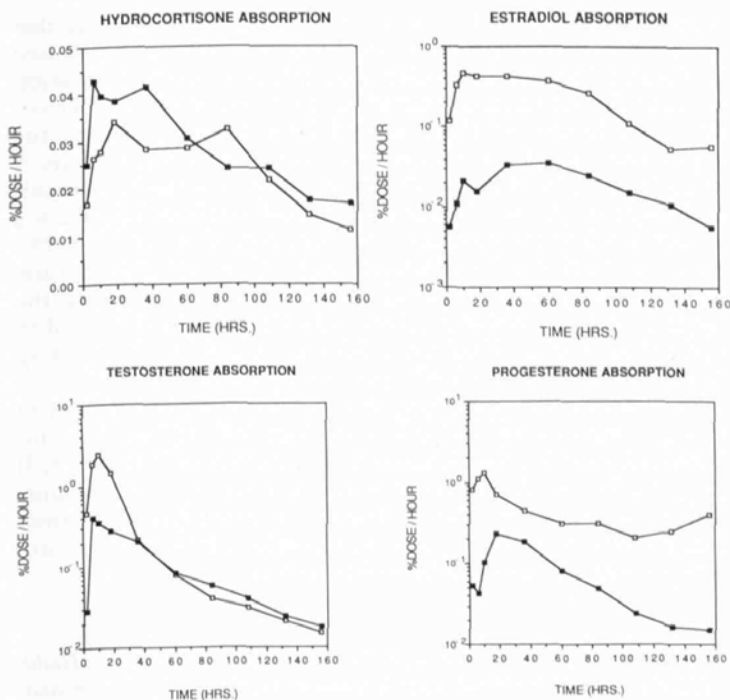


Figure 1. Urinary excretion rates (mean % dose per hour) as a function of time following topical application of four steroids under occluded (*open square*) and "protected" (*filled square*) conditions. A, hydrocortisone; B, estradiol; C, testosterone; D, progesterone.

cluded and "protected" measurements; for hydrocortisone, on the other hand, a linear graph is presented and the occluded and "protected" results essentially superimpose.

The multiple-dose measurements, which were performed under occlusion, are summarized in Table III. Again, total recoveries of applied radioactivity were good. An analysis of variance showed that for each of the steroids there was no significant difference ($p > 0.05$): a) in the percentage dose absorbed dermally between the first and eighth doses under occlusion and b) between the multidose absorption figures and the percentage dose absorbed following a single dose under occluded conditions (Tables I and III) [with the possible exception of estradiol for which marginally significant differences ($p = 0.04$) in percutaneous absorption between the first and eighth doses of the multidose regimen and between the first dose of the multiple application study and the single acute dose study were found].

Finally, in Table IV, partition coefficients of the steroids between each of three oil phases (octanol, isopropyl myristate, tetradecane) and water are reported.

Table IV. Oil-Water Partition Coefficients of Steroids Studied

Steroid	$\log K_{O/w}^a$	$\log K_{I/w}^b$	$\log K_{T/w}^c$
Hydrocortisone	1.61	-0.19 ± 0.02	-2.17 ± 0.03
Estradiol	2.49	2.33 ± 0.04	-0.027 ± 0.003
Testosterone	3.32	1.98 ± 0.002	0.68 ± 0.02
Progesterone	3.87	2.62 ± 0.005	2.27 ± 0.11

^a $K_{O/w}$ = Octanol-water partition coefficient [9,10].

^b $K_{I/w}$ = Isopropyl myristate-water partition coefficient (mean \pm standard deviation; $n = 6$).

^c $K_{T/w}$ = Tetradecane-water partition coefficient (mean \pm standard deviation; $n = 6$).

DISCUSSION

The experiments reported in this paper highlight three issues: 1) the accountability of the applied chemical dose and the potential utility of the technique for measurement of topical bioavailability; 2) the effect of occlusion on the *in vivo* skin permeation of steroids; and 3) the relationship between percutaneous absorption and the relative lipophilicity of the penetrant.

The mass balances achieved in this work are generally high and often approach 100%. A conventional *in vivo* approach [2-6] would have only revealed the percent dose absorbed columns in Tables I-III. Disposition of the remainder of the applied radioactivity would remain unknown. The importance of repeating the washing procedures and chamber analysis at the end of the 7-d experimental period is indicated in the improved accountabilities observed in the "protected" (Table II) and multiple-dosing (Table III) studies. Further support for this contention has recently been observed in our laboratory for a series of para-substituted phenols [11], for which, again, essentially complete mass balance has been recorded. It is pertinent to note that in Table II hydrocortisone, the *least* lipophilic steroid, is significantly measurable in the stratum corneum at 7-d post-dosing. The amount recovered is clearly relevant when considered in relation to the level of percutaneous absorption. The persistence of hydrocortisone in the stratum corneum for this prolonged period suggests chemical-tissue interaction of appreciable strength. Although the nature of this "binding" phenomenon is not revealed by these experiments, the effect clearly goes beyond simple depot behavior. This hypothesis is reinforced by the fact that the more lipophilic estradiol is barely detectable in the stratum corneum at the end of the experiment (Table II). In addition, the recent investigation [12] using para-substituted phenolic penetrants has revealed the same pattern: phenols with more polar para-substituents (e.g., $-\text{NH}_2$, $-\text{NHCOCH}_3$, $-\text{NHCOCH}_2\text{H}_5$) show prolonged stratum corneum residence, whereas more lipophilic analogs (p-CN, p-I) do not.

In Fig 2, the percentage dose absorbed for each steroid is plotted as a function of penetrant octanol/water partition coefficient; results obtained under occluded and "protected" conditions are compared.

Table III. Disposition of Topically Applied ^{14}C -labeled Steroids Following Multiple Dose Under Occluded Conditions

Steroid	Dose ^b	Absorbed ^c	Percentage of Applied Dose ^a				Total
			1st HTC ^d	1st Wash ^e	2nd HTC ^f	2nd Wash ^g	
Hydrocortisone	1st	3.5 ± 1.3	23 ± 7.7	53 ± 11	3.5 ± 1.4	2.6 ± 0.8	85 ± 4.3
	8th	3.1 ± 1.0	32 ± 5.4	33 ± 7.5	7.4 ± 0.8	4.8 ± 1.7	81 ± 2.5
Estradiol	1st	38 ± 7.9	47 ± 12	14 ± 6.8	0.6 ± 0.8	0.5 ± 0.6	100 ± 3.9
	8th	22 ± 7.1	37 ± 9.9	21 ± 5.2	0.4 ± 0.2	0.5 ± 0.2	81 ± 6.0
Testosterone	1st	51 ± 10	46 ± 9.1	1.7 ± 1.0	0.2 ± 0.1	$.06 \pm .06$	99 ± 4.3
	8th	50 ± 9.5	37 ± 9.7	4.3 ± 5.4	0.2 ± 0.2	$.06 \pm .04$	92 ± 17

^a Mean \pm standard deviation ($n = 5$, except for hydrocortisone 8th dose, for which $n = 4$).

^b The 1st and 8th doses of a daily dosing regimen, lasting 14 d, were ^{14}C -radiolabeled.

^c Values corrected for incomplete renal elimination [3].

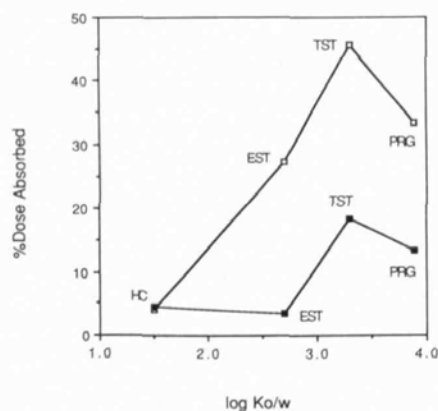


Figure 2. Percutaneous absorption of four steroids (mean % dose absorbed) as a function of octanol/water partition coefficient ($K_{o/w}$) under occluded (open square) and "protected" (filled square) conditions.

With the exception of hydrocortisone, unpaired t-tests reveal that there is significantly ($p < 0.01$) more penetrant absorbed under occlusion than under protected conditions. Although it is generally accepted dogma that occlusion increases percutaneous absorption, quantification of the effect in vivo is scant [13,14]. It is also believed, on the whole, that occlusion increases transdermal penetration for all compounds, but that, in particular, more water-soluble materials will exhibit greatest enhancement. However, our results show that the least lipophilic steroid, hydrocortisone, appears unaffected by occlusion. This observation also contradicts an earlier study [15] which showed a clear promotion of absorption for hydrocortisone when the application site was occluded with thin plastic film (Saran Wrap). However, in this previous experiment, the skin site was not washed until 4 d post-dosing, during which time the occlusive protection remained continuously in place. There is, in addition, some evidence to suggest that continued frictional contact combined with skin flexing produces a "rubbing" effect which may cause an elevation in percutaneous absorption [16,17]. While the plastic film remains in direct contact with the skin surface, the HTC does not. The occlusion-induced enhancement in absorption seen for the lipophilic steroids may be understood by a consideration of the steps involved in percutaneous penetration. Following application, the chemical must i) diffuse from the skin surface through the stratum corneum, ii) partition from the stratum corneum into the much more aqueous in nature viable epidermis, iii) diffuse through the epidermis and upper dermis, and iv) encounter the cutaneous microvasculature and gain access to the systemic pool. Occlusion leads to hydration of the stratum corneum and must, therefore, exert its effect(s) on one or both of the first two steps. If hydration simply decreased the viscosity of the stratum corneum transport pathway (now believed to involve the intercellular lipid-filled channels [18,19]), then the penetration of all chemicals should be equally enhanced by occlusion. An alternative possibility is that the stratum corneum-viable epidermis partitioning step is altered. Hydration of the stratum corneum will reduce the effective partition coefficient of the penetrant between the stratum corneum and viable epidermis (because the two tissue phases now appear more similar). The effect of this decrease will be to increase the kinetics of transfer of penetrant from stratum corneum to viable epidermis, a change that should become progressively more apparent as the lipophilicity of the absorbing molecule increases [20].

The importance of the partitioning step discussed above is further implied by the dependence of percutaneous absorption on steroid lipophilicity (Fig 2, Table IV). Penetration does not continue to increase with increasing lipophilicity. This attenuation in absorption implies a shift in the rate-determining step from stratum cor-

para-substituted phenols are comparable [11]. The possibility that the parabolic form of percutaneous absorption versus log K is caused by decreased surface availability as a result of increased association between the penetrant and the HTC has been considered. We believe that this explanation is not valid for two reasons: First, the dependency of HTC-recovered dose on penetrant lipophilicity is weak. Second, literature data for the absorption of the four steroids under open application, i.e., non-protected, conditions [3] show a similar trend: hydrocortisone $1.9 \pm 1.6\%$; estradiol, $10.6 \pm 4.9\%$; testosterone, $13.2 \pm 3.0\%$; progesterone, $10.8 \pm 5.8\%$. In this case, no consistently available absorptive surface was accessible to the applied compounds. Interestingly, only the result for estradiol in this earlier study is significantly different from the corresponding absorption values in Table II ("protected" conditions).

In summary, this paper presents evolving improvements in *in vivo* percutaneous absorption methodology. The approach is complementary to the recently described experiments of Rougier et al [21-26]. The results demonstrate mass balance and dose accountability, a means to study the effects of occlusion on skin penetration, and, in the long term, the potential to define chemical structure-percutaneous absorption relationships in man.

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