Percutaneous Absorption of Corticosteroids: Age, Site, and Skin-Sectioning Influences on Rates of Permeation of Hairless Mouse Skin by Hydrocortisone

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Abstract □ As part of a long-range plan to decipher the mechanism of mass transfer of corticosteroids across skin, the permeation of [^{3}H]hydrocortisone through hairless mouse skin was characterized by *in vitro* diffusion cell techniques. Age and anatomical site-related behaviors were explored mainly with whole skin but also with skin stripped of its horny layer. Permeability of the mouse skin was low shortly after birth and increased during the singular normal hair cycle exhibited by the SKH-hr^{-1} mouse strain. The return to a hairless state over a period of 20–35 d was accompanied by decreased permeability of hydrocortisone. A permeability coefficient of $\sim 2 \times 10^{-4}$ cm/h was found for the mature mouse. For whole skin, there was no difference in the permeabilities of dorsal and abdominal skin sections, but for stratum corneum-free membranes and dermal membranes, the abdominal site appeared to be more permeable, which is consistent with its thinner dimensions. Totally stripped skin and isolated dermis are ~500 and ~1000 times more permeable than intact skin, respectively; the unaltered stratum corneum of the hairless mouse is thus shown to be the major barrier to the mass transfer of hydrocortisone.

Keyphrases □ Absorption, percutaneous—hydrocortisone, influence of age, site, and skin sectioning on permeation rates, hairless mouse skin □ Hydrocortisone—percutaneous absorption, influences of age, site, and skin sectioning on permeation rates, hairless mouse skin

In previously published studies on the absorption through skin of hydrocortisone (the prototype of the corticosteroids), it was noted that the process is highly sensitive to the manner of treatment of the skin sample. The first evidence of its absorption was skin atrophy on repetitive applications to rat skin (1). When ^{14}C-labeled hydrocortisone became available, direct proof of its passage through skin in the form of radioisotope collected in urine after topical applications to human volunteers was gathered (2). By such methods, it was possible to show that only tiny fractions of the applied steroid passed through intact skin (2, 3), but that absorption through diseased and inflamed skin was far more facile (4). Occlusion was demonstrated to improve absorption by several times that without occlusion (3), and stripping of skin had exaggerated effects of enhancement (3, 5). By using a similar technique in monkeys, the influences of the amount of hydrocortisone applied to a specified area and the frequency of application have been described (6), and the important conclusion that absorption of hydrocortisone through skin is increased on long, continuous application has been reached (7). Along these lines, hairless mouse epidermal sections excised from animals treated with UV light and acetic acid–retinoic acid (vitamin A), all of which were under circumstances that induced epidermal hyperplasia, were measurably more permeable to hydrocortisone than were the skin samples used as controls (8). Excised skin (human) has also been used to study vehicle effects (9, 10). Hydrocortisone has also been shown in human volunteers to be a relatively feeble inhibitor of induced inflammation when directly compared with its intermediate-chain-length 21-alkyl esters (11).

All of the mentioned absorption-related phenomena and influences can be put to stringent, quantitative testing by *in vitro* diffusion cell methods. Yet, to date, only Scheuplein *et al.* (12) have characterized the permeabilities of such compounds, finding human epidermal permeability coefficients of 3×10^{-6} cm/h for hydrocortisone and 1×10^{-5} cm/h for cortisone. It is the specific purpose of this study to obtain, by using the hairless mouse model system, baselines on the absorption of hydrocortisone as a prelude to factoring the degree of permeation dependency of corticosteroid pharmacological responses.

EXPERIMENTAL SECTION

Chemicals—The tritiated hydrocortisone[1] used in these studies had a stated radiochemical purity of >99% and a specific radioactivity of 95.2 Ci/mmol. Sodium chloride irrigation[2] was used as an isotonic medium in which to prepare the hydrocortisone solutions and as the medium of the receptor compartment of the diffusion cell. [^{3}H]Hydrocortisone stock solutions, with a radiochemical activity of ~100 μCi/mL, were prepared. These solutions were diluted roughly 15-fold during the course of charging the donor compartment of the cell. Final concentrations of hydrocortisone in the chamber of the diffusion cell were $<10^{-5}$ M.

Membrane Preparation—The basic techniques for prepartion of the membranes and for running of the permeation experiments have been previously reported (13–16) and, therefore, are only briefly described herein. In most studies, the membranes were full-thickness skin sections from the abdominal and dorsal surfaces of hairless mice, SKH-hr^{-1} strain[3]. Male hairless mice of known ages were sacrificed by spinal cord dislocation. Rectangular sections of skin, several centimeters in each dimension, were excised from the animal, and the adhering fat and other visceral debris were removed. After trimming each excised skin section into an oversized, rough circle, the tissue was placed in a diffusion cell. Stretching of the skin, as evidenced by distortion or expansion of the circular marking, was corrected, and the cell halves were made fast with a spring clamp. The area available for diffusion between compartments was ~0.6 cm^2.

For some experiments, the skin surface, while still on the animal carcass, was stripped repeatedly of its stratum corneum cells by fastening adhesive tape[4] smoothly and securely to the surface with light pressure from the fingers. Attempts were made to obtain adhesion over the entire covered surface before the tape was abruptly removed. This procedure was repeated many times on each site with fresh pieces of adhesive tape used for each stripping.

Dermal sheets were prepared for the permeation studies by soaking excised skin sections for ~24 h, at which time the epidermis could be separated from the dermis by peeling carefully with the blade of a spatula. The isolated dermis layer was mounted in the diffusion chamber.

Diffusion Cells—Small glass diffusion cells, the dimensions of which have been reported previously, were employed (13–16). The cells were assembled with the freshly excised mouse skin between the half-cells. The assembled cells were then immersed in constant-temperature baths. The cell contents were stirred at 150 rpm.

Permeation Procedure—Each half-cell was partially filled (~1 mL) with saline, evacuated with a syringe, and filled with 1.4 mL of saline. The contents were mixed for 5 min to ensure temperature equilibration. Samples (100 μL) were withdrawn from each side; the samples were assayed to ensure that no residual radioactivity remained from previous runs. At this point, the donor

[1] Lot no. NET-396; New England Nuclear Corp., Boston, Mass.
[2] Abbott Laboratories, North Chicago, Ill.
[3] Skin Cancer Hospital, Philadelphia, Pa.
[4] Scotch Brand cellophane tape; 3M Co., Minneapolis, Minn.

0022-3549/84/0900-1287$01.00/0

Table I—Permeability Coefficients and Lag Times of Hydrocortisone as a Function of Age and Anatomical Site

Mouse Age, d	n	Weight, g	Site[a]	$P_I \times 10^4$, cm/h[b]	Range of P_I	$t_{L,I}$, h[c]	Range of $t_{L,I}$	$P_{II} \times 10^4$, cm/h[b]	Range of P_I	$t_{L,II}$, h[c]	Range of $t_{L,II}$
5	3	3.67 ± 0.52	A	1.0 ± 0.3	0.7-1.2	7.6 ± 1.0	6.8-8.8	4.1	2.9-5.2	21.5[d]	18.3-24.6
			D	1.2 ± 0.1	1.1-1.3	9.9 ± 0.6	9.2-10.4	2.4 ± 0.2	2.3-2.6	17.8 ± 2.2	16.1-20.3
			C	1.1 ± 0.2	—	8.7 ± 1.5	—	3.1 ± 1.2	—	1.93 ± 3.4	—
15	5	7.8 ± 0.44	A	3.7 ± 1.3	2.5-5.2	4.4 ± 1.6	2.5-6.4	—	—	—	—
			D	4.5 ± 2.4	3.1-8.7	7.8 ± 2.4	5.2-11.4	—	—	—	—
			C	4.1 ± 1.9	—	6.1 ± 2.6	—	—	—	—	—
20	5	10.4 ± 0.65	A	3.4 ± 1.5[b]	2.3-5.5	5.3 ± 0.7	4.3-5.9	—	—	—	—
			D	4.4 ± 1.6	3.0-5.8	6.2 ± 1.5[d]	3.6-7.0		—	—	—
—			C	3.9 ± 1.3	—	5.8 ± 1.2	—	—	—	—	—
35	2	21.5 (21,22)	A	3.1	3.0-3.1	5.9	5.7-6.2	15.7	13.0-18.4	24.7	23.7-25.8
			D	3.1	3.0 3.2	7.4	6.7-8.1	20.8	18.5-23.1	25.9	25.5-26.4
			C	3.1 ± 1.0	—	6.7 ± 1	—	18.3 ± 4.1	—	25.3 ± 1.2	—
516	3	40.7 ± 1.5	A	2.4 ± 0.5	1.9-2.8	6.9 ± 0.7	6.1-7.4	—	—	—	—
			D	1.6 ± 0.4	1.3-2.0	8.6 ± 3.2	6.1-12.2	—	—	—	—
			C	2.0 ± 0.6	—	7.7 ± 2.3	—	—	—	—	—
657	2	41.0 (41,41)	A	1.7	1.2-2.1	11.8	9.3-14.2	—	—	—	—
			D	1.7	1.2-2.2	11.0	9.6-12.5	—	—	—	—
			C	1.7 ± 0.6	—	11.4 ± 2.4	—	—	—	—	—
80-100[e]		—	—	~3.0	—	~10	—	~90	—	~45	—

[a] A, D, and C represent abdominal, dorsal, and combined sites, respectively. [b] P_I and P_{II} are the permeability coefficients in phases I and II, respectively. [c] $t_{L,I}$ and $t_{L,II}$ are the lag times of phases I and II, respectively. [d] Average of one less value than the number of mice is indicated; an anomalous value was discarded. [e] Independent studies with an HPLC assay with nonradiolabeled hydrocortisone are to be presented in another report.

Table II—Permeability Coefficients of Hydrocortisone through Stripped Skin and Dermal Sections

No. of Strippings	Mouse Age, d	n	Site[a]	$P \times 10^4$, cm/h ± SD[b]	Range of $P \times 10^4$, cm/h	Average Ratio (D/A) of P[c]	Average P over Both Sites $\times 10^4$, cm/h
25	62	3	A	1080 ± 150	990-1250	0.58	855
			D	630 ± 210	390-790		
50	65	3	A	1310 ± 110	1190-1390	0.70	1110
			D	900 ± 55	850-950		
100	65	2	A	1560 ± 190	1360-1740	0.72	1340
			D	1110 ± 66	1060-1160		
Dermis	150	1	A	1890	—	0.81	1710
			D	1530	—		

[a] A and D represent abdominal and dorsal surfaces, respectively. [b] P is presented to allow direct comparisons to be made with data in Table I. [c] Average of the individual ratios for each mouse.

compartment was charged with 100 μL of the stock solution. Saline (100 μL) was added to the receiver. At predetermined intervals, samples were withdrawn from the respective compartments, transferred to vials containing scintillation cocktail[5] (10 mL), and assayed on a liquid scintillation counter[6].

The moment of charging the donor half-cell marked the zero time point for the diffusional run. Two minutes elapsed before the initial donor sample was withdrawn to allow for uniform mixing, and the concentration of this sample was used as the initial donor cell concentration. The overall procedure was designed to maintain an essentially fixed concentration across the membrane during the course of a run. The permeation entered pseudo steady state, and the permeability coefficients of the steroids were readily ascertained.

Analysis of Data—The data were plotted as counts collected in the receptor compartment as a function of time. Correction was made for sampling, which in all cases was done by replacing with saline. The permeability coefficient for a given run was calculated from:

$$J_T = P \cdot A \cdot \Delta C = V\frac{dC}{dt}$$

where J_T is the total pseudo-steady-state flux (in cpm/s) across the skin, P is the permeability coefficient (in cm/s); A is the diffusional area (in cm^2); ΔC is the concentration differential across the membrane, which was taken to be equal to the donor phase concentration, (in cpm/mL); V is the half-cell volume and volume of the receptor compartment (in mL); and dC/dt is the steady-state slope in terms of cpm/mL/s.

RESULTS

Some representative permeation plots obtained from runs with full-thickness skin sections taken from mice at 5, 20, 35, and 516 d of age are shown in Fig. 1. All runs were carried out for ~40 h. At 5 and 35 d, such plots were of a biphasic nature. Two lag times and two permeability coefficients were recorded in such instances. Data from runs such as these are summarized in Table I.

Table II is a compilation of data obtained on processed skins, *i.e.*, skins stripped of stratum corneum and dermis isolated by soaking overnight in saline. All data in Tables I and II were collected at 37°C.

DISCUSSION

Effect of Age—The permeabilities of the SKH-hr^{-1} hairless mouse skin to alkanols have previously been shown to pass through a cycle over the first 3 weeks of life (17), which is coincident with the development of a coat of fur. Similar behavior is evident for hydrocortisone, even within the limited number of age points available (Fig. 2). At 5 d (before the first appearance of hair), the permeability was low. By 15 d (slightly past midcycle and about the point in time of the first shedding of the coat), the permeability coefficient was ~3.7 times larger than the value at 5 d, and it remained high to near the end of the hair cycle (20 d). The overall averages at the latter two time points are statistically larger than the greater 5-d average (Student t test; $p < 0.01$). Based on permeability coefficients measured in the early phase of permeability of skins from 35-d-old mice and older, the permeability of hydrocortisone through the intact skin declined slightly after shedding of the furry coat, which was more or less complete at 25 d of age. The permeability coefficients of hydrocortisone through unbroken, mature mouse skin of the specified strain is on the order of 2×10^{-4} cm/h.

Permeability of Processed Skin Sections—In these studies, some skin samples were stripped 25, 50, and 100 times with adhesive tape and others were soaked overnight to remove the entire epidermal layer from the skin, leaving sheets comprised strictly of dermis and a thin film of protective tissue covering the undersurface of the dermis. The overall averages for the permeability coefficients of these processed tissues (Table II) are plotted against the number of strippings (Fig. 3). The isolated dermis represents infinite stripping. This plot indicates a systematic increase in permeability as the

[5] Aquasol; New England Nuclear Corp.

[6] Model 200 or 9000 scintillation counter; Beckman Instruments Co., Fullerton, Calif.

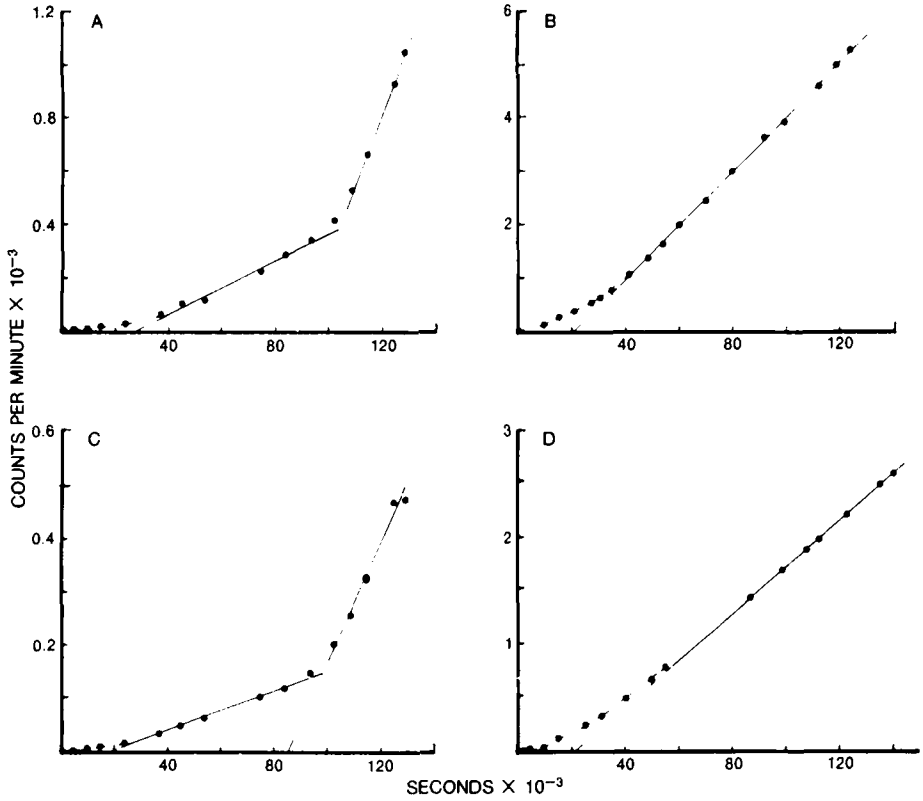

Figure 1—*Representative plots of counts per minute* versus *time for the permeation of hydrocortisone through the skins of hairless mice, aged 5 d (A), 20 d (B), 35 d (C), and 516 d (D).*

strippings become more numerous, but even at 100 strippings, the skin appears to be less permeabile than the dermis alone. The bulk of the barrier impairment is accomplished with 25 strippings, however, and the permeability coefficient is increased by roughly 300 times relative to the intact skin at this point. Seventy-five more tape strippings only doubled the coefficient again, whereas removal of the entire epidermis produced only a 2.5-fold additional increase. Past work with alkanols has indicated that 25 strippings effectively removed the entire stratum corneum. At first impression, the data obtained with hydrocortisone suggest that this may not be so. However, the stripping procedure is laborious and it is suspected that wear and tear of the resident living epidermal matrix, and possibly the dermis, may be responsible for increased permeability after exaggerated stripping rather than further removal of horny cells. As with all previous permeating species, the isolated dermal membrane is from 1.5 to 2 times more permeable than stripped skin, which is consistent with a resistance in the viable epidermis of the same order of magnitude as that of the dermis, which is much thicker. Finally, the absolute permeability coefficients of skin stripped 25 times (~0.085 cm/h) and isolated dermis (~0.17 cm/h) are roughly one-half the values found for the alkanols (14, 18) and for phenol (19) with tissue prepared in a comparable manner from mice of comparable age. On the other hand, neomycin, a larger molecule than hydrocortisone, is less permeable than the corticosteroid (20). There is a readily evident molecular size dependency to diffusion across these processed skin sections.

Anatomical Site as a Permeability Variable—Among all the studies, dorsal and abdominal skin sections from the same animals were studied in tandem. As far as we could determine, the permeabilities of full-thickness skin sections taken from these locations were the same. In contrast, stripped skin sections and isolated dermal sections from the abdomen appear to be one-third more permeable than comparably prepared sections from the back of the animals. Given that the intact skin section is several hundred times less permeable than the least-permeable sample of processed tissue, the implication is clear that the horny layers on the two sites have essentially the same barrier property. When the stratum corneum is removed, back sections evidence slightly greater diffusional resistance because they are, based on previous measurement (17), 40–50% thicker at 65 d of age.

Hairless Mouse–Human Comparison—Scheuplein *et al.* (12) have reported 3×10^{-6} cm/h as the 25°C value for the permeability coefficient of hydrocortisone through human epidermal membranes under similar experimental conditions. Considering that the 37°C permeability coefficient for hairless mouse skin lies somewhere between 1.1×10^{-4} and 4.1×10^{-4} cm/h (Table I, early phase or P_1 values), it appears as if this tissue in its normal state is many

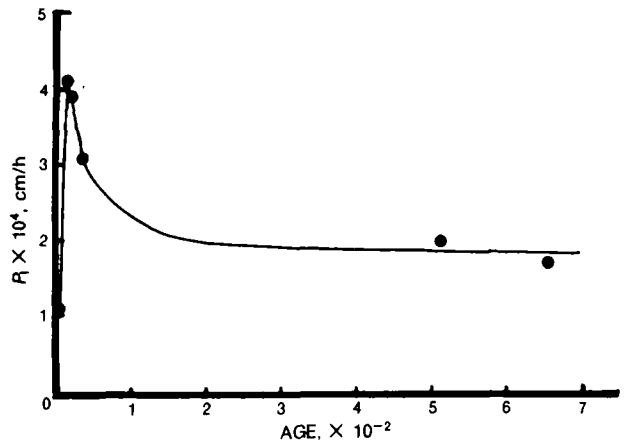

Figure 2—*Permeability coefficients (P_1 values) of hydrocortisone through hairless mouse skin as a function of mouse age.*

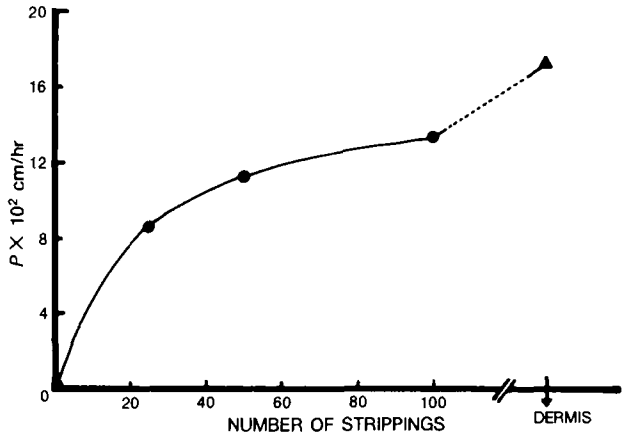

Figure 3—*Permeability coefficients of hydrocortisone through hairless mouse skin as a function of number of strippings. Data are also presented for the dermis. All data points represent average values of the abdominal and dorsal permeability coefficients.*

times more permeable to hydrocortisone than human skin. Assignment of an exact factor to this difference is made difficult, as the representativeness of the human epidermal value is uncertain, with there being no statistical data provided, nor are the temperature sensitivities of the permeation of hydrocortisone through the tissues known (but they can be assumed to be large). Nevertheless, this appears to be the first clear demonstration that to some permeating species, intact human and hairless integuments offer quantitatively different mass transfer resistances, with human skin being at least one logarithmic order less permeable. Previous diffusion cell-generated data for the alkanols (13, 14, 16) and phenol (19) have left the impression that the two skin types were, for practical purposes, identical in permeability.

Biphasic Character in Some Permeability Plots—A most unusual feature of some of these data is the biphasic quality of the permeability plots obtained with mice 5 and 35 d old. At 35 d of age, this aspect of the data was unequivocal. Like other researchers (8), we at first assumed that the skin membranes were deteriorating. However, when the permeability coefficient of a single section of skin was repetitively assessed approximately every 12 h over a 100-h period, using a previously outlined "hydration" protocol (16), the permeability coefficient remained constant at 2.5×10^{-4} cm/h. Subsequently, additional evidence has been gathered on skin of older animals which shows these same qualities (21), and it now appears that if a run is long and continuous, two distinct phases of permeation will be evident, with the second, more permeable, phase being as reproducible in its time of onset and permeability rate as the first. However, the onset of the second phase is at >40 h with the older skin sections, which is, by chance, the arbitrarily selected duration for the experiments reported here and which explains why the second phase was not apparent with the older skin sections. Also, the permeability coefficient of the second phase seems to increase dramatically with mouse age (consider the data for 5- and 35-d-old mice presented here). We suspect it was not evident after 5 d but not until 35 d because permeability in the early phase dominated events at 15 and 20 d. The physicochemical implication that this represents two separate and parallel permeability pathways through the stratum corneum with different dependencies on animal age is clear. This concept is mechanistically important and is thus being put to rigorous experimental testing.

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ACKNOWLEDGMENTS

This work was presented in part at the 127th Annual Meeting of the American Pharmaceutical Association, Washington, D.C., April 1980 (Basic Pharmaceutics Section Abstract No. 100).

Determination of Minoxidil in Bulk Drug and Pharmaceutical Formulations by Ion-Pairing High-Performance Liquid Chromatography

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Received June 8, 1983, from *Control Analytical Research and Development, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication September 23, 1983.

Abstract □ An ion-pairing liquid chromatographic method with UV detection is described for the determination of minoxidil in bulk drug, compressed tablet, and topical solution formulations. The chromatographic system consists of a microparticulate octadecylsilica column and a mobile phase composed of sodium dioctylsulfosuccinate in aqueous methanol (pH 3). The bulk drug and the topical solution samples are prepared by the dissolution of the drug in internal standard solution. Sample preparation for the compressed tablet formulation involves dissolving the drug from an aliquot of pulverized sample and centrifuging to remove insoluble excipients. Quantitative recovery of minoxidil from formulation excipients was demonstrated; assay precision was <1% *CV*.

Keyphrases □ Minoxidil—compressed tablets, topical solution and bulk drug, HPLC □ HPLC—minoxidil, compressed tablets, topical solution, and bulk drug

Minoxidil (I), 2,4-diamino-6-piperidinopyrimidine 3-oxide, a potent, orally active antihypertensive agent, is commercially available as a compressed tablet formulation[1]. There are, as yet, no published methods for the determination of minoxidil in bulk drug or dosage forms. A simple, precise, and specific assay method for minoxidil in these matrices was needed for quality control activities. For use in accelerated stability studies, potential decomposition products of minoxidil must be separated from the drug peak. Minoxidil is quite stable, decomposing only at high temperatures to form 2,4-di-

[1] Loniten, The Upjohn Co., Kalamazoo, Mich.

0022-3549/84/0900-1290$01.00/0