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Percutaneous Penetration of Hair Dyes

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Summary. Scalp penetration of 7 hair dyes (oxidative and direct) that occurs under conditions of hair dye usage was evaluated for both rhesus monkey and man using ^{14}C labeled materials by quantifying their absorbtion via urine assays. Both species showed a remarkably similar pattern of dye penetration. The extent of scalp penetration is slightly higher for direct dyes but in neither case does it exceed 1% of the applied dose.

Key words: Hair dyes – Percutaneous penetration

We have recently reported [2], on the extent to which hair dyes penetrate the scalp under practical conditions of hair coloring. These hair-dyeing experiments performed with rhesus monkeys and human volunteers revealed remarkably similar patterns of dye permeation in both species and indicated that less than 0.2% of the applied dye dose penetrated the skin. We have expanded the study to include additional dyes from both the permanent (oxidative) and semipermanent color categories and have explored several aspects of the recovery of the absorbed dyes as well as the mechanism of their penetration. This report summarizes the results obtained so far.

Materials and Methods

In general, the methodology employed to study dye absorption was based on the procedure developed by Feldmann and Maibach [1] and involved quantifying absorption on the basis of the percentage of radioactivity excreted in the urine following the application of a known amount of labeled compound.

Hair Dyes

Commercially available hair-dye products were labeled with radioactive materials either purchased from ICN Pharmaceuticals (Irvine, CA, USA) or synthesized in our own laboratory. Oxidative-dye formulations were enriched as follows: p-phenylenediamine (PPD; ring ^{14}C; sp. act., 203 μCi/mg) was added to Nice'n Easy (124) Blue Black containing 2.7% of PPD; resorcinol (ring ^{14}C; sp. act., 273 μCi/mg) was added to Miss Clairol Black Velvet containing 1.225% of resorcinol; 4-amino-2-hydroxytoluene (methyl ^{14}C; sp. act., 159.1 μCi/mg) was added to Clairesse (211) Light Auburn containing 0.69% of 4-amino-2-hydroxytoluene. The following semipermanent hair dyes were radioactively labelled: N^4,N^4-bis-(2-hydroxyethyl)-N^1-methyl-2-nitro-p-phenylenediamine [H.C. Blue 1; ring ^{14}C; sp. act., 113 μCi/mg] was added to Loving Care Lotion (795) Darkest Brown containing 1.48% of H.C. Blue 1; N^4,N^4-bis-(2-hydroxyethyl)-N^1-hydroxyethyl-2-nitro-p-phenylenediamine [H.C. Blue 2; ring ^{14}C; sp. act., 378.6 μCi/mg] was added to Loving Care Lotion (795), Darkest Brown, containing 1.77% of H.C. Blue 2; 2-nitro-phenylenediamine (2-nitro-PPD; ring ^{14}C; sp. act., 0.576 μCi/mg) was used in a preparation of Miss Clairol Creme Formula Red Fashion Fire Silver (a discontinued product) containing 1.36% of 2-nitro-PPD; 4-amino-2-nitrophenol (ring ^{14}C; sp. act., 6.4 μCi/mg) was used in a preparation of Miss Clairol Creme Formula Sparkling Sherry containing 0.433% of 4-amino-2-nitrophenol.

Hair-Dyeing Procedure

The process instructions specific for each hair-color product were followed. The net weights of a single application of the hair-coloring products vary between 3 (semipermanent dyes) and 4 fl oz (oxidative, permanent dyes; 2 fl oz dye solution is mixed with 2 fl oz 6% aqueous hydrogen peroxide). This is sufficient to color up to 120 g hair, while the average weight of female scalp hair 4 in. long is about 60 g. The lotion/hair ratio commonly operative during hair coloring is thus 1.5–2.0, and the latter value was chosen to determine the quantity of dye mixture used in the studies with rhesus monkeys.

Human Volunteers. The coloring was performed on one subject at a time. The subject was seated in a chair with his head resting on a specially constructed sink support for comfort and the easy collection of rinse water. The dye mixture was applied to dry hair, worked gently into the hair mass over a period of 5–8 min, and then left on the hair for an additional 20 (permanent color) or 30 (semipermanent color) min. In the latter case, a plastic turban was wrapped around the hair for the dyeing period. The dyed hair was thoroughly rinsed, towel blotted, dried, and either clipped with an electric clipper or left.

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Rhesus Monkeys. The animals were anesthetized with 0.2 ml ketamine and comfortably placed on a laboratory bench top in a supine position. The head of each monkey rested on a specially designed sink support to facilitate the coloring process and assure quantitative collection of the rinse water. The dye lotion (total of 5 g in the case of oxidative dyes consisting of 2.5 g dye solution and 2.5 g 6% aqueous hydrogen peroxide) was worked into the dry scalp hair until all of the dye mixture had been used (3 min). The operator wore vinyl disposable gloves. Twenty minutes were allowed for the dyeing process to proceed (30 min in the case of semipermanent dye, in which case a plastic turban was also used). After dyeing, the hair was rinsed with a microshower until the rinsing water was free of color. The excess water remaining on the hair was blotted with paper towel, and the dyed hair was cut off with electric clipppers.

Urine Collection

Human Volunteers. The subjects were given plastic urine containers for each time period i.e. 0–4, 4–8, 8–12, and 12–24 h after application, and then every 24 h for as long as required.

Rhesus Monkeys. After the dyeing procedure had been completed, the monkeys were restrained in ophthalmological chairs, thus preventing them touching the scalp area. Urine samples were collected at 6, 12, and 24 h and, thereafter, at 24-h intervals for 7 days. For both species, the total urine weight at the end of each time period was recorded, and an aliquot was removed for analysis.

Radioactivity Determination in Urine

All urine samples were filtered and assayed in PPD-triton-toluene using a liquid-scintillation spectrometer. A ^{14}C-toluene internal standard (100,000 cpm) was added to each counting vial to determine the extent of quenching. The counting cocktail was 81% efficient, and the background was 22 cpm. Most specimens were also counted using the wet-washing method [1]. The assay values listed in the tables have been corrected for incomplete excretion due to either parenteral injections or oral administration.

Radioactivity Determination in Dyed Hair and Stratum Corneum

The samples of hair of the horny layer were digested overnight in counting vials, each of which contained 1 ml Unisol. The digested samples were decolorized by the addition of 50% H_2O_2, and each was diluted with 15 ml Unisol complement. The clear samples were equilibrated in a counting chamber at 4° C before counting on a Packard-Tricarb liquid-scintillation spectrometer. Three samples of hair and one sample of stratum corneum from each subject were analyzed; three radioactivity determination were performed for each sample.

Results and Discussion

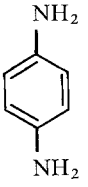
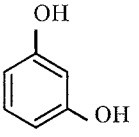
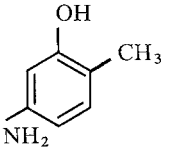
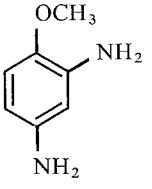
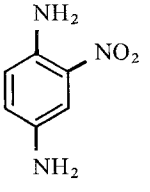
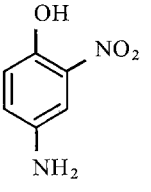
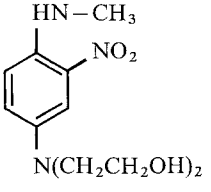
Two methodological approaches (denoted as Application Only and Application and Wear) reflecting different experimental objectives were used in this study. In the first method, the hair was removed immediately following the completion of the coloring procedure; in the other method, the hair was left. The first approach allowed us to evaluate the extent of skin penetration by hair dyes which resulted from the hair-coloring procedure alone. This was the methodology utilized in our earlier study on dye absorbtion [2]. The second approach stemmed from the fact that people color their hair to wear it as such, and because the dyed hair represents a reservoir of dye moieties which, through a variety of routes, may become bioavailable.

Application Only

The data concerning the total urinary excretion of radioactive dye components are given in Table 1. In most cases, they reflect the assays obtained during 144 h following the application of the dye; however, in a few cases, a time span of 96 h was used, as after longer periods, the counts were at the background level. The assay data for 2,4-diaminoanisole (DAA) and PPD are taken from our previous study [2].

Two headings in Table 1, namely, $T_{1/2}$ and dose excretion (%) require some clarification. The urinary excretion was found to follow first-order kinetics quite satisfactorily, and thus, the time required for 50% excretion ($T_{1/2}$) was chosen as an additional and suitable quantifying parameter. Regarding the dose, it should be pointed out that, in the process of hair dyeing, the coloring material is usually applied to hair in the form of a viscous lotion which the user of the product distributes uniformly throughout the hair mass. As a result, the individual hair fibers and the surface of the scalp are coated with a thin layer of the coloring liquid. It is reasonable to assume that only the liquid that is in direct contact with the scalp serves as a dye reservoir available for skin penetration. The size of this reservoir depends not only on the total amount of the product used but also on the retention of the lotion by the hair; this, in turn, is a function of fiber weight and its surface area as well as the viscosity of the lotion. The product-to-hair weight ratio is an important parameter in this respect. Unless the product is applied sparingly (product-to-hair ratio of much less than 1; a situation not usually encountered in hair dyeing), the thickness of the liquid film found on the scalp during coloring is many times (5–10 times) greater than the thickness of the horny layer of the scalp. Under such conditions, the extent of scalp penetration reaches a limiting value for the given dye system, and any further increase in the quantity of the applied product is without any measurable effect on dye diffusion [3]. Nonetheless, at constant penetration, an increase in the product-to-hair ratio has a mathematical consequence, i.e. a corresponding decrease in the percentage of the absorbed dose. During this study and our earlier work [2], every attempt was made to maintain a constant product-to-hair ratio 2) and thus to make the dose excretion values intercomparable.

Table 1. Parameters of hair-dye penetration in man and the rhesus monkey.

Hair-color category	Dye		Hair coloring		Urine assays	
	Name	Structure	Species	Number of subjects	Total dose excretion (%)[a]	Half time ($T_{1/2}$) of urinary excretion (h)
Permanent (oxidative)	p-Phenylene-diamine (PPD)		Man	5	0.190 (0.06)	16
			Rhesus monkey	3	0.182 (0.06)	22
	Resorcinol		Man	3	0.076 (0.03)	31
			Rhesus monkey	3	0.177 (0.03)	31
	4-Amino-2-hydroxyy-toluene		Man	3	0.200 (0.10)	24
	2,4-Diamino-anisole (DAA)		Man	3	0.022 (0.01)	18
			Rhesus monkey	2	0.032 –	20
Semipermanent (non-oxidative)	2-Nitro-PPD		Man	3	0.143 (0.04)	24
			Rhesus monkey	3	0.551 (0.10)	24
	4-Amino-2-nitrophenol		Man	3	0.235 (0.08)	10
	HC Blue 1		Man	5	0.151 (0.12)	18
			Rhesus monkey	3	0.127 (0.03)	40

[a] Values in parentheses represent standard deviation

We have previously [2] on the remarkable equivalence of the cutaneous absorption of PPD, DAA and HC Blue 1 in the rhesus monkey and man. Neither 2-nitro-PPD nor resorcinol appears to follow this pattern strictly; both dyes penetrate the scalp of the rhesus monkey more than that of man. In absolute terms, the differences are small, but the fact that the respective $T_{1/2}$ values of the urinary excretions are identical for both species lends additional credence to this observation.

Taken as a whole, the excretion data for the various dyes (except DAA) differ very little from each other, thus connoting a similar amount of skin penetration. This is in spite of substantial differences in the chemical structure of the dyes, the nature of the dye bases, and the reaction pathways responsible for color formation. The observed effect is, however, somewhat fortuitous, as the dyes are present in their respective formulations at different concentrations. A better perception of their penetration potential can be deduced from the flux values which were calculated for individual dyes from the 24-h excretion data, in which case the quantity applied in each case was normalized to 10 $\mu M/cm^2$ (Table 2).

There was an approximately ten fold spread in the flux, with DAA at the low end of the scale, while 4-amino-2-nitrophenol exhibited the highest potential. No apparent correlation to either molecular weight or the chemical structure was evident. Admittedly, all of the dyes studied have a low molecular weight [the mol. wt. of resorcinol is 104, and that of HC Blue 1 is 250], and they carry no charge under conditions of hair coloring. Also ranking of the dyes in Table 2 was not reciprocated by their solubility characteristics. The membrane/vehicle partition coefficients (which reflect the solubility properties of materials in media of differing polarities) are considered to be important factors in determining the flux of materials through the stratum corneum but, surprisingly, their utility in this case was minimal (Table 3).

Thus, while on the one hand, dyes with similar partition coefficients showed an almost tenfold difference in flux, on the other hand, dyes with a small difference in flux were separated by a decade of partition coefficients.

The octanol/water partition coefficients also seemed to be at odds with those determined for stratum corneum/water, and there was no evidence of a pattern that could be persuasively interpreted. The data almost imply that the lipid domain in the horny layer is the unlikely site of the retention of lipophilic dyes. That the distribution of dyes in the stratum corneum is highly complex is further evidenced by the fact that the delipidization of stratum corneum by chloroform/methanol increased the values of the partition coefficients of the dyes irrespective of whether they were hydrophilic or preferred a nonpolar environment. Clearly, the removal of lipids augments the reservoir capacity of the horny layer. It would be presumptuous, however, to assume that such an increase simply translates into faster or more extensive diffusion, as the latter critically depends on the binding of the dyes to the stratum corneum, and there is no information to show how delipidization affects the binding characteristics.

A useful (albeit perhaps only incremental) insight into the mechanism of scalp penetration by hair dyes can be gained from the $T_{1/2}$ values of urinary excretions. The results of monitoring urinary recoveries of dyes administered orally or by parenteral injection show (Maibach H. I. and Wolfram L. J. unpublished observation) that the elimination of these materials from either the rhesus monkey or man is rapid, yielding $T_{1/2}$ values of 4 h or less. This was clearly not the case for the urinary dye recoveries following hair dyeing, where the $T_{1/2}$ values varied between 10 and 40 h, suggesting that only trivial amounts of dye had penetrated the stratum corneum

Table 2. Flux of hair dyes through human scalp

Dye	Flux ($mol/cm^{-2}\ h^{-1}$)
DAA	9.2×10^{-11}
Resorcinol	2.2×10^{-10}
4-Amino-2-hydroxytoluene	4.5×10^{-10}
2-Nitro-PPD	4.7×10^{-10}
HC Blue 1	4.9×10^{-10}
PPD	6.3×10^{-10}
4-Amino-2-nitrophenol	8.3×10^{-10}

Table 3. Partition coefficients of hair dyes between octanol/H_2O and stratum corneum/H_2O[a]

Dye	Partition coefficient		
	Octanol/H_2O	Intact stratum corneum/H_2O	Delipidized stratum corneum/H_2O
DAA	0.7	–	–
Resorcinol	7.0	3.6	7.7
4-Amino-2-hydroxytoluene	25.4	8.0	21.1
2-Nitro-PPD	3.6	13.2	28.9
HC Blue 1	10.8	2.8	7.3
PPD	0.2	4.0	7.3
4-Amino-2-nitrophenol	10.1	5.0	9.1

[a] Guinea-pig stratum corneum

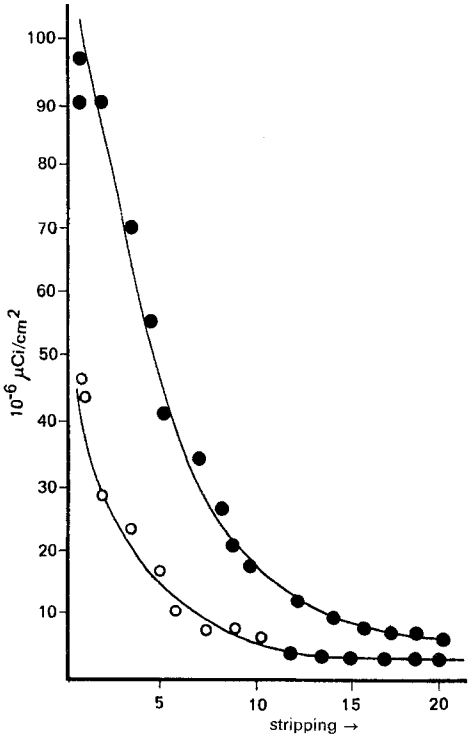


Fig. 1. Distribution of radioactive PPD in the horny layer. (●) Immediately after coloring; (○) 16 h after coloring

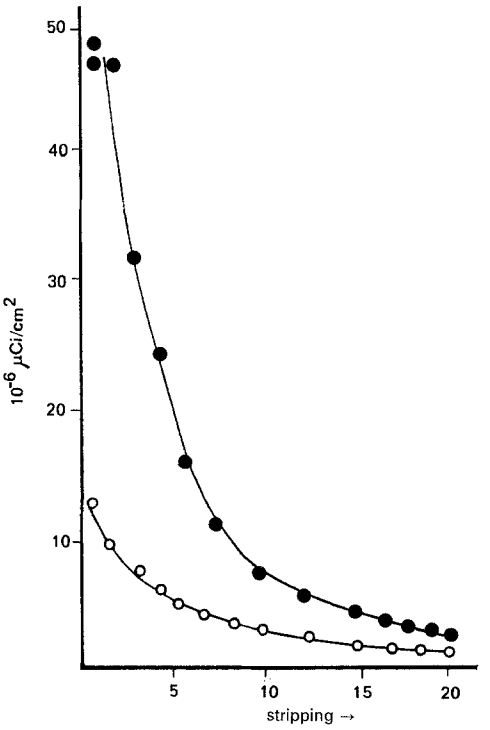


Fig. 2. Distribution of radioactive HC Blue 1 in the horny layer. (●) Immediately after color application; (○) 18 h after color application

during the actual process of hair coloring. It follows that the bulk of the urine-recovered dye must have been taken up into the horny layer and then slowly released into the circulation. Some penetration of the hair follicles and/or sweat ducts might also have occurred, but this shunt mechanism – judging again by high $T_{1/2}$ values – seems of less importance. Direct experimental support for the magnitude of the horny-layer reservoir was obtained by applying a measured quantity of dye formulation to the forearms of human volunteers (thus mimicking the dyeing procedure) and then removing the sequential layers of stratum corneum as far as the glistening layer by stripping with adhesive tape. The application areas were large enough to allow the stripping of adjacent regions 16 or 18 h after the color application. Figures 1 and 2 illustrate the results obtained for PPD and HC Blue 1, respectively. The change in the concentration profiles of both dyes with time is a dramatic demonstration of their mobility in the horny layer and serves as an independent confirmation of the observed kinetics of scalp penetration.

In view of the comparable mobility of both dyes, it is tempting to conclude that the diffusing moiety of oxidative dyeing is primarily, if not exclusively, unchanged PPD. Although the formation of permanent colors involves coupling reactions which are associated with the progressive decrease in the concentration of the oxidative precursors (PPD, DAA, resorcinol, etc.), it has been shown [3] that as much as 50% of the originally present precursors is recovered unchanged on completion of the hair-coloring process.

The magnitude of the dye reservoir in the horny layer was readily estimated from the cumulative radioactivity of the isolate strips. For PPD, we obtained a value of 6 µg/cm^2 and, for HC Blue 1, we obtained a value of 4 µg/cm^2. The standard dye dose used in hair coloring with Nice' n Easy (124) is 1,620 mg PPD, and with Loving Care (795), it is 1,330 mg HC Blue 1. Assuming the average surface area of the scalp to be 600 cm^2, the dose absorbed during hair dyeing yields a value of 0.22% for PPD and 0.18% for HC Blue 1. The correspondence between these values and the results obtained from the urinary assays is striking and strongly supports the previously expressed view that the bulk of dye penetration takes place from the horny-layer reservoir formed as a result of the hair-coloring process.

Application and Wear

The hair-coloring procedures employed in this part of the study were identical to those already described; however, the collection of urine and the radioactive

Table 4. Dose absorption of hair dyes in man under conditions of use (application + 30-day wear)

Dye	Number of subjects	Cumulative dose absorption (%)					$T_{1/2}$ (h)
		1st day	10th day	20th day	30th day	(SD)	
PPD	3	0.19	0.31	0.34	0.34	(0.12)	26
2-Nitro PPD	4	0.19	0.42	0.62	0.75	(0.30)	150
HC Blue 1	4	0.15	0.28	0.30	0.50	(0.15)	138
HC Blue 2	4	0.01	0.07	0.094	0.09	(0.02)	52

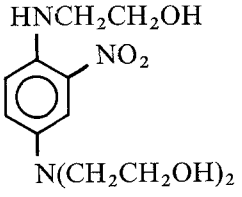
assays continued for as long as 30 days following the dye application. The results of the assays, both total and interim, are given in Table 4. The $T_{1/2}$ values of urinary excretions are also included.

The results fall into a pattern that could be anticipated from the mechanism of hair coloring that is characteristic of a given class of dyes. In the case of oxidative (permanent) dyes (based on PPD and its couplers), the color-forming reactions convert the small, mobile, and colorless molecules into much bulkier dye moieties trapped within the structure of the hair. There is little chance for these materials to diffuse out of the hair even when the latter become fully swollen during shamponing. On the other hand, the direct (semipermanent) dyes, on their deposition in the fiber, do not undergo any changes in size and retain some mobility which translates into a potential for outward diffusion. Thus, while the hair acts as a repository for both types of dye, their bioavailability is clearly different. This arrangement is experimentally verified by the urinary-excretion data. There was only a marginal increase in the dosage absorption of PPD when compared to the Application-Only values, and most of the increase was generated within 2 days of the color application. On the other hand, HC Blue 1 and 2-nitro-PPD registered a four- to fivefold increase, with measurable absorption values spread over several weeks. This trend was also reflected in the $T_{1/2}$ values of urinary excretion – a trivial change for PPD, but a substantial increase for both HC Blue 1 and 2-nitro-PPD.

The excretion data shown in Table 4 not only reflect scalp penetration but also include dye which had become bioavailable through other ports of entry (dermal as well as oral) – a real-life situation. In this sense, the $T_{1/2}$ values do not have the same meaning as those in Table 1, where they exclusively refer to scalp permeation.

The higher mobility of semipermanent dyes, as compared to their oxidative counterparts, implies faster depletion of the hair reservoir. This is fully attested to by the results of the radioactive assays of the dyed hair. Over the 30-day wear period, the hair colored with permanent (oxidative) dyes lost approximately 10% of its original dye content, while losses of well over 60% were recorded for hair colored with semipermanent dyes.

In Table 4 the results presented for HC Blue 2 appear to be at odds with the remainder of the data in the table and the arguments which have just been put forward. In its chemical structure



HC Blue 2 is a close homolog of HC Blue 1. It remains unaltered during hair dyeing, yet in its urinary-excretion characteristics (particularly during the first 24 h), it is more like some oxidative-dye precursors, such as DAA or resorcinol, than its semipermanent counterparts. HC Blue 2 is much more water soluble than HC Blue 1 (octanol/water partition coefficient, 1.6) and, in its partition coefficient (1.1), shows a lower preference for stratum corneum than any of the dyes shown in Table 3.

A clue to the unusual behavior of this dye was provided by skin-stripping experiments. Figures 3, 4 show the dye-content profiles of human stratum corneum stripped from forearms dyed with compositions containing either HC Blue 1 or HC Blue 2. The stripping was done immediately after dyeing and again 6 h later. The radioactivity of the dye lotions was almost identical, as were the assays of stratum corneum strips harvested immediately after dyeing [29.7×10^{-4} μCi for HC Blue 1; 30.3×10^{-4} μCi for HC Blue 2]. Obviously, both dyes diffused at comparable rates while the dye lotions were present. However, the assays of the 6-h strips revealed that, while the activity of HC-Blue-1-dyed skin decreased to 24.5×10^{-4} μCi (16% loss), the activity of HC-Blue-2-dyed tissue remained unchanged. Clearly, the HC Blue 2 was bound to the stratum corneum much more strongly than the HC Blue 1, yet such a conclusion

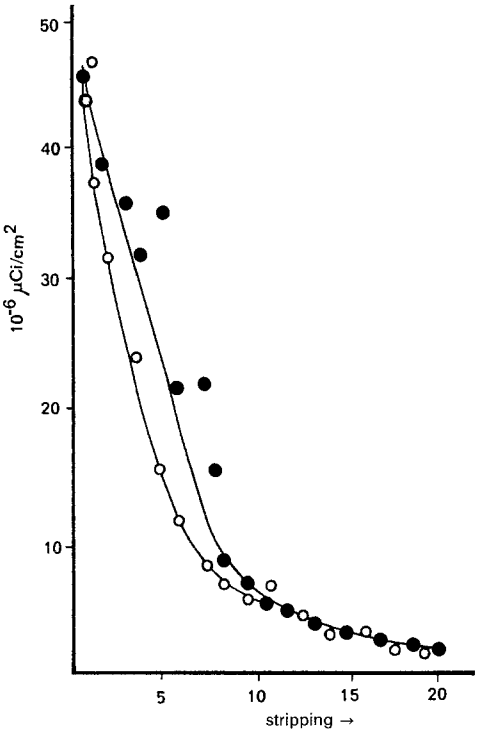


Fig. 3. Distribution of radioactive HC Blue 1 in the horny layer. (●) Immediately after color application; (○) 6 h after color application

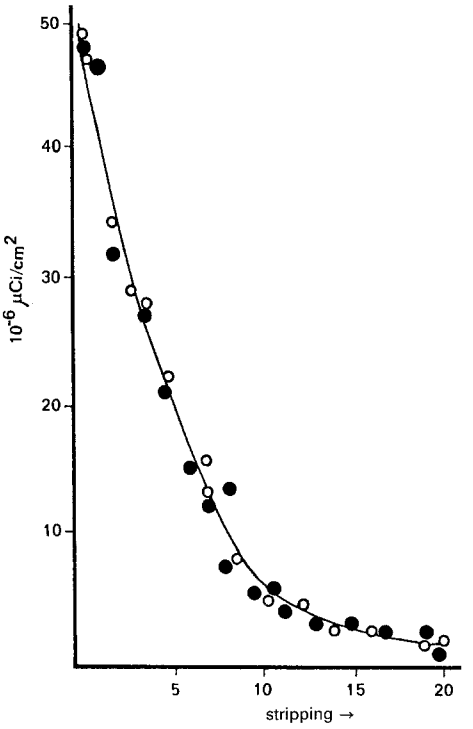


Fig. 4. Distribution of radioactive HC Blue 2 in the horny layer. (●) Immediately after color application; (○) 6 h after color application

could hardly be arrived at on the basis of its partition coefficient.

There is little doubt that an increase in the tenacity of binding is inversely related to the dye mobility within the horny layer and thus adversely affects dye diffusion into viable epidermis. With the diffusion process markedly slowed down, the natural process of desquamation assumes an important role. The bulk of the dye reservoir is located in a few of the uppermost layers of the stratum corneum, and their loss by desquamation can lead to a rapid and precipitous drop in the quantity of bioavailable dye and thus in the total extent of skin penetration. From the results presented here, it appears that HC Blue 2 exemplifies such a behavior.

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