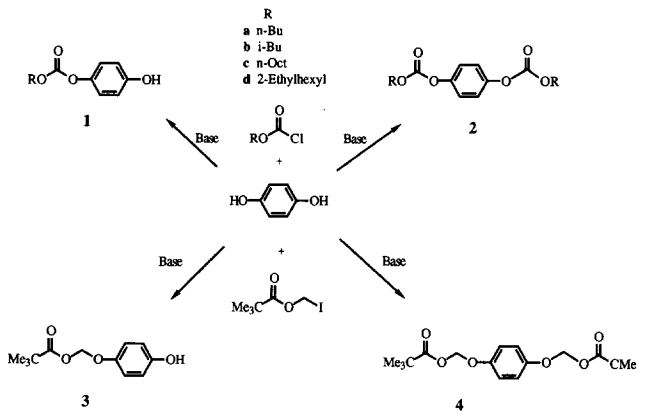
Chemistry

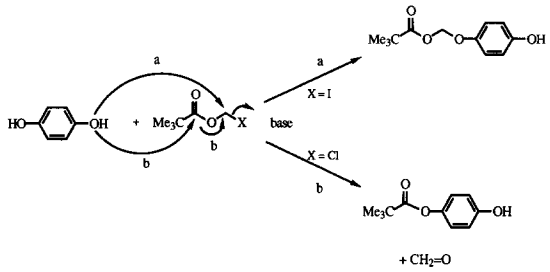
Two groups of HQ derivatives were prepared (scheme 1): mono-**1** and bis-carbonates **2**, and mono-**3** and bis-POM ethers **4**. In addition, HQ monobutyrate **5** [9] was also prepared for comparative purposes. The carbonates **1** and **2** were readily obtained by reacting HQ with one or two equivalents of the corresponding alkyl chlorocarbonates. However, in order to obtain monoalkyl ethers **3** it became necessary to use a 3:1 ratio of HQ:alkylating agent. The monoalkylation of hydroquinone has been described [10]. The alkylation reactions were carried out with iodomethyl pivalate rather than with chloromethyl pivalate. In the latter case, considerable amounts of the corresponding mono- and bis-pivalate esters of HQ were obtained. Pivaloyloxymethyl (POM) derivatives of carboxylic acids are well known [11, 12] and are readily obtained by reaction of the corresponding acids with chloromethyl pivalate. Chloromethyl pivalate can react with nucleophiles at one of the two available electrophilic centers, namely, the carbonyl or the methylene groups, respectively (scheme 2). Weak nucleophiles, such as carboxylates, react preferentially at the methylene group (path a), whereas strong nucleophiles, such as phenolates, readily attack the carbonyl group (path b). In the former case, the desired POM ethers are obtained, whereas in the latter case, the undesired pivalate esters are produced. By replacing the chloride with iodide in the starting halomethyl pivalate, it is possible even with phenolate nucleophiles to direct the attack at the methylene group [13].

Results and discussion

In an attempt to reduce the undesired side effects observed when high concentrations of HQ are used, a



Scheme 1.



Scheme 2.

series of HQ derivatives were prepared and evaluated as potential prodrugs of HQ. These lipophilic derivatives were expected to be absorbed transdermally with greater efficiency than HQ itself. Based on the assumption that the absorbed compounds behaved as prodrugs and underwent effective *in vivo* hydrolysis, smaller molar concentrations of the HQ derivatives could be expected to bring about a degree of depigmentation equivalent to that obtained with pure HQ, resulting in a concomitant reduction in the incidence of ochronosis.

An interesting and unexpected finding noted at the very first visit, *ie*, even before subjects began applying the cream, was that the subjects' right hands, their dominant hands, were darker than their left. The mean measurements for the right hand indicated a 0.19% greater degree of darkness than the left hand ($P < 0.001$). There were only three left-handed subjects amongst the 72 volunteers, a number statistically insufficient to determine whether their left hands were darker than their right. Side effects were not reported by any of the subjects during the study. Five of the initial 72 subjects were dropped from the study due to poor attendance at follow-up visits.

Depigmentation of the skin on the right hand, *ie*, the hand which received HQ only, began soon after the initial application. A comparison of 'low-dose' and 'high-dose' subjects showed that the degree of depigmentation was less in the 'low-dose' subjects (fig 1). Fifty-nine percent of the maximum attained depigmentation developed within the first 4 weeks (fig 1, high dose), and the maximum was attained at 12 weeks. Beyond 12 weeks, however, the depigmenting effect was gradually lost and progressive darkening or repigmentation of the skin occurred. The repigmentation effect always appeared at 12 weeks, irrespective of the amount of HQ used. At 25 weeks, a cross-over point was reached at which time the 'low-dose' users exhibited more depigmentation than the 'high-dose' users. The high-dose group comprised those subjects using 0.6 g or more of HQ per month, and low-dose users were those subjects applying 0.3 g

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Skin-depigmenting prodrugs of hydroquinone

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Summary — Novel mono-**1** and bis-carbonates **2**, and mono-**3** and bis-pivaloyloxymethyl ethers **4** (bis-POM-ethers) of hydroquinone (HQ) evaluated as skin-lightening agents, showed that: 1) they cause an initial depigmentation of the skin; 2) the depigmenting effect is not maintained over prolonged periods of time; and 3) prolonged use results in a shift of the chromatic locus, suggesting the formation of a new chemical product in the skin. HQ was a more potent depigmenting agent than any of the derivatives tested.

skin depigmentation / prodrugs / hydroquinone

Introduction

Oliver and coworkers [1] discovered that the monobenzyl ether of hydroquinone (HQ) caused leukoderma during the occupational use of rubber gloves. Subsequent studies revealed that HQ itself causes mild skin depigmentation *via* a mechanism involving inhibition of tyrosinase, which in two oxidative stages catalyzes the conversion of tyrosine into L-DOPA and dopaquinone [2]. At low concentrations, HQ does not cause leukoderma and is therefore used for the treatment of melanosis. HQ is unstable under oxidizing conditions and is frequently used in combination with anti-oxidants. The problem of exogenous, or cosmetic, ochronosis was first recognized in 1969, and subsequently highlighted by Finlay *et al* [3] in 1975, when 35 cases of exogenous ochronosis where described in South African black women. All had been using strong HQ-containing skin-lightening creams for several years. Five years later, by 1980, cosmetic ochronosis was reported to have reached epidemic proportions [4]. Legislation was consequently introduced to limit the concentration of HQ to a maximum of 2%. Despite this reduction in concentration, there are still numerous questions regarding HQ which remain to be answered. 1) Is 2% HQ safe, or does it still exhibit hyperpigmentation and ochronotic properties? 2) HQ-containing skin creams have been used by blacks in the USA for many years without any untoward effects. The first report of cosmetic ochronosis in the USA only appeared in 1983 when Cullison *et al* [5] described a single incidence of exogenous ochronosis. In 1985 two additional cases were described [6], and by 1988 the fifth and sixth cases were reported [7]. It is significant to note that the formulation and concentration of HQ in USA skin-lightening creams is identical to the 2% concentration of creams manufactured in Southern Africa. Yet, despite this, the incidence of ochronosis in the USA is insignificant when compared to the number of cases in Southern Africa. It can thus be postulated that some other factor may also be responsible for hyperpigmentation and ochronosis. 3) HQ has been used for almost 30 years in the therapeutic management of melanoma, ephelides and lentigines. The most widely accepted HQ formulation used by dermatologists, 'Kligman and Willis's formula', contains 5% HQ [8] – a concentration far in excess of that currently permitted in commercial preparations. Yet, despite the therapeutic use of huge concentrations of HQ, no iatrogenic cases of hyperpigmentation or ochronosis have been reported. 4) Would the application of an HQ-prodrug also cause cosmetic ochronosis? In the course of our investigations, various carbonate and ether prodrugs of HQ were synthesized and were evaluated as skin-depigmenting agents by a prospective clinical trial using reflectance photometry. The depigmenting potency of fatty acid esters of HQ has been recently described [9].

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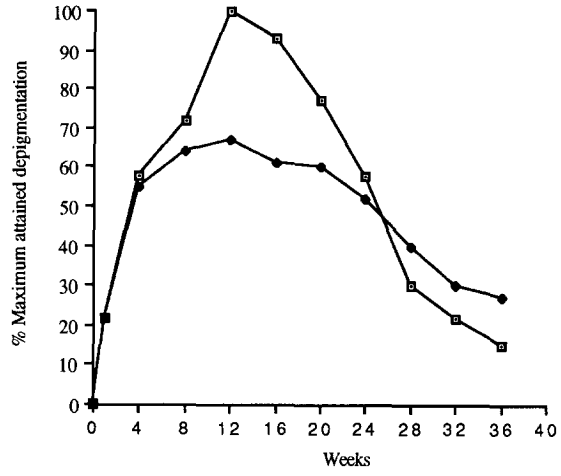

Fig 1. Percent relative depigmentation of high- and low-dose hydroquinone. —□—, high dose; —◆—, low dose.

or less of HQ per month (0.6 g of HQ corresponds to a standard 30 g tube of the commercial cream).

The results thus far indicate the intensity of the pigmentation. The readings obtained with tristimulus filters, however, indicating the actual color of the skin, showed a progressive shift in the chromatic locus during the course of the study. Comparative depigmentation rates of the left hand, *ie*, the hand exposed to the prodrug preparations, are shown in figure 2.

The maximum depigmentation induced by prodrugs **Ic** and **4** after 8–12 weeks was considerably smaller than that reached by HQ, and therefore testing of these 2 compounds was consequently terminated.

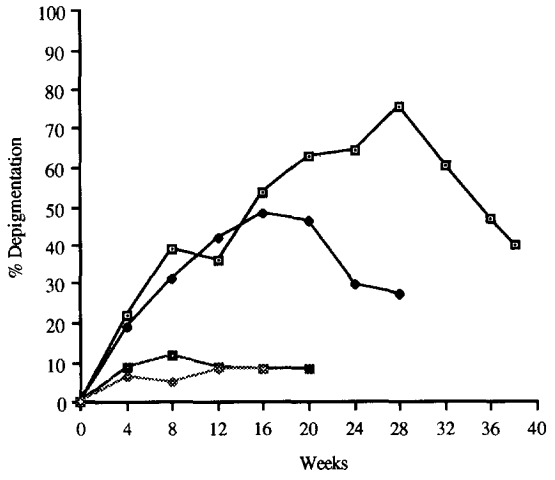

Fig 2. Percent induced depigmentation relative to hydroquinone. —□—, **2c**; —◆—, **5**; —■—, **4**; —◈—, **1c**.

Compounds **2c** and **5** were studied for 28 and 38 weeks, respectively, when **2c** attained 76% of the depigmentation induced by HQ, and **5** 48%. As with HQ, the depigmenting effect of these two derivatives was not maintained and progressive darkening of the skin ultimately occurred. These two substances also exhibited a shift in the chromatic locus.

Although HQ-induced hyperpigmentation and ochronosis invariably occurs on the face, a control site was required for this study. For obvious cosmetic reasons, it was decided not to use the two sides of the face for the study. The dorsal surfaces of the hands were thus chosen as test sites, since they have relatively thin skin, somewhat similar to that of the face. Furthermore, possible minute variations in skin color due to seasonal tanning would not affect the study, as any such tanning would affect each hand to the same degree.

The observation that the subject's dominant hands were darker than their non-dominant hands has not been noted before. Possible reasons for this could be a slightly greater use of the dominant hand with consequent greater exposure to the tanning effect of the sun. Alternatively, and perhaps more likely, the difference could be due to greater use of the dominant hand with consequent increased blood flow and hence increased melanocyte metabolism.

HQ cream

The maximum attained depigmentation in this study occurred at 12 weeks for HQ, several weeks longer than that reported by Kligman and Willis [8]. This difference, however, could possibly be due to the higher 5% concentration of HQ used in their study. Figure 1 clearly shows that 2% HQ, if applied for a sufficiently long period, will ultimately lose its depigmenting capability and the skin will tend to repigment. Kligman and Willis, however, reported that depigmentation persisted in those subjects who continued HQ application for 6 months. It is possible, however, that tretinoin and dexamethasone, which were included in Kligman and Willis's HQ formula, somehow maintain the depigmentation effect, whereas a standard skin-lightening cream that does not have these ingredients will not. This could perhaps explain why the therapeutic use of HQ, even at higher concentrations, has not been found to cause ochronosis.

The change in the chromatic locus that was noted for the HQ preparation suggests that the loss of skin depigmentation is not only the result of decreased HQ efficacy, but may also be due to the accumulation of a newly-formed substance within the skin. A recent publication [14] indicates that "the chemical composition of the ochronotic material and the pathogenesis of the paradoxical effect of hydroquinone are unknown".

Skin biopsies to determine the nature of this substance were not performed in any of the subjects. Thus, it is not possible to conclude whether this newly-formed substance is a precursor of ochronotic pigment. The chemical changes that occur in cosmetic ochronosis have, however, been discussed in several reports [3, 15–18].

The comparative depigmentation rates in high-dose and low-dose users indicates that hyperpigmentation is dose-related and will occur mainly in those subjects using greater quantities of HQ. Subjects using lower quantities of HQ clearly did not show the same potential for hyperpigmentation. Hardwick *et al* [19, 20] have indicated that Southern Africa blacks are more likely to overuse HQ, and will thus be more prone to develop ochronosis.

The study on the HQ preparation has clearly shown that the problem of cosmetic hyperpigmentation is primarily the result of improper use of skin-lightening creams, and confirms Findlay's belief [21] that: "It is not the high concentration in the container that really matters. What does matter is the uncontrolled use, several times daily over several years, of highly concentrated products."

HQ prodrug creams

All four HQ derivatives tested showed the same basic properties as HQ. These properties can be summarized as follows: 1) they cause an initial depigmentation of the skin; 2) the depigmenting effect is not maintained over prolonged periods of time; and 3) prolonged use results in a shift of the chromatic locus, suggesting the formation of a new chemical product in the skin.

The tested derivatives of HQ are expected to be subject to enzymatic hydrolyses, and prolonged exposure to them did not maintain their depigmenting effect and progressive darkening of the skin ultimately occurred in a way analogous to that observed with HQ, thereby strongly supporting the notion the compounds behave as HQ-prodrugs. The depigmenting ability of the derivatives is clearly not as effective as that of pure HQ. A possible explanation may be that, although the prodrugs are expected to be absorbed percutaneously more readily because they are more lipophilic than HQ, the actual amount of HQ administered in prodrug form is small, as its molecular weight is considerably smaller than that of the derivatives.

Experimental protocols

Chemistry

^{1}H-NMR spectra 300 MHz were obtained on a Brucker WH-300 spectrometer in $CDCl_3$. Chemical shifts were expressed in ppm downfield from Me_4Si used as the internal standard. Chemical ionization mass spectra were obtained on a Varian Mat 731 spectrometer. Progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel (Merck, 5554) and flash chromatography was carried out on silica gel (Merck, 9385).

General procedure for the synthesis of (1-alkyl)-(4-oxy-phenylene) carbonates ***1***

To a solution of hydroquinone (0.36 mol), pyridine (0.12 mol) and 4-dimethylaminopyridine (0.012 mol) in anhydrous EtOAc (400 ml) at 0°C, was added dropwise an appropriate alkyl chloroformate (0.12 mol) and the mixture was further stirred at room temperature for 24 h. The precipitate was filtered, the filtrate was flash-concentrated and the residue taken up in hexane was washed with water until no unreacted hydroquinone could be detected by TLC (hexane/ether, 1:1). The organic phase was dried ($MgSO_4$), filtered, evaporated and the residue was triturated with pentane or flash-chromatographed to give the products as white solids or oils.

(1-Butyl)-(4-oxy-phenylene) carbonate ***1a***

Obtained from butyl chloroformate in 50% yield, mp: 60–62°C. ^{1}H-NMR ($CDCl_3$) δ: 0.99(t, J = 8 Hz, 3H, Me), 1.44 (sextet, J = 8 Hz, 2H, $MeCH_2$), 1.73(quintet, J = 8 Hz, 2H, $MeCH_2CH_2$), 4.25(t, J = 8 Hz, 2H, OCH_2), 6.73 and 6.98 (AA'XX' system, J = 9 Hz, 4H, ArH). MS (CI) *m/e*: 211 (MH^+). Anal $C_{11}H_{14}O_4$ (C, H).

[1-(2-Methylpropyl)]-(4-oxy-phenylene) carbonate ***1b***

Obtained from isobutyl chloroformate in 50% yield, mp: 46–48°C. ^{1}H-NMR ($CDCl_3$) δ: 1.00(d, J = 6.5 Hz, 6H, 2 x Me), 2.05(m, 1H, CH), 4.03(d, J = 6.5 Hz, 2H, CH_2), 6.74 and 7.00 (AA'XX' system, J = 9 Hz, 4H, ArH). MS (CI) *m/e*: 211 (MH^+). Anal $C_{11}H_{14}O_4$ (C, H).

(1-Octyl)-(4-oxy-phenylene) carbonate ***1c***

Obtained from octyl chloroformate in 68% yield, mp: 40–41°C. ^{1}H-NMR ($CDCl_3$) δ: 0.9(m, 3H, Me), 1.26–1.40(m, 10H, 5 x CH_2), 1.73(quintet, J = 6 Hz, 2H, OCH_2CH_2),4.24(t, J = 6 Hz, 2H, OCH_2), 4.93(broad s, 1H, OH), 6.80 and 7.03(AA'XX' system, J = 8 Hz, 4H, ArH). MS (CI) *m/e*: 267 (MH^+). Anal $C_{15}H_{22}O_4$ (C, H).

[1-(2-Ethylhexyl)]-(4-oxy-phenylene) carbonate ***1d***

Obtained from 2-ethylhexyl chloroformate in 62% yield as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.88–0.98(m, 6H, 2 x Me), 1.28–1.48-(m, 9H, CH and 4 x CH_2), 4.18(d, J = 6 Hz, 2H, OCH_2), 6.74 and 7.0(AA'XX' system, J = 9 Hz, 4H, ArH). MS (CI) *m/e*: 267 (MH^+). Anal $C_{15}H_{22}O_4$ (C, H).

1,4-Bis-alkyl-phenylene carbonates ***2***

General procedure. Compounds **2** were prepared as described for **3** from hydroquinone (1 mol), pyridine (1 mol), 4-dimethylaminopyridine (0.01 mol) and an appropriate alkyl chloroformate (2 mol).

1,4-Bis-(1-butyl)-phenylene carbonate ***2a***

Obtained from butyl chloroformate in 38% yield, mp: 53–54°C. ^{1}H-NMR ($CDCl_3$) δ: 0.96(t, J = 8 Hz, 6H, 2 x Me), 1.44(sextet, J = 8 Hz, 2H, 2 x $MeCH_2$), 1.73(quintet, J = 8 Hz, 4H, 2 x $MeCH_2CH_2$), 4.25(t, J = 8 Hz, 4H, 2 x OCH_2), 7.02(s, 4H, Ar). MS (CI) *m/e*: 311 (MH^+). Anal $C_{16}H_{22}O_6$ (C, H).

1,4-Bis-[1-(2-methylpropyl)]-phenylene carbonate ***2b***
Obtained from isobutyl chloroformate in 52% yield as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.00(d, J = 6.5 Hz, 12H, 4 x Me), 2.05(m, 2H, 2 x CH), 4.03(d, J = 6.5 Hz, 4H, 2 x CH_2), 7.02(s, 4H, Ar*H*). MS (CI) *m/e*: 311 (MH^+). Anal $C_{16}H_{22}O_6$ (C, H).

1,4-Bis-(1-octyl)-phenylene carbonate ***2c***
Obtained from octyl chloroformate in 64%, yield, mp: 40–41°C. ^{1}H-NMR ($CDCl_3$) δ: 0.9(m, 6H, 2 x Me), 1.26–1.40(m, 20H, 10 x CH_2), 1.74(quintet, J = 7 Hz, 4H, 2 x OCH_2CH_2), 4.25(t, J = 7 Hz, 4H, 2 x OCH_2), 7.20(s, 4H, Ar*H*). MS (CI) *m/e*: 423 (MH^+). Anal $C_{24}H_{38}O_6$ (C, H).

1,4-Bis-[1-(2-ethylhexyl)]-phenylene carbonate ***2d***
Obtained from 2-ethylhexyl chloroformate in 71% yield as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.88–0.98(m, 12H, 4 x Me), 1.28–1.48(m, 18H, 2 x CH and 8 x CH_2), 4.18(d, J = 6 Hz, 4H, 2 x OCH_2), 7.18(s, 4H, Ar*H*). MS (CI) *m/e*: 423 (MH^+). Anal $C_{24}H_{38}O_6$ (C, H).

1-Oxy-4-pivaloyloxymethyloxybenzene ***3***
To a mixture of hydroquinone (26.4 g, 0.24 mol) and anhydrous K_2CO_3 (5.5 g, 0.04 mol) in dry acetone (120 ml), was added dropwise iodomethyl pivalate [12] (19.58 g, 0.08 mol). The mixture was stirred at room temperature for 48 h and progress of the reaction was monitored by TLC (hexane/ether, 2:1). The precipitate formed was filtered and the filtrate was flash-evaporated. The residue was taken up in ether, washed repeatedly with water until no unreacted hydroquinone could be detected by TLC. The organic phase was dried over $MgSO_4$, filtered, evaporated and the residue was chromatographed on silica gel eluted with hexane/ether, 2:1, to give 4.3 g (24 %) of product, mp: 90–91°C. ^{1}H-NMR ($CDCl_3$) δ: 1.20(s, 9H, *t*-Bu), 5.7(s, 2H, CH_2), 6.75 and 6.95(AA'XX' system, J = 9 Hz, 4H, Ar*H*). MS (CI) *m/e*: 225 (MH^+). Anal $C_{12}H_{16}O_4$ (C, H).

Bis-pivaloyloxymethyloxybenzene ***4***
Compound **2** was obtained as described for **1a** from hydroquinone (1 mol), K_2CO_3(2 mol) and iodomethyl pivalate (2 mol). The product was obtained in 36% yield upon trituration of the residue with pentane, mp: 50–52°C. ^{1}H-NMR ($CDCl_3$) δ: 1.20(s, 18H, 2 x *t*-Bu), 5.7(s, 4H, 2 x CH_2), 6.98(s, 4H, Ar*H*). MS (CI) *m/e*: 339 (MH^+). Anal $C_{18}H_{26}O_6$ (C, H).

Biological evaluation

The reflectance photometer is a high-precision instrument that can accurately measure minute changes in color. By using the photometer on the skin, small changes in color that may occur during evolution of dermatosis can be measured with great accuracy. This apparatus by far surpasses the results achievable by clinical assessment or by the use of highly variable, and hence unreliable, color photography.

The photometer used in this study was the Photovolt M 575 (Seragen Electronics, Indianapolis, IN, USA) and is comprised of a sensor unit and a processing unit or photometer. The basic principles of reflectance photometry may be summarized as follows. The sensor unit of the photometer consists of a light source as well as the measurement photocells. The light source bounces a light beam off the skin and the resultant reflectance is measured by the photoelectric cells. The sensor unit transmits these measurements to the photometer where they are processed and converted into a digital reading. Various filters can be placed between the skin and the sensor unit. By using special standardized 'Tristimulus' filters and plotting the readings on chromatic charts, not only the brightness of the object, but the chromatic locus, or actual color, of that object can be determined.

Patients and methods
The HQ prodrugs were initially submitted to the South African Bureau of Standards for irritancy testing (method SABS 671). A 48-h patch test was also performed on human volunteers. Three of the prodrugs, **1b**, **2b** and **3**, were found to be skin irritants and no further testing was performed with these agents. Of the remaining compounds, four prodrugs, **1c**, **2c**, **4** and **5** [9], were used in the study.

Seventy-two South African black volunteers (signed consent form issued by the Ethics Committee of the University of Witwatersrand Medical School), 69 women and 3 men, aged 19 to 58 years (mean 29.7 years) were studied. The subjects were divided into 4 groups of 18 – one group for each of the 4 prodrugs. First, their hands were thoroughly cleaned so that gloss from surface oils and sweat would not influence the readings. After standardization of the photometer, the skin on the dorsum of each hand was measured with the reflectance photometer. Three Tristimulus filters were used, and 3 measurements were taken for each filter. A total of 1296 readings were thus performed at every visit. Each subject was then instructed to apply, twice a day, a prodrug preparation to the dorsum of the left hand only, and a 2% HQ preparation to the right hand only. Both HQ and prodrug creams contained *p*-aminobenzoic acid (PABA) as a sunscreen. The subjects were seen after 1 week, and then at 4-week intervals. At the end of each 4-week period, the dorsa of the hands were again measured by reflectance photometry, the empty or half-empty tubes were collected and weighed, and the amount of prodrug or HQ cream used during that 4-week period was calculated.

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