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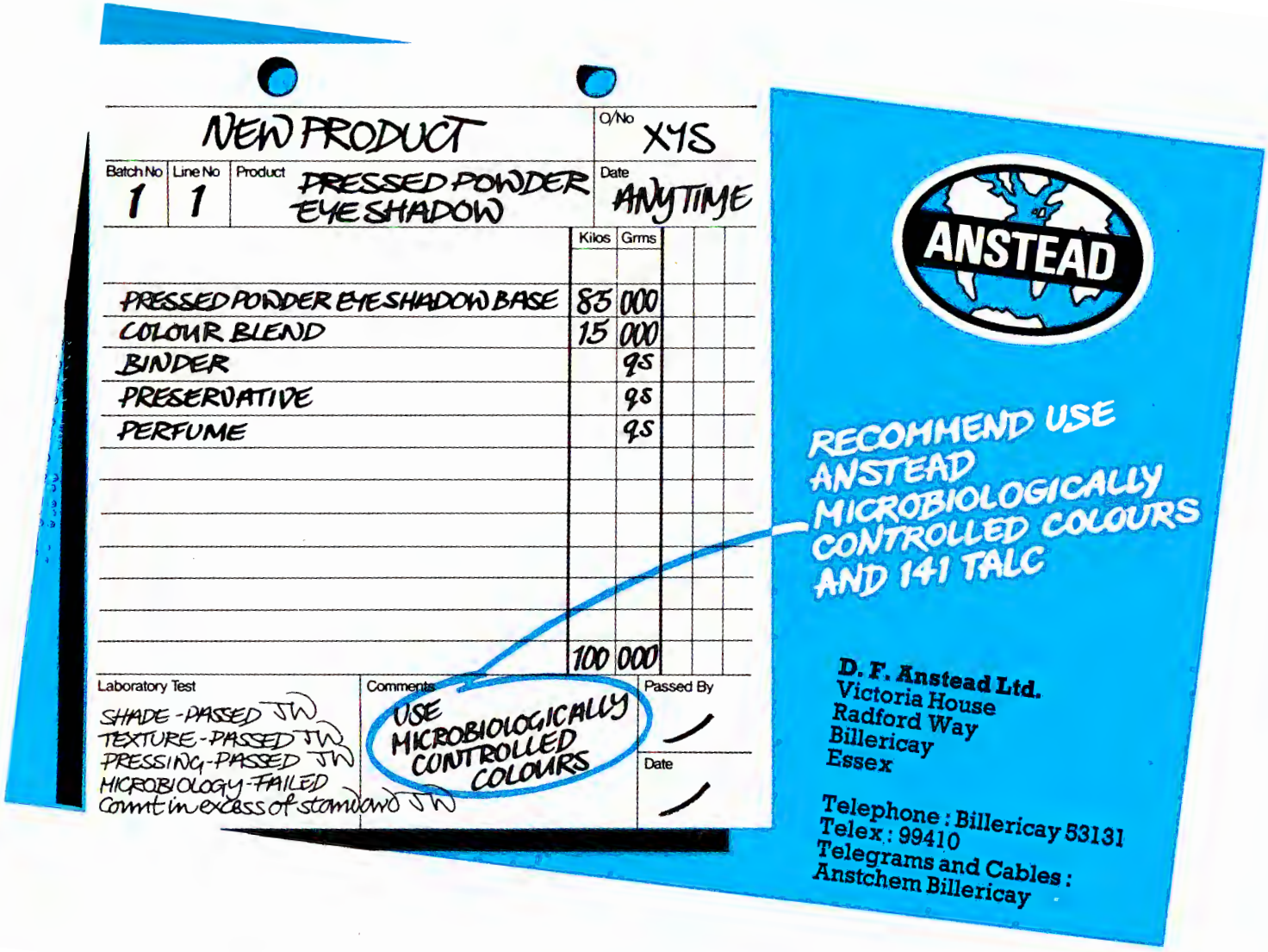
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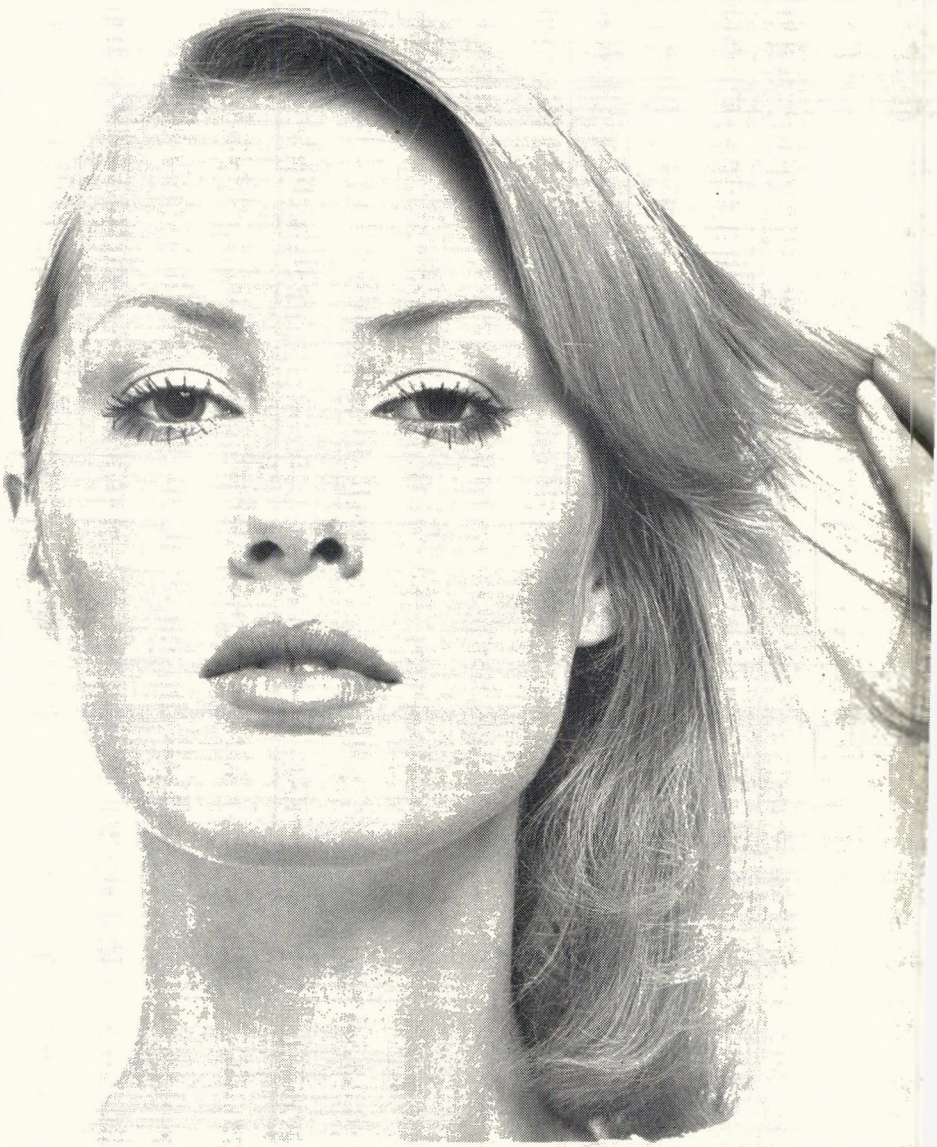

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Synopsis—Skin pigmentation shows a regularity in its geographical distribution. There appears a strong clinical component to the variation in each of the four quadrants of longitude, apparently independent of a strong continental component. Quantitative studies, based on reflectance spectrophotometry, have resulted in a better understanding of the genetics and biological significance of skin colour variation. The genetic component of normal pigmentation variation within a population is polygenic. Heritability estimates derived from a Sikh sample centre around 60–80%. The genetic basis of differences between populations appears to reside in some four gene pairs for the difference between Europeans and Africans, and two gene pairs for the difference between Indians and Europeans.

Quantitation of the geographical distribution shows a remarkably close relationship of mean pigmentation with environmental variables, and in particular with latitude, the biological significance of which appears to reside in protection against ultraviolet radiation. It appears that skin colour is also involved in thermoregulation. New hypotheses envisage a role of pigmentation in, among others, disease protection and intracellular metabolism.

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Synopsis—Total lipids from human scalp have been collected from individuals (both sexes and of different ages), and examined by differential scanning calorimetry.

Melting endotherms have been plotted between −100 and +50°C. The curves tend to change with the age of the subject. The shape of the curve is a physicochemical property of sebum, from which a diagnosis, seborrheic or not seborrheic, may be obtained.

This new parameter has been correlated with other properties of sebum.

The stability of disodiumsulphosuccinated undecylenic monoethanolamide in shampoo formulations: O. W. WHYMARK. *Journal of the Society of Cosmetic Chemists* **28** 343–349 (1977)

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Journal of the Society of Cosmetic Chemists

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Nail disorders caused by external influences: PETER D. SAMMAN. *Journal of the Society of Cosmetic Chemists* **28** 351–356 (1977)

Synopsis—Damage to nails caused by physical or chemical trauma is outlined. A surprising number of cases are due to the patients themselves either by biting, fiddling with the nails, attempting to remove non-existent parasites and occasionally deliberately to obtain sympathy. Nail cosmetics are generally very well tolerated but deformities occur at times and are described. The wearing of footwear can also cause trouble. Chemical damage is rather uncommon but frequent contact with water or soap and water is responsible for much damage to nails.

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A physical method for qualitative examination of human sebum

PIERRE BORE *and* NOEL GOETZ *Research Laboratories, Société L'OREAL, 1, Avenue de Saint-Germain, 93600 Aulnay-Sous-Bois, France*

Presented on 7 June 1976 at IXth IFSCC Congress, Boston, U.S.A.

Synopsis

Total **lipids** from human **scalp** have been collected from individuals (both sexes and of different ages), and examined by **differential scanning calorimetry.**

Melting endotherms have been plotted between −100 and + 50°C. The curves tend to change with the age of the subject. The shape of the curve is a physico-chemical property of sebum, from which a diagnosis, **seborrheic** or not seborrheic, may be obtained.

This new parameter has been correlated with other properties of **sebum.**

Introduction

The film of scalp lipids is composed of: sebum resulting from sebaceous gland excretion; lipids proceeding from the Malpighi (1) layer epidermal cells and waste fatty fractions.

Due to the preponderance of scalp sebaceous glands, these three elements are normally referred to collectively as sebum.

This human sebum has been studied, with special regard to: its composition (1) (2) (3) (4) (5) (6); the factors which influence its production (7) (8) (9) (10) (11); the mechanism of its formation (relation squalene-cholesterol).

Some authors have been interested in composition variations according to age and sex (12) (13) (14) (15) (16).

Our purpose was to define a seborrheic state through consideration of physico-chemical properties corresponding with this state.

If one attempts to define seborrheic state from a cosmetic point of view, one can say that it is characterized by: an exacerbation of the fatty and oily aspect of the scalp; the presence, on hair, of a more or less thick, shiny, greasy lipidic film which fixes dust and gives an unpleasant heavy aspect to the hair.

This subjective evaluation proves to be insufficient to start searches for the anti-seborrheic properties of certain molecules and to measure the evolution of a seborrheic state during a treatment. Our studies on the seborrhea evaluation led us to consider physical and morphological differentiations of sebum. J. L. Burton (17) in 1970, in his study of the acneic phenomenon made some physico-chemical measures of sebum such

as: density; viscosity; surface tension; melting point. The extracted amounts of sebum being small, measurements were delicate. In particular viscosity was calculated from the Washburn's equation from capillarity measurement.

We have tried to measure the melting endotherms of sebum through the Differential Thermal Analysis technique. The results of this investigation were submitted to correlation studies with some physico-chemical parameters and led us to define some properties of sebum corresponding to a non pathogen seborrheic state.

Collection methods

To obtain sufficiently reliable results it was necessary to obtain appreciable quantities and quantitative samplings of sebum. At present the known methods are: direct suction of the sebaceous gland; application to the skin of a sampling rod (7) (8) (9); application of a solvent on the skin surface (18) (19); application to skin of absorbing matter (10); friction of hair followed by washing with solvent (12) (20).

The amounts of sebum obtained are determined by gravimetry when the sample is large enough; other more or less approximate methods have been proposed when the sample is not large enough e.g. turbidimetry (19), sebograph – sebum-meter (7) (9), colorimetry (21), Acid Number – Iodine Number (19), Oxidation (19). The amounts of sebum obtained through point sampling (1 mm^2) have poor reproducibility. These variations prove the heterogeneity of the distribution of sebaceous glands. The composition of the mixture collected depends, to a certain extent, on the method of extraction used (22). In the method now to be described the total amount of sebum present on scalp and hair is collected. The principle is based on the quantitative emulsification of sebum by a surfactant mixture whose composition has been devised to effect a predetermined detergency:—

Sodium laurylsulfate	4·3 g
betaine derivative	2·0 g
$R - \overset{\overset{CH_3}{\vert}}{\underset{\underset{\oplus\ CH_3}{\vert}}{N}} - CH_2COO^{\ominus}$ (R = coprah)	
lactic acid	0·0235 g
de-ionized water qs	100 ml

The extraction is achieved in two stages:

(1) Exactly 15 ml of the surfactant solution are poured on to the wet hair; a light massage is used with gloved hands until foam is obtained. It is then rinsed with 1000 ml of de-ionized water and the whole is carefully collected in a measuring vessel.

(2) The preceding operation is repeated with 10 ml of the surfactant solution. It is rinsed and collected again in the measuring vessel and the total volume of aqueous sebum suspension is measured exactly. The whole or a part of the suspension carefully homogenized is extracted by peroxide free diethyl ether. The ether solution carefully washed with water and dehydrated with sodium sulfate, is evaporated. The sebum is evaluated by gravimetry. The amounts of sebum collected from a given person, at constant time intervals, are reproducible within limits of $t \pm 15\%$. A detailed analysis of the sebum so

collected leads to results consistent with those published in the literature and shows that the sebum so collected is not altered.

THERMAL ANALYSIS MEASUREMENTS

Definition—Apparatus—Methodology

Human sebum is in the solid crystalline form at $-100°C$; it is wholly liquid at $+50°C$. Between these two temperatures the physical state of sebum is not well defined. Some constituents are in the crystalline form, others in the liquid form. One cannot exclude the presence of other physical forms such as liquid crystals or emulsions. Differential thermal analysis records the curve $\Delta H = f(T)$.

Between $-100°C$ and $50°C$, this curve can be called, as a first approximation, the sebum melting curve. This melting signal is very widely spread on the temperature axis and we are therefore led to measure weak ΔH values against units of temperature.

It is therefore necessary to work at high sensitivity and the apparatus, under these conditions, is also sensitive to specific heat (Cp) variations.

From *Fig. 1*, the baseline of the melting curve strongly slopes towards the 'endo' direction. The form of this graph represents the evolution of Cp as a function of temperature. This aspect of the thermal analysis curve has not been exploited.

Measurements have been achieved on a thermal analyser Dupont® 900, fitted with a DSC cell (Differential Scanning Calorimetry).

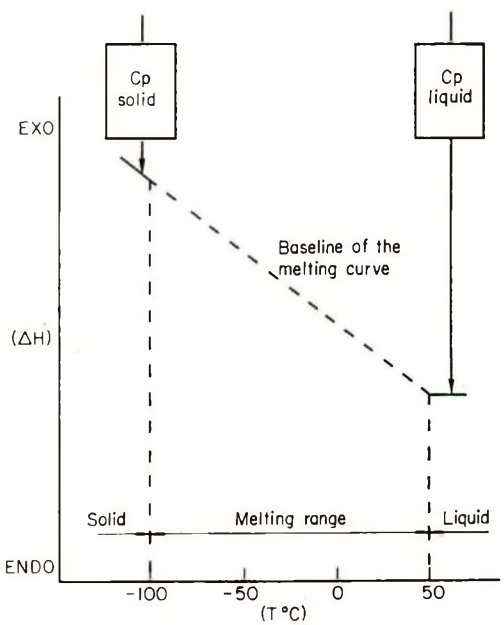

Figure 1. Localization of the melting curve of sebum on the differential scanning calorimetry plot.

The following experimental conditions were adopted

Amount of sample	: 30 mg
Sensitivity on temperature scale	: 50°C inches^{-1}
Sensibility on T scale	: 0,2°C inches^{-1}
Sample atmosphere	: dry N_2
Starting temperature	: -100°C
Final temperature	: $+50$°C
Heating rate	: 10°C m^{-1}

Each sample was submitted to two fusions from which only second plots were retained. Indeed, a difference is often observed between the first and second plot which depends on the thermal history of the sample. On the other hand, it was shown that the plot does not change between the second, the third, and subsequent fusions. The crystallization plots were not recorded, because the device does not allow a linear temperature programmed from $+50$°C to -100°C.

Exploitation of differential scanning calorimetry signals

Fig. 2 shows a typical plot of human sebum melting. The signal is very complex. Within the scope of the present study, we shall not attempt to develop a theoretical interpretation of this curve. In order to obtain from the plot anything other than a purely visual observation, a processing system capable of obtaining comparative numerical results for several individuals must be chosen.

If one tries to achieve from the plot anything other than a purely visual observation one must choose a processing system capable of obtaining comparative numerical results for several individuals.

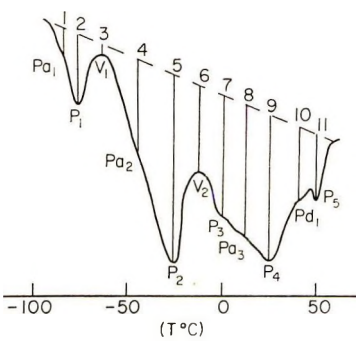

Figure 2. Typical melting curve of human sebum, with the points used for calculations.

It is possible, for example, to integrate the plot (by cutting out and weighing) and exploit the integral plot that would result from it, and which would represent the melted fraction as a function of temperature.

Finally 11 specific points were chosen that could be found again on every plot. The plots were characterized by the ordinate of these points (with respect to the baseline). This is a way of exploiting but not integrating curves. Therefore the chosen points are not equidistant on the temperature axis but they take into account the peculiarities of the plot. As *Fig. 2* shows these points are: Slopes up and slopes down (Pa and Pd); peaks (P); valleys (V). The ordinates were normed with respect to their sum; we set up this sum equal to 100.

The validity of the research depends on the reliability of the device giving the plots. The plots in *Fig. 3* were recorded successively starting from the same sample. It is obvious that the apparatus can give the plots with satisfactory reproducibility.

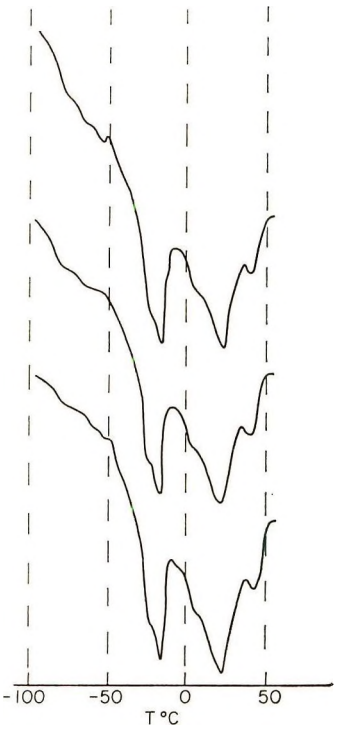

Figure 3. Reproducibility test for the apparatus. Successive plots from the same sample.

SAMPLING

The previously described method of sampling was well accepted by the subjects, allowing us to carry out a large number of operations on heads without any difficulty.

The research was performed on forty-seven subjects of both sexes with natural hair (not dyed, not bleached) whose ages ranged from 5 to 65.

For each subject many samplings were carried out at various time intervals.

Results

The melting plots for human sebum are all in the same temperature range (from $-100°C$ to $50°C$).

The same signals are always encountered, but the relative ratios of some of them in respect to the others vary widely from one subject to the other.

In *Fig. 4* the melting curves of three subjects are reproduced. Obviously the plots are typical of each subject. The relationship, subject-appearance of the melting curve is constant in time—for a given subject the same pattern is met with from sebum sampled at several weeks intervals.

Investigations of the plots obtained for the population studied permitted the following conclusions to be drawn: none of the characteristics of the thermal analysis curve

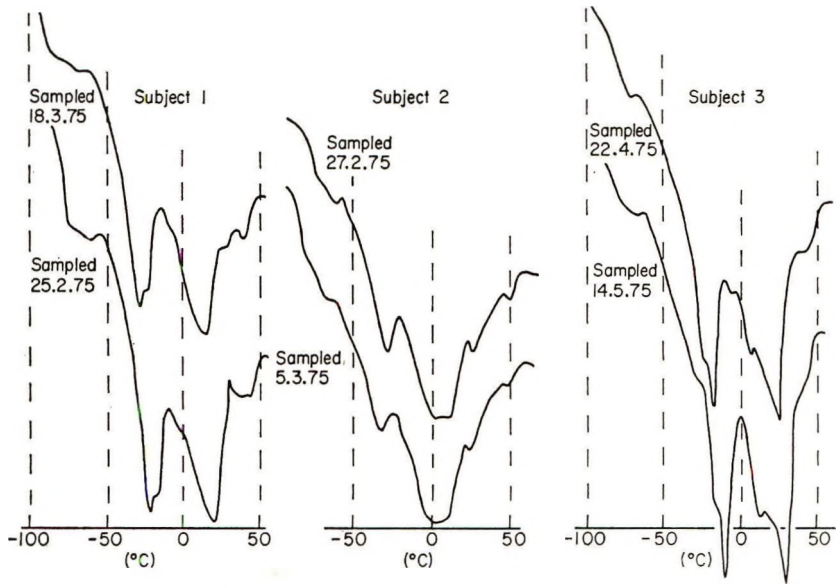

Figure 4. Characteristic melting curves from three different subjects.

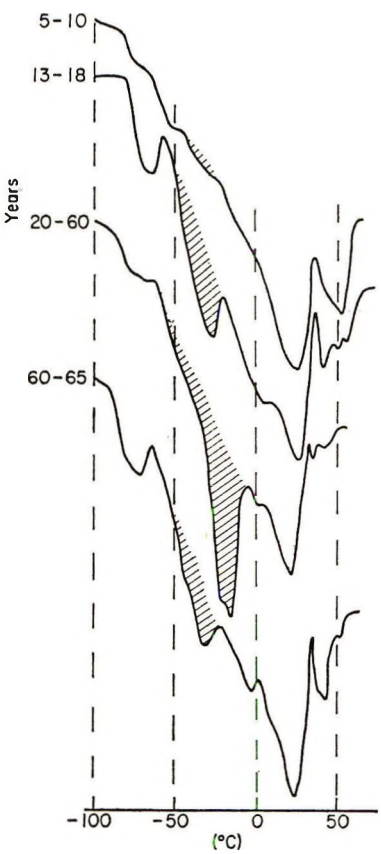

Figure 5. Examples of melting curves showing the evolution of sebum with age.

depended on the sex of the subject; the thermal analysis curves varied with the age of the subject as is shown by the plots reproduced in *Fig. 5*. Note that the area of the melting signal below $-15°C$, increases very drastically at puberty, increases less quickly from 20 years on, then decreases gradually with ageing.

This trend is emphasized by the plots of *Fig. 5* which are individual cases that we chose in each age group.

A method was then sought to check whether this trend was confirmed within the total population.

The melting plots were characterized by eleven numbers which are the ordinates (normed in respect to their sum) of the previously defined points.

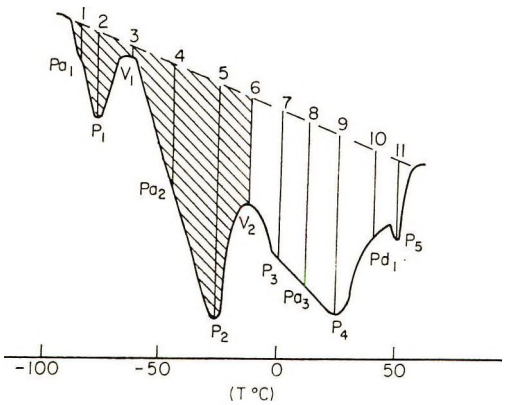

Figure 6. Typical melting curve of human sebum. Calculation of the parameter Σ_6.

The area of the curve located below the temperature of $-15°C$ is represented by the sum of the first six ordinates. This value will be afterwards symbolized by Σ_6 (*Fig. 6*)

$$\begin{aligned}\Sigma_6 = &\text{ normed ordinate of } Pa_1\\ &+ \text{ normed ordinate of } P_1\\ &+ \text{ normed ordinate of } V_1\\ &+ \text{ normed ordinate of } Pa_2\\ &+ \text{ normed ordinate of } P_2\\ &+ \text{ normed ordinate of } V_2.\end{aligned}$$

The value Σ_6 has not a definite physical meaning: recourse to this term is an arbitrary way of defining the melting curve. One consequence is that the larger the sebum fraction melted at $-15°C$, the higher Σ_6.

The term Σ_6 was determined for the whole population. It varies between 26 and 57. *Fig. 7* shows the average values of Σ_6 in the different age groups.

Within the whole population, the trend previously illustrated by individual cases is repeated: a sharp increase of Σ_6 at puberty with a tendency to decrease with increasing age.

Discussion

The population was submitted to a clinical examination. The clinician and the subject answered three questions concerning the observation: of the state of the hair; of the state of the scalp; of the frequency of shampooing required for good set of the hair-styling.

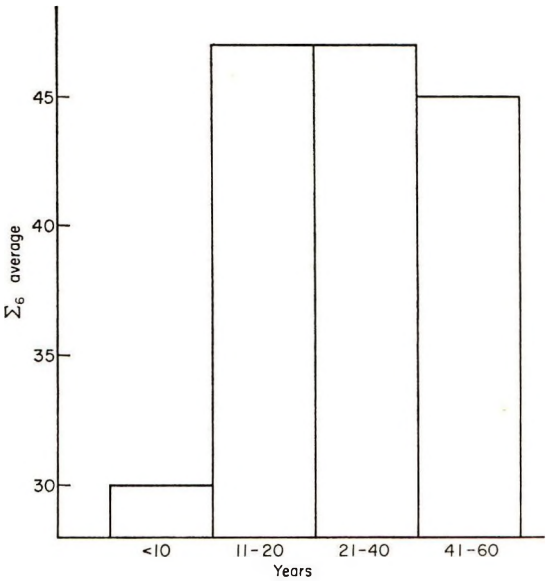

Figure 7. Average values of Σ_6 in the different age groups.

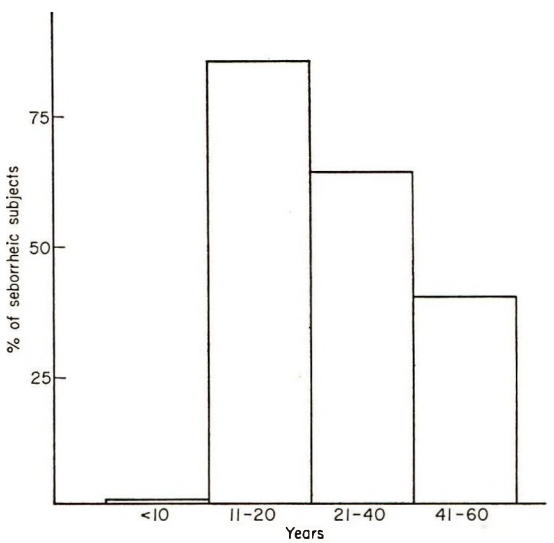

Figure 8. % of seborrheic subjects in the different age groups.

The answers distinguished a certain percentage of seborrheic individuals. The diagnosis of this clinical examination was formulated from a strictly cosmetic point of view. *Fig. 8* shows how seborrheic persons were distributed in the different age groups.

It is clear that persons affected with seborrhea are encountered in all age groups, except children under 8 years. Between 13 and 18 years of age 90% of the investigated subjects were affected. The percentage of seborrheic persons then decreased with age and, if this evolution is compared to that of Σ_6, as shown in *Fig. 8*, the similarity is evident.

The whole of the panel was classified according to increasing value of Σ_6. From this classification, the histogram in *Fig. 9* was drawn based on the conclusions of the clinical examination.

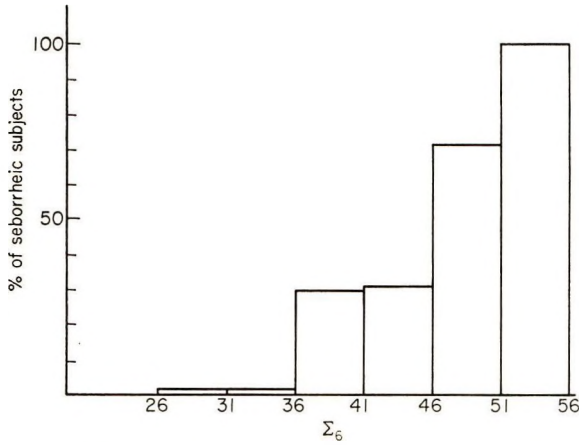

Figure 9. Distribution of seborrheic subjects according to values of Σ_6.

It is to be noted that the percentage of seborrheic subjects increases with Σ_6. For Σ values included between 26 and 36 no cases of seborrhea were found.

As a corollary, above $\Sigma_6 = 51$ only seborrheic individuals were found.

These two groups represent 20% of our population. For these subjects the determination of Σ_6 gives a perfect correlation with the diagnosis.

Between these two limits, the probability of finding seborrheic subjects increases with the value of Σ_6.

This fact shows that for $36 < \Sigma_6 < 51$ there is no absolute and perfect correlation between the conclusion of the clinical examination—which is subjective—and the parameter Σ_6 which is measurable.

This finding is not surprising; the clinical examination includes a complex reality where there is simultaneously: the quantity of sebum secreted per unit time (possibly corrected by a factor related to the mass and to the surface state of the hair), the quality of sebum which is related to its physical properties (spreading, viscosity, melting plot . . .). The method used only included the qualitative parameter.

There are other measurable parameters which define this notion of quality of sebum especially viscosity. Other parameters directly bound to composition are: percentage of squalene; iodine number; the ratios of the quantities of palmitic and palmitoleic acids.

These parameters were determined for the whole population, to see whether they are in correlation with Σ_6.

The correlation is positive with iodine number and percentage of squalene, negative with viscosity and the ratio

$$\frac{\text{quantity of palmitic acid}}{\text{quantity of palmitoleic acid}} \quad (\textit{Fig. 10})$$

These correlations are readily explicable: a high Σ_6 shows that the melting signal is important in the low temperature range. In other words, at ambient temperature, the larger is the liquid fraction of sebum the higher is Σ_6. Similarly high Σ_6 values mus

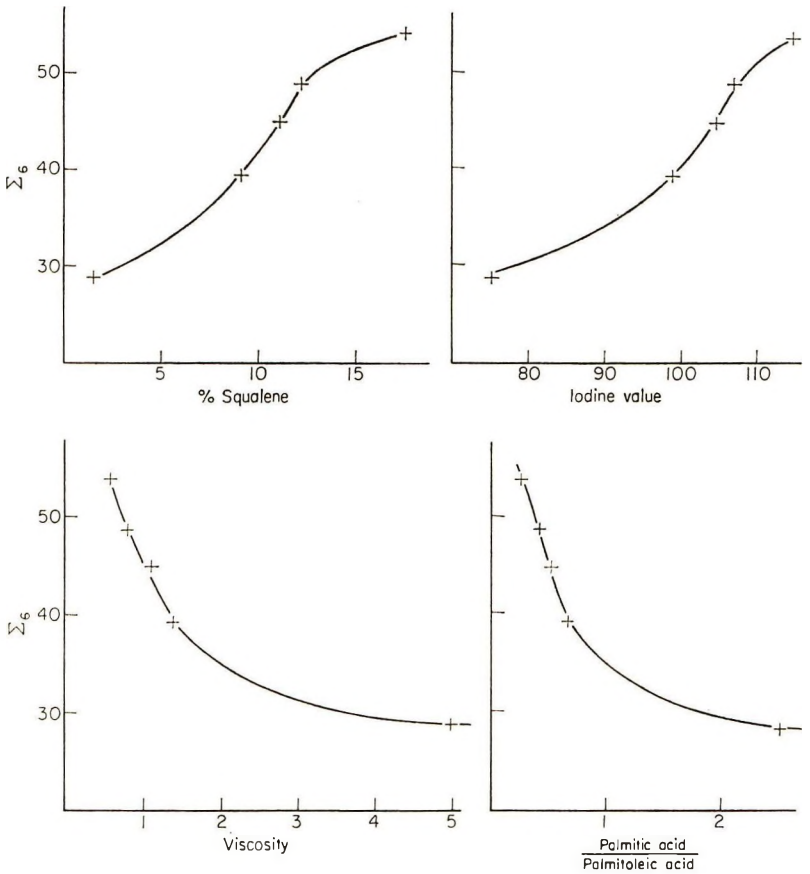

Figure 10. Correlations between Σ_6 and some physico-chemical parameters.

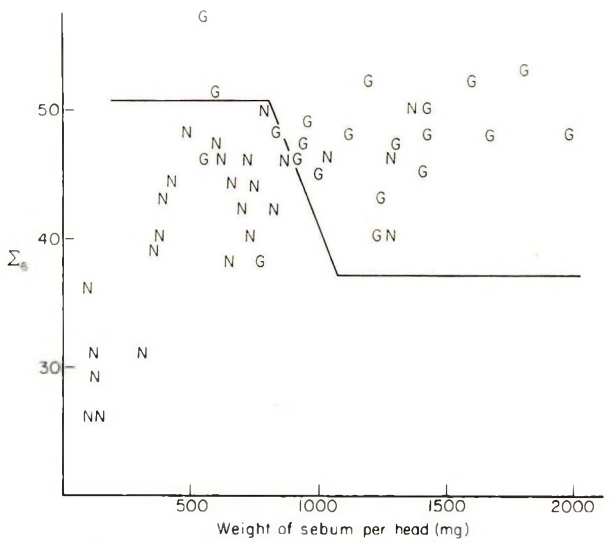

Figure 11. Relation between quantitative and qualitative parameters.

correspond with a low viscosity. Squalene, a polybranched polyunsatured hydrocarbon is a low viscosity low melting oil, consequently, the richer the sebum is in squalene, the higher is Σ_6.

The correlations of Σ_6 with the two other variables: iodine number; ratio palmitic acid/palmitoleic acid; illustrate in a similar manner the relation between the fluidity of sebum and the proportion of unsaturated compounds which compose it.

Independently of these qualitative criteria, a quantitative parameter was also determined, this being the mass of sebum extracted from the head. *Fig. 11* shows the relation between the quantitative and qualitative parameters; also indicating by G, seborrheic subjects; N, non-seborrheic subjects.

The results of the differential scanning calorimetry allow us partially to define the borders of this field: if Σ_6 is lower than 36 the quality of sebum is such that the subject is certainly not seborrheic; on the contrary if Σ_6 is higher than 51 the quality of sebum is such that the subject is certainly seborrheic. If Σ_6 lies between these limits, the clinical symptom arises from a joint contribution of the qualitative and quantitative parameters.

Conclusions

The method represents a new approach to the qualitative estimation of human sebum.

Considering the correlations which have been established the following physico-chemical parameters of sebum appear to define the limits of a seborrheic or non-seborrheic state:

Physico chemical parameter	Seborrheic state	Non-seborrheic state
Σ_6	> 51	<36
% squalene	> 12	< 9
Iodine number	>102	<80
Palmitic Acid / Palmitoleic Acid	$< 0{\cdot}70$	> 1
Viscosity 25°	$< 0{\cdot}7$*	$> 1{\cdot}5$†

* Newtonian

† Plastic or pseudo plastic

In practice these physico-chemical measurements are not all easily accessible. Thus the determination of the percentage of squalene requires the use of a gas-liquid chromatographic procedure. The determination of C_{16} acids requires a preliminary saponification then precise chromatography on a capillary column. The viscosity, when determined by a cone and plate process as used in this work, requires many samples. The applied constraint and the temperature at which the measurement is carried out, are chosen in an arbitrary manner before the measurement and their choice has an effect upon the significance of the observed values.

On the other hand, the iodine number is a parameter easy to determine with good precision but the results lie in a narrow range of values which are difficult to interpret

The new method supplies more information since by following the thermodynamics of fusion it is possible to measure objectively slow or small changes in sebum, for example in the course of a corrective treatment. This method is easy and can be applied to small amounts of sample (30 mg). One could also consider its application for the study of acnea.

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Human pigmentation: its geographical and racial distribution and biological significance

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Synopsis

Skin pigmentation shows a regularity in its geographical distribution. There appears a strong clinal component to the variation in each of the four quadrants of longitude, apparently independent of a strong continental component. Quantitative studies, based on reflectance spectrophotometry, have resulted in a better understanding of the genetics and biological significance of skin colour variation. The genetic component of normal pigmentation variation within a population is polygenic. Heritability estimates derived from a Sikh sample centre around 60–80%. The **genetic** basis of differences between populations appears to reside in some four gene pairs for the difference between Europeans and Africans, and two gene pairs for the difference between Indians and Europeans.

Quantitation of the geographical distribution shows a remarkably close relationship of mean pigmentation with environmental variables, and in particular with latitude, the biological significance of which appears to reside in protection against **ultraviolet radiation**. It appears that skin colour is also involved in thermoregulation. New hypotheses envisage a role of pigmentation in, among others, disease protection and intracellular metabolism.

Introduction

Of all biological variation in man, skin pigmentation differences are the most conspicuous and, after sex, the most emotive. Recognized from early prehistoric times, anecdotes on the origin of variation in skin pigmentation occur in the mythology and folklore of the ancient civilizations. Thus the Babylonians, the dark people, are thought of as the descendants of Ham, the 'curse of darkness' being the consequence of Ham's disobedience to Noah (Genesis 9: 29). In Greek mythology differences in skin colour originated from the catastrophe that occurred when Phaeton took the reins of the sun chariot from his father Helios, but was unable to steer accurately so that at some places the sun passed too close to the earth burning the inhabitants black, while at others it rose too high resulting in the inhabitants turning pale.

Early attempts to classify races were based on skin colour, following Blumenbach who in 1775 distinguished five races, yellow (Mongolian), brown (Malaysian), red (American), black (Ethiopian), and white (Caucasian), and such attempts continued right through the nineteenth century and into the twentieth century until the dynamic nature of the race process and the fruitlessness of classification for classification's sake was realized.

In the earliest days, therefore, pigmentation was identified with continental group or race of man. But the awareness of colour differences that had become widespread among

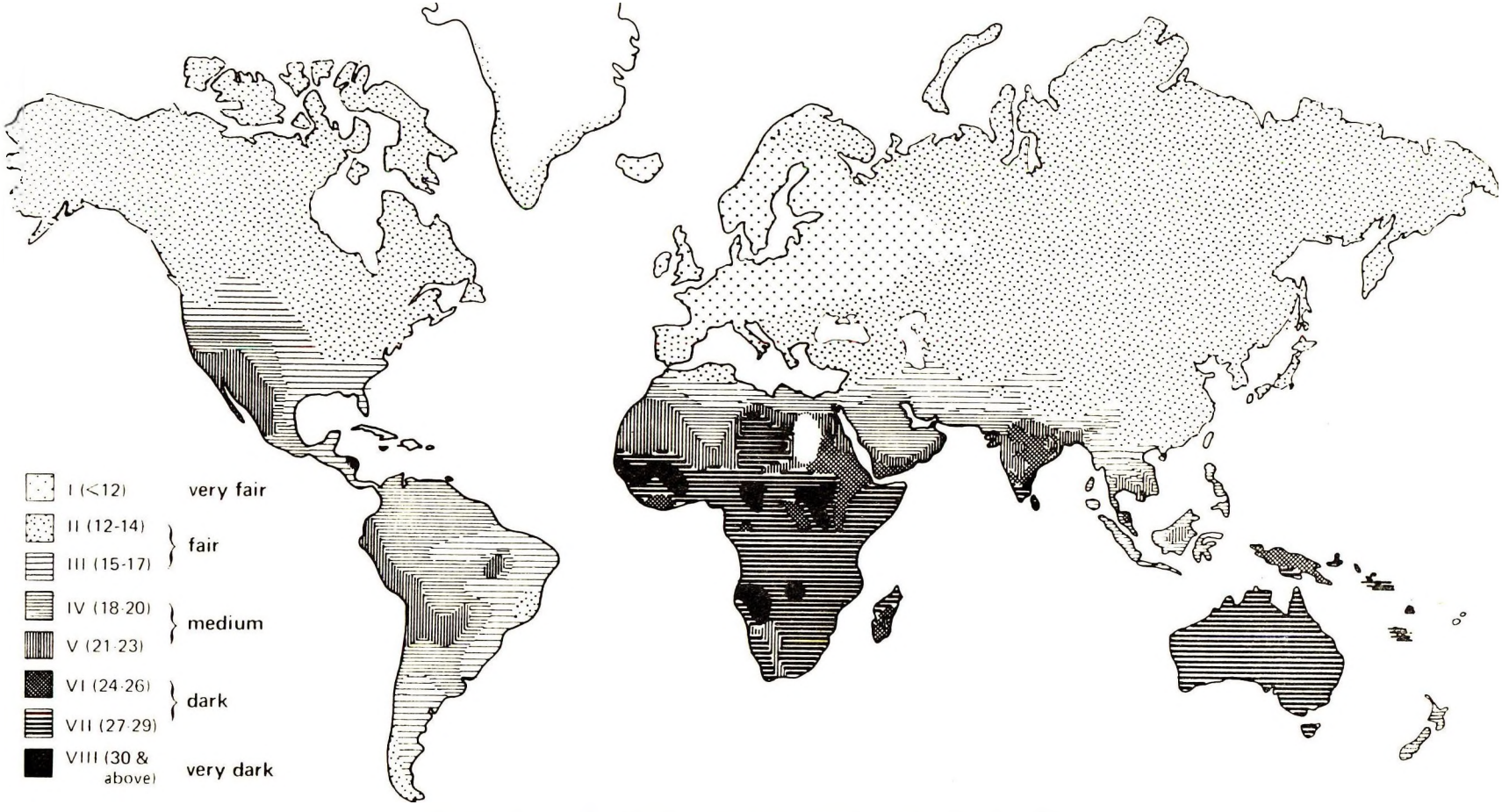

Figure 1. Geographical distribution of pigmentation (von Luschan's scale).

laymen and investigators alike led to the accumulation of descriptive observations of skin colour. These were of necessity highly subjective, but they were the foundations for the more systematic approach to colour measurement of later years. The earliest though crude approach to actual measurement of pigmentation was by arbitrary categorization of the whole range of colour into a number of shades, and subsequently colour scales were developed, such as those devised by Broca and von Luschan, respectively of paper and coloured porcelain tiles, comparison with which improved the accuracy of description. These continued to be developed until the postwar period, the latest being that of Gates (1). With all their disadvantages, nonetheless they provided the basis for a somewhat more objective comparison of human skin colours.

As a result it was possible to draw outline maps of the geographical distribution of pigmentation (von Luschan's scale) such as in *Fig. 1*. The general regularity of geographical distribution of pigmentation is clear even from these early studies. First there is a strong continental component to the variation, e.g. Europe is distinguished from Asia. Secondly there is a strong clinal component. There is no doubt about the tendency for darker skins to occur in tropical regions, and lighter in temperate, and this holds in all four quadrants of longitude; even in the Americas the generalization holds though the intensity of the gradient is rather less than in the Old World. But then on closer inspection there are further generalizations that appear to emerge. In Africa deepest pigmentation occurs not at the lowest latitudes, but appears to coincide with the great horseshoe of open savanah grasslands, while in north-west Europe pigmentation reaches a particularly low level for its latitude. Fleure's (2) discussion drew attention to obvious exceptions to the pigmentation gradient, but his attempts to explain them, in the absence of agreement on the selective function of pigmentation, essentially remain mere speculation.

In 1951, Weiner (3) described a portable reflectance spectrophotometer (EEL), suitable for field work. This made it possible to obtain, outside the confines of a laboratory, objective and accurate measures of skin colour on human populations in their native environments in the form of reflectance measures at standard wavelengths. For the first time it was possible to quantitate pigmentation. Thus it was possible to obtain information from family studies in order to investigate the inheritance of skin pigmentation, from experimental studies to investigate its physiology. The way was open to examine a variety of problems of its biology.

GENETICS

Though there is some phenotypic variation in skin colour due for example to suntanning or some disease states, there is no doubt of the fundamental genetic basis of skin colour differences, both between and within races. Take a negro child born in Africa and bring him up in Britain, or a white child born in London and raise him in Africa, and they remain respectively dark and light. There are some individual major genes, which segregate in Mendelian fashion, which affect skin colour, for example a child with phenylketonuria will show appreciably lightened pigmentation, as is dramatically illustrated in a Yemeni family (*Fig. 2*); phenylketonuria is a recessive condition, the child possessing two deleterious genes, one from his father and one from his mother, which cause deficiency of phenylalanine hydroxylase and inhibit the metabolism of phenylalanine, affecting all subsequent steps in the metabolic sequence. Such major genes are few, there is no indication in normal families of skin colour segregation such as would be explained by a major

gene effect, and it is generally thought that the genetic component of pigmentation variation is polygenic. But we have no good family studies made within a single population that allow this hypothesis to be tested, and this is a sad gap in our genetic knowledge, which my colleagues and I have endeavoured to fill.

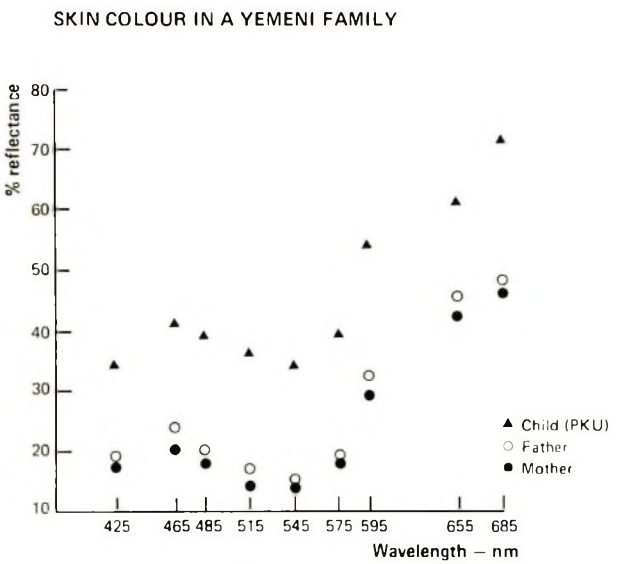

Figure 2. Effect on skin colour of phenylketonuria homozygosity.

We first looked at 141 husband and wife pairs of Sikhs (4) and showed that there was no assortative mating for skin colour at the upper inner arm site. Hence a straightforward analysis of intrafamilial correlation and regression allows estimates of heritability to be made (5). Reflectance data were collected from these 141 families, in which the number of children per family varied from one to five. We examined the regression of children on each parent at nine wavelengths, first counting each child separately, secondly using the mean of all children in a sibship, and thirdly making a weighted estimate according to the family size. The regression coefficient of each child on father ranged from 0·256 $\pm$ 0·053 to 0·399 $\pm$ 0·053 (wavelengths 545 and 685 nm). For child on mother the range was from 0·440 to 0·571 (the same wavelengths), while the regression coefficient on midparent ranged from 0·618 $\pm$ 0·068 to 0·807 $\pm$ 0·062. The regression coefficients using the sibship mean, and the weighted mean, were similar both in general level and in the curious discrepancy between the estimates on the father and on the mother. The sib/sib correlations ranged from 0·476 $\pm$ 0·055 to 0·591 $\pm$ 0·047. Breaking down the offspring by sex makes little difference. The general levels are compatible with polygenic inheritance, but there are clearly some problems of interpretation. The regression coefficients on midparent at the longer wavelengths are somewhat lower, and the sib/sib correlations at the longer wavelengths somewhat higher, than would be expected for a character competely under polygenic control, with no environmental contribution to the variance. The lower regressions on the fathers seem to imply some environmental involvement, while the fact that some sib/sib correlations are rather high again suggests the operation of some environmental factors. On account of these difficulties heritability estimates have to be accepted with critical caution.

Heritability using the data on fathers ranges from 54 to 84%, on mothers from 65 to 100%, and on midparent from 59 to 78%. I am inclined to regard the estimates from the mother as being the least reliable.

Within a population then, a polygenic hypothesis appears reasonable, and on this skin colour is of moderately high heritability. But the genetic basis of difference between populations is something quite different, and to elucidate this studies of hybrid populations are necessary. One method of analysis (6) is to compare observed distributions of pigmentation (by discrete classes) with the binomial distributions expected were a given number of genes involved. This approach assumes that the reflectance measurement is linearly related to the number of genes for pigmentation, that the genes are of equal effect, that they are additive, and that the population is in genetic equilibrium and in a state of random mating. One also needs to know the relative contributions of the parental populations to the hybrid. Stern, using United States negro data, concluded that relatively few genes are involved in the skin colour differences between Europeans and Africans, and his best estimate was four–five gene pairs. Harrison *et al.* (7) applied a similar method to Brazilian negroes and again found that relatively few gene pairs were involved, three to four giving the best fit.

A different method was used by Harrison & Owen, (8), in their investigation of the Afro-European hybrid population in Liverpool. They partitioned the quantitative variation into components and calculated the number of effective factors responsible for the interparental difference. Their calculations indicate the number of effective factors (= genes) again to be three to four.

The authors of these studies are fully aware of the difficulties attending the methods they use, and quite properly are cautious in the presentation of their results. But one cannot overlook the similarity that appears to be emerging in the results of these several studies, i.e. that the number of effective factors or genes or chromosome sections responsible for the pigmentation differences between Africans and Europeans are few in number, of the order of three or four.

A similar method of analysis has been applied to Indian/European hybrids (5). Our material relates only to first generation (F1) hybrids, so clearly one of the assumptions of the model, genetic equilibrium, is not fulfilled. Moreover, the two parental populations do not present the complete contrast in skin pigmentation that was the case in the African/European mixtures, so one cannot consider the genes for less and more pigmentation as being equal in proportion in the hybrids. In these circumstances the analysis is to be regarded only as exploratory. The best fit of the observed and the expected distributions in these hybrids seems to be accomplished at one to two pairs of factors or genes, and as the number of gene pairs increases there is a worsening of fit. While it seems entirely reasonable to expect the number of differences between European and Indian to be fewer than between European and African, these results must be regarded as tentative.

Interesting indirect supporting evidence as to the small number of genes involved in population differences comes from simulation studies by Livingstone (9, 10). He finds that with four loci the evolution of the range of human skin colour differences would take about 800 generations with no dominance, even with relatively slight differences in fitness (6% maximum) and migration between populations. In the subsequent model he also took into account the effects of population size, genetic drift and allele fixation. This work shows that observed differences are not incompatible with reasonable estimates of selection in man and duration of modern human evolution.

GEOGRAPHICAL DISTRIBUTION

Equally fascinating are the pronounced geographical gradients in skin colour, and these too can be further examined using the results of reflectance spectrophotometry. So far some 130 samples, approximately half of each sex, are available to represent the world's indigenous populations. Results are not all technically comparable, for though most were obtained with the EEL spectrophotometer, others were obtained with other models where the wavelengths used are not the same; for only a proportion of the samples are the data given at all nine wavelengths available on the EEL instrument (425, 465, 485, 515, 545, 575, 595, 655, 685 nm); the skin sites examined are not always the same; the samples vary in size, relate to individuals of different ages. The data are certainly too few for mapping. However, they can be used for an examination of those environmental variables with which pigmentation variation is most closely related.

The available data were scrutinized for comparability, and seventy-seven samples were regarded as sufficiently comparable for analysis. Each sample was located sufficiently precisely for values of the following environmental variables to be assigned to it: latitude, mean annual temperature, mean maximum temperature (average of the highest each year), mean minimum temperature (average of the lowest each year), maximum mid-day humidity (highest monthly mean), minimum midday humidity (lowest monthly mean), and altitude. These geographic and climatic variables are of course not independent of each other, as the zero order correlations show; there is for example an obvious influence of latitude on temperature, and a close correlation of mean temperature with maximum and minimum temperature. On account of these intercorrelations, our analysis (11) took the form of an examination of zero order correlation coefficients of reflectance readings with each environmental variable to show the general associations, and subsequent examination by stepwise regression in an endeavour to identify the order of importance in which these environmental variables contribute to the variation in skin pigmentation.

At the upper inner arm, most samples are available at wavelength 685, fewest at wavelengths 485 and 575. Association with latitude predominates (*Table 1*), accounting for between 88% (485 nm) and 70% (685 nm) of the total variance in mean reflectance. The second important variable appears to be mean temperature which, considered alone, would account for between 83% (485 nm) and 36% (655 nm) of the total variance. The order of the remaining variables differs from wavelength to wavelength, but in general the extreme temperature readings come next, then the humidity, while altitude shows the lowest correlations. Associations of reflectance with latitude and humidities are consistently positive, with temperatures and altitude negative. The association with geographical variables is clearly very strong, reflectance increasing with increasing latitude, decrease in temperature, and increasing humidity.

Taking into account the intercorrelation of the geographical variables in order of importance (i.e. proportion of the total variance accounted for) by stepwise regression, the order of the variables changes (*Table 2*). The contribution of latitude alone (since it predominates in the zero order analysis) remains unchanged, accounting for between 77 and 88% of the total variance for all wavelengths except 685 nm where it accounts for 70%. The addition as an independent second variable of maximum temperature at wavelengths 425, 465, 515 and 545 accounts for a further 10–21%, or the addition of mean temperature at wavelengths 485 and 575 for 11%. At the three longer wavelengths maximum humidity moves up into second place, accounting for some 2–13%.

Table I. Correlation coefficients (zero order) of environmental variables with mean reflectance at the upper inner arm
Male and female samples

Wavelength:	425 nm	465 nm	485 nm	515 nm	545 nm	575 nm	595 nm	655 nm	685 nm
Latitude	0·895	0·932	0·938	0·907	0·880	0·928	0·928	0·889	0·835
Mean temperature	−0·824	−0·795	−0·913	−0·797	−0·836	−0·898	−0·898	−0·598	−0·681
Minimum temperature	−0·533	−0·581	−0·616	−0·575	−0·596	−0·657	−0·658	−0·599	−0·590
Maximum temperature	−0·556	−0·481	−0·687	−0·511	−0·616	−0·680	−0·654	−0·101	−0·400
Minimum humidity	0·347	0·239	0·623	0·317	0·400	0·622	0·590	0·103	0·281
Maximum humidity	0·403	0·264	0·506	0·284	0·318	0·478	0·446	0·210	0·331
Altitude	−0·238	−0·285	−0·372	−0·255	−0·265	−0·371	−0·347	−0·405	−0·236
	$N = 61$	N = 45	$N = 32$	$N = 45$	$N = 55$	$N = 32$	$N = 36$	$N = 51$	$N = 77$

Table II. Percentage contribution to the variance of skin colour at the upper inner arm by environmental variables

Contributions to variance (%)

Wavelength 425 nm		Wavelength 465 nm		Wavelength 485 nm		Wavelength 515 nm		Wavelength 545 nm	
Latitude	80·1	Latitude	86·9	Latitude	88·0	Latitude	82·3	Latitude	77·4
Max. temp.	15·9	Max. temp.	9·9	Mean temp.	11·7	Max. temp.	12·3	Max. temp	21·4
Altitude	3·3	Altitude	2·7			Altitude	2·6	Min. humidity	0·8
Min. temp.	0·1	Min. temp.	0·3			Min humidity	0·9		
						Max. humidity	0·6		
						Min. temp.	0·5		
						Mean temp.	0·1		
	$N = 61$		$N = 45$		$N = 32$		$N = 45$		$N = 55$

Wavelength 575 nm		Wavelength 595 nm		Wavelength 655 nm		Wavelength 685 nm	
Latitude	86·0	Latitude	86·2	Latitude	79·0	Latitude	69·7
Mean temp.	11·1	Max. humidity	13·3	Max. humidity	1·7	Max. humidity	6·6
				Max. temp.	0·9	Max. temp.	4·0
				Mean temp.	0·6	Min. temp.	1·6
				Min. temp.	0·1	Mean temp.	1·2
						Altitude	0·7
						Min. humidity	0·2
	$N = 32$		$N = 36$		$N = 51$		$N = 77$

The predominance of latitude and the subordinate roles of the other variables is clearly shown by the first order partial correlation coefficients at the four wavelengths for which there are most samples. The correlation with latitude diminishes very slightly, if at all, after exclusion of the effects of maximum temperature or maximum humidity, diminishes rather more but still remains appreciable after the exclusion of mean temperature. Clearly the latitudinal correlation owes little to these three variables. Excluding the effects of latitude, the correlations with mean temperature diminish very markedly, those with maximum temperature or maximum humidity rather less.

The regression equations of reflectance on the relevant environmental variables at each wavelength (both sexes combined) show that these account for a remarkably high proportion of the total variance. Only for the two longest wavelengths does this drop below 97%, though exact comparison is precluded by the different number of samples available at each wavelength.

There is no doubt at all about the dominating influence of the latitudinal associations, nor of the appreciable independent contribution of maximum or mean temperature at wavelengths 425 to 475 nm. Humidity makes a negligible contribution to the variance at wavelengths below 595 nm, the effect being restricted to maximum humidity at the three longest wavelengths. The remaining environmental variables make negligible contributions. Particularly interesting is the contrast between the shorter and longer wavelengths in the importance of temperature and humidity.

These results indicate a remarkably close relationship of mean pigmentation with environmental variables, and in particular a dominating association with latitude. It is reasonable to argue therefore, that some factor associated with latitude has a strong biological influence. Of the environmental variables associated with latitude, the amount of ultraviolet radiation received at the earth's surface varies inversely with latitude, while temperature is also strongly associated; the effect of both at a given locality is, of course, modified by other variables such as cloudiness or altitude. Temperature is already taken into account in the present analysis, the latitude association of reflectance values is independent of it, so it seems most likely that ultraviolet radiation is the factor responsible for the latitudinal association of pigmentation.

BIOLOGICAL SIGNIFICANCE

Protection against ultraviolet radiation

Darwin (12) suggested selective advantage of the Negro's black skin through the protection it afforded against the harmful rays of the sun. The ultraviolet parts of the spectrum in particular, while at minimal exposures they have some beneficial effects, at greater doses promote injury in the form of sunburn and carcinogenesis. The carcinogenic wavelengths lie between 2537 and 3341Å, while erythema is induced increasingly rapidly at wavelengths of less than 3200, reaching a maximum at 2800Å. However, the relationship of pigmentation to solar radiation is not simple. Much of the radiation reaching the surface of the body does not reach the deeper melanin-containing layers. It is scattered, reflected, and absorbed in the outer horny layer of the skin (though in dark skins this layer too contains some melanin). However, some radiation reaches the deeper layers. When sheets of the stratum corneum from Africans and Europeans in Nigeria, shown to be similar in thickness to each other, were placed on a photographic plate and

exposed to ultraviolet irradiation, the European specimen showed pronounced penetration, whereas the degree of penetration in the darker African skin was much less (13). Comparing darker Europeans with fair, Mackie & McGovern (14) found that the fairer absorbed more radiant energy in the dermis, while the darker absorbed the greater part in the basal layer. Deeper penetration of ultraviolet radiation into the dermis in less melanized whites has also been demonstrated in several more recent studies (15, 16, 17). Moreover, the positive results of ultraviolet therapy in whites with rickets show that active rays do penetrate deeply enough to affect at least the superficial capillary circulation or to stimulate the nerve endings. Other experimental studies of differential penetration have shown that while transmission of invisible rays is reduced, wavelengths of 3650Å penetrate white human skin 2·2 mm thick. Pronounced penetration into the corium and subdermal layers requires rays of over 4000Å. A considerable proportion of ultraviolet rays at 2500Å reaches the corium, but rays at 2700–2800Å are practically all absorbed in the epidermis, and Negro skin absorbs particularly in the region from 2537 to 2800Å. While the photoprotective role of melanin is a complex phenomenon, involving attenuation of radiation by scattering, effective absorption, and dissipation of the damaging rays as heat, the barrier of melanin in the basal epidermal layers is obviously effective.

There are other lines of evidence besides the experimental. The fate of those dwellers in the tropics who have little or no melanin is instructive. Adult albinos in tropical areas develop a thickening (absent in young children) of the horny layers of the skin, a less efficient barrier than an abundance of melanin. Second, there is the evidence of cancer development. The rarity of cutaneous cancers in dark-skinned as compared with light-skinned subjects has often been noted. Those Europeans who are affected tend to be outdoor workers whose face and dorsum of hand (exposed areas) are the most frequent sites, while in whites in the United States there is a latitudinal north-to-south gradient in the increase of these tumours. The selective effect of protection against these tumours is likely to be small, for they are relatively infrequent and they occur mainly in the later years of life after reproduction has ceased. Another type of tumour is the melanoma, also rare but selectively more severe since it can occur in young adults; this is a pigmented tumour which in Europeans arises from pigmented spots in the epidermis, and in Negroes from relatively unpigmented regions of the body. While cases are rare in Europe, there were 395 melanoma cases in 20 000 white Australian patients, all of whom had lived a long time in Australia, and this high prevalence could well be related to excessive exposure to sunlight. Melanomas are said to be rarer in Negroes, yet malignant melanoma, next to squamous-cell cancer, is the commonest form of malignant disease seen in east and central Africa, and in Bengal it is much more common than in Europe. Most of the squamous-cell cancers are due to malignant changes in chronic tropical ulcers of the legs and the epitheliomata associated therewith, while the malignant melanomas occur mostly on the foot on its unprotected plantar surface. The extent to which radiation is involved, in that injury exposes the deeper tissues to it, is not clear. There is, however, considerable confirmatory evidence from animal experiments on the relation of pigmentation to cancer.

The protective function of melanin it seems is twofold. First in the short-term the melanized epidermis protects the deeper layers of the dermis against immediate damage, and secondly in the long-term it affords protection against cancer. Short-term damage can be prevented to some extent by secondary pigmentation in the form of sun tan, but the long-term effects seem affected more by primary pigmentation.

Thermoregulation

The results of the present analysis suggest also that thermoregulation may also be invoked as a selective mechanism for skin pigmentation, though in a subordinate but measurable role. Although melanin may bestow on deeply melanized skin the potential selective advantage of protection against the carcinogenic effects of ultraviolet rays, at the same time it imposes an additional heat load, because of the greater absorption and relatively less reflection of solar energy. Black skin absorbs 80% or more of incident visible light, while light skin absorbs only about 60% or less. Black and white skin reflectance curves differ less at the shorter infrared wavelengths (700–1000 μ), though again the black absorbs slightly more. About 95% of the infrared part of the radiation responsible for thermal effects is absorbed by a depth of 2 mm of skin surface, and 99% of it by a depth of 3 mm, thus suggesting that the differential response of heat load from solar energy resides in the pigmented parts of the epidermis (18). It is therefore to be expected that black skin would be heated more than white skin by the absorption of sunlight. The expected energy absorption has been calculated for white and for black skin exposed to radiant energy of similar spectral distribution to sunlight at the surface of the atmosphere; relatively untanned black skin absorbed 34% more energy than white skin. Since the passage of sunlight through the atmosphere filters out more energy in the infrared region than in the visible, the contrast in heat load from the visible spectrum may well be greater by the time sunlight has reached the earth's surface. In the American desert the heat absorption of unclothed white men exposed to sunlight amounted to 140 calories per hour on the average, a considerable heat load when it is remembered that the resting heat production is usually taken as 90 calories per hour. Under hot desert conditions, therefore, the additional heat absorption of a Negro due to skin colour may be 40 calories per hour or more, a potentially heavy strain on the heat-regulating mechanism under conditions of severe stress. Baker (19) reported a greater rise in rectal temperature accompanied by greater sweating in Negroes than in White soldiers while exercising in the nude, a difference which disappeared when exercising in clothing. Hence disadvantage of the pigmented skin in thermoregulation can be imagined in heat stress either where there is little chance of replenishing water lost in sweating, or in conditions of high humidity where evaporative heat loss is very difficult; indeed, in humid heat stress conditions, it was found that in nineteen matched pairs of negro and white males heat casualties claimed twenty-two victims and only one of them was Negro. With this the distribution of pigmentation in Africa appears to conform. But on the world scale the temperature association appears to be the wrong way round. Perhaps the deeper pigmentation triggers earlier activation of heat loss mechanisms such as sweating, or perhaps the heat load is insufficient for the disadvantages to operate. Another possibility comes from the earlier discussion. Dark skins are much less susceptible to sunburn damage than are light skins. Severe sunburn damage to the skin involves the sweat glands, disturbs their functioning and hence heat regulation. Under conditions in which efficient sweating is critical, the survival value of any feature such as pigmentation which may prevent sweat gland damage will be great. Though not understood fully, acclimatization to heat as a component of the adaptive role of melanin against solar radiation appears reasonable.

Another possibility is that pigmented skin may act as a more efficient dissipator of the solar heat load by radiation (20). Here the evidence is conflicting, and several studies (e.g. 21, 22) suggest small differences of about 2–3% in the emissivity coefficients of black

and white skins between 5000 nm and 8000 nm. But even a small physical effect of this magnitude may be significant from the point of view of adaptation or survival.

Vitamin D synthesis

The slight pigmentation of European populations poses a different problem. The role of melanin in vitamin D synthesis was suggested by Murray as long ago as 1934. Vitamin D is synthesized in the epidermis from a precursor 7-dehydrocholesterol, by photoactive reaction to UVR, medicated by melanin and keratin in the epidermis. Excessive or deficient synthesis can result respectively in hypervitaminosis or rickets in children. Fremon & Loomis (23) suggested that while the heavily melanized skin of the Negro screens out excessive UVR by absorption, and so prevents excessive amounts of vitamin D, the less melanized and keratinized skin of the northern Asians and north-west Europeans enables the available amounts of ultraviolet light to be fully utilized for synthesis of adequate quantities of vitamin D. On this hypothesis there would be dual selection for heavy pigmentation to prevent toxic overdoses of vitamin D where there is heavy ultraviolet radiation, and for slight pigmentation in regions of low ultraviolet. The first half of this hypothesis does not hold, for example Fremon (23) found no cases of hypervitaminosis amongst the Whites in the tropics. But selection for fair skin may well have occurred in north-west European conditions of coolness, heavy cloud cover, and relatively little radiation and indeed the suggestion from recent genetic studies of overall dominance of the genes from the white parent over those from the black parent carries the implication that selection for depigmentation may have been strong. In conditions of minimal sunshine where dietary vitamin D is low, increased access of these rays to the deeper skin layers could well be selectively advantageous in preventing the development of rickets. There is little direct evidence, but it is interesting that black children dwelling in the great cities in the northern part of America and Pakistani children in Glasgow are more susceptible to rickets than are white children. Certainly the occurrence of rickets amongst the more deeply pigmented inhabitants of some cities of the United Kingdom is today causing concern (24). This may indicate something more than a dietary deficiency. It may be due to the fact that, of the lessened amount of ultraviolet light reaching the skin in the temperate latitudes, again diminished by atmospheric pollution and indoor living, what is left after absorption by the melanin of black skin is insufficient to stimulate the production of vitamin D in the dermal layers.

Disease protection

An intriguing suggestion is that of Wasserman (25, 26), who argues that the main selective factor in the evolution of darker pigmentation in the tropics is disease and not climate. The primary need for survival in tropical peoples must have been protection against many infective and parasitic diseases. In their defence, the tropical dwellers evolved a mechanism of increased reticuloendothelial activity and elevated gammaglobulins. These features are related inversely to the size and acitvity of the adrenal cortex, so that through the decrease in adrenal cortical activity there would be increased MSH and ACTH levels which in turn result in increased pigmentation. Thus according to this hypothesis the dark skin pigmentation is only a byproduct of successful adaptation of a more efficient reticuloendothelial system. Such a hypothesis apart from ignoring other adaptive values of melanin, is certainly inadequate to explain the lighter pigmentation of whites.

New roles

Recently new concepts of the protective role of melanin have emerged. Melanin acts as an efficient photosensitizer in the presence of incident radiation. It has been suggested that melanin effectively eliminates those cells genetically damaged on exposure to ultraviolet. Ebling & Rook (27) propose a phototoxic role of melanin in addition to its photoprotective role, Riley (28) speculates on the phototoxic role of melanin as a substitute for photoprotection, suggesting that by their lighter skin the Caucasoids in the tropics suffer more from skin cancer more through failure to remove the genetically damaged cells than through the absence of the melanin buffer.

Also on account of its stable free radical nature, which gives a free electron receptor site on its molecule, melanin can combine with many other substances. It is able to form lipofuscin, a lipomelanin important in ageing (29). It forms complexes with chloropromazine, a drug which not only promotes melanin transport (30), but is also effective in treatments of some mental disorders and leaves symptoms of Parkinsonism and hyperpigmentation as side effects. Melanin moreover, is phagocytized by leucoytes, can therefore circulate in the body (31, 32), and indeed melanin granules have been reported in 75% of skin-draining lymph nodes of Bantu but in only 20% of Caucasoid lymph nodes. Wasserman (33) therefore suggests a possible role for melanin in intracellular metabolism. That some of the new concepts of the role of melanin may be true finds support from Ebling & Rook (27), who point out that it occurs in many organs besides the epidermis and this may have a variety of roles other than environmental adaptation.

Conclusion

Whatever the mechanisms, the close correlation with geographical factors suggests a very strong adaptive role. It seems that protection against ultraviolet radiation in areas where this is intense, and increased synthesis of vitamin D where there is minimal ultraviolet radiation, provide the two most important selective roles for heavy and light melanin pigmentation.

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The stability of disodiumsulphosuccinated undecylenic monoethanolamide in shampoo formulations

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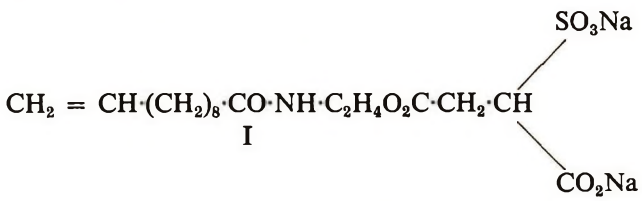
Synopsis

Disodiumsulphosuccinated undecylenic monoethanolamide (DSUM) though stable in the pH range 5·0 t 6·5, undergoes hydrolysis at pH 7 forming **undecylenic monoethanolamide.** An adaptation of an establishe colorimetric method based on the formation of iron (III) hydroxamates by reaction of the ester group i the molecule has been shown to offer sufficient specificity for DSUM for use in stability studies. Simu taneous screening with thin layer chromatography confirmed the specificity.

Introduction

Disodium sulphosuccinated undecylenic monoethanolamide (1) (DSUM) is used i shampoo formulations, at concentrations of about 1% w/w, as an antidandruff agent an preservative. The commercially available material is probably a mixture of related com pounds but consists essentially of undecylenic monoethanolamide sulphosuccinate (I).



I

DSUM can be determined in shampoo formulations based on triethanolamine laur sulphate by a colorimetric procedure depending on the formation of iron (III) hydro xamates. The procedure employed is a modification of that described by Bergmann (2 for amides and by Goddu, LeBlanc & Wright (3) for esters. It has been found satisfactor when used as part of a production control scheme, providing a suitable 'blank' shampo containing no DSUM is available as a comparison. The 'blank' is necessary to provide measure of the colour obtained in the absence of DSUM due to other shampoo in gredients. When this assay procedure was applied to samples of shampoos stored fo periods of several months, it appeared that the DSUM content was decreasing with tim It therefore became important to establish whether the results reflected a decrease i

)SUM content or a change in some other ingredient of the formulation. Thus the eason for the loss of DSUM, the routes of breakdown of DSUM and the specificity of he analytical method all required investigation. The results of these investigations are eported in this paper.

:outes of breakdown of DSUM—preliminary studies

'he most probable route of breakdown of DSUM was thought to be by hydrolysis. Both ıe ester and amide functions in the molecule are susceptible to hydrolytic attack. *Fig. 1* epicts two routes of breakdown by hydrolysis. The products of alkaline hydrolysis are I and III. A white precipitate (a yellow oil if the reaction mixture is hot) is formed. The ıfrared spectrum of this material clearly showed the presence of an amide group ıbsorption bands centred at 3450, 1633 and 1550 cm^{-1}) and the presence of hydroxyl and double bond could also be inferred, though less certainly. Bands due to sulphonate 1250 cm^{-1}) and ester groups (1740 cm^{-1}) present in the original material were missing. 'his information is consistent with structure II, undecylenic acid monoethanolamide. It hen follows that the other product is III, trisodium sulphosuccinate which is water oluble and was not isolated from the reaction mixture. When II is treated with alkaline ydroxyammonium sulphate and subsequently with iron (III) chloride solution as in the olorimetric assay procedure (see below), no colour is produced. It is clear, then, that the ster function in DSUM is alone responsible for the chromogenic response in the assay rocedure, the amide function playing no part in colour formation since the conditions re not sufficiently vigorous (2), (4). Acid hydrolysis leads to a loss of surface activity in ilute solutions (0·1% w/v) as measured by a biphasic cetrimide titration in which an in- icator (methylene blue) is distributed between chloroform and dilute sulphuric acid olution to give an equal depth of colour at the end point. This loss of surface activity mounted to 50% at pH 2·65 in 24 h, and was accompanied by a loss of ester function, ıeasured by the colorimetric procedure (see below) of less than 10%. This shows that 'hile the ester function is not readily attacked under cold acidic conditions some other art of the molecule does undergo change.

:xperimental

OLORIMETRIC DETERMINATION OF DSUM IN SHAMPOO

'eagents

.ll reagents were of analytical reagent grade where possible.

Hydroxyammonium sulphate

5M Sodium hydroxide solution

5M Hydrochloric acid

'on (III) chloride stock solution

A 0·444M solution in 0·1M hydrochloric acid.

'on (III) chloride reagent

Dilute 5·0 ml Iron (III) chloride stock solution, filtered if necessary, and 1·0 ml 3·5M hydrochloric acid to 25 ml with distilled water.

.lkaline hydroxylamine Reagent

Mix equal volumes of M hydroxylamine sulphate solution and 3·5M Sodium hydroxide. Prepare a fresh solution daily.

Procedure

Dilute about 5 g of shampoo, (W_{sa}g,) weighed to ± 0·02 g to 50 ml in a standard flask. To a 4·0 ml aliquot in a 25 ml standard flask add 5 ml alkaline hydroxylamine reagent, swirl gently and stand at room temperature for exactly 5 min. (Use a stop watch and time the 5 min-period from the start of the addition of reagent.) Then add 5·0 ml hydrochloric acid, swirl and immediately add 1·0 ml iron (III) chloride reagent. Swirl to mix and dilute to the mark with 95% ethanol. Mix well, allow the gas evolution to subside and transfer to a 40 mm cell. Measure the absorbance (A_{sa}) of the solution within 3 min at 530 nm using water as the reference taking care to ensure there are no bubbles in the light path. Using a sample (W_{blk} of the base shampoo containing the same ingredients as the sample but lacking the DSUM carry out the same determination to obtain a blank result (A_{blk}).

Calibrations

Primary calibration with DSUM. This calibration is only required initially.

Prepare solutions of DSUM in distilled water to cover the concentration range 0·25 to 1·25 mg ml^{-1}. Take 4 ml aliquots and apply the colorimetric procedure described above. Plot a graph of the net absorbance readings *vs* DSUM (mg) taken. The plot should be a straight line passing through the origin. Calculate the slope (F) of the line in terms of DSUM (mg) per absorbance unit.

Secondary calibration with ethyl acetate. This calibration should be performed simultaneously with the primary calibration and on each occasion that DSUM is determined.

Dissolve 1 g ethyl acetate (W_{std}) accurately weighed, in 20 ml ethanol in a 50 ml standard flask. Dilute to the mark with ethanol. Mix well and transfer 2·0 ml to a 250 ml standard flask containing 150 ml distilled water and dilute to the mark with distilled water. Mix well and transfer 4·0 ml to a 25 ml standard flask and continue as in procedure from 'Add 5 ml alkaline hydroxylamine reagent . . .' measuring the absorbance (A_{std}) of the resulting solution in a 40 mm cell at 530 nm.

Calculation

DSUM in sample = $50/4\ (A_{sa} - A^*_{blk})\ F'$mg

Where A_{sa} = absorbance of sample solution at 530 nm

A^*_{blk} = absorbance of shampoo blank solution at 530 nm

corrected for weight difference = $\dfrac{W_{sa}}{W_{blk}} \times A_{blk}$

F' = Calibration factor which is calculated by allowing for changes in sensitivity of the method due to reagent differences, temperature differences etc. using the secondary calibration results:—

$$F' = F \times \frac{W_{std_1}}{A_{std_1}} \times \frac{A_{std_2}}{W_{std_2}}$$

in which the subscripts 1 and 2 refer to secondary calibrations performed at the same time as primary calibration and determination respectively.

THIN LAYER CHROMATOGRAPHIC EXAMINATION OF SHAMPOO

Reagents

TLC development solvent: 15 ml 1,4-dioxan, 75 ml chloroform and 15 ml carbon tetrachloride. Prepare a fresh mixture daily.

Hydrolysis products of DSUM

Alkaline hydrolysis product

Weigh 10 g DSUM. Add 100 ml M sodium hydroxide solution and reflux for 30 min. Cool and filter. Wash the precipitate with distilled water until free from alkali. Recrystallize from aqueous acetone. The yield is about 3 g.

Acidic hydrolysis product

Weigh 10 g DSUM, add 100 ml 3M hydrochloric acid and reflux for 1 h. Extract the oil or precipitate with diethyl ether. Dry by the addition of anhydrous sodium sulphate, filter and evaporate to dryness. The yield is about 2·5 g.

Precoated silica gel 60 TLC plates (E. Merck) 10 × 20 cm.

Ethanolic sulphuric acid solution

Add cautiously 10 ml concentrated sulphuric acid to 80 ml ethanol (74 o.p. spirit). Cool and dilute to 100 ml with ethanol.

Procedure

Extract 4·0 g of the sample with three separate 10 ml portions of diethyl ether. Combine the ether extracts and wash once with 20 ml distilled water. Discard the aqueous wash and dry the ether extract over anhydrous sodium sulphate. Filter, evaporate on a water bath to a small volume, transfer the residue to a 5 ml standard flask using diethyl ether, and dilute to the mark. Prepare 0·4% w/v solution of the alkaline hydrolysis product and a 0·33% w/v solution of the acid hydrolysis product. These solutions, when 5 µl is spotted directly on to the TLC plate, are equivalent to 100% degradation of the DSUM. Dilute these standard solutions to provide solutions corresponding to 25%, 50% and 75% degradation. Apply 5 µl portions of the sample extract and these diluted solutions to the plate. Develop the plate to about 15 cm above the origin line, and dry in an oven at 100°C. Spray with ethanolic sulphuric acid. Dry at 140°C for 15 min. View the plate under U.V. illumination (365nm), and compare the intensities of the standard spots with those from the sample to estimate extent of degradation. Allow for the apparent degradation simultaneously detected in an extract from a freshly prepared aqueous 1% solution of DSUM.

Results and discussion

Assay procedure

The colorimetric assay depends upon measurement of an unstable colour. All assays were therefore performed in duplicate working rapidly to ensure that absorbance measurements were complete within 3 min of the addition of the iron (III) chloride reagent. The conditions described above have been found to give optimum colour stability. The intensity of the colour produced has been found to vary with the batch of prepared reagent used. For this reason ethyl acetate was used as a convenient standard substance to monitor the sensitivity given by a batch of reagent. This avoided the use of DSUM as a

working standard which would not have been satisfactory since its long term stability was unknown.

Results obtained with this procedure over several months have shown that in routine use the 95% confidence limits (2 σ) are $\pm$ 0·05% w/w DSUM at a nominal 1% w/w concentration.

From the results of the preliminary studies it was apparent that the colorimetric assay procedure is specific for DSUM in the presence of its decomposition products formed by alkaline hydrolysis. If acidic hydrolysis occurs, the method may not produce valid results as the decomposition may proceed only as far as the formation of (IV) and (V), (*Fig. 1*), the latter containing an ester group which would respond to the colorimetric assay in a similar way to DSUM. This aspect of method specificity was investigated in conjunction with a storage test using TLC to detect degradation products.

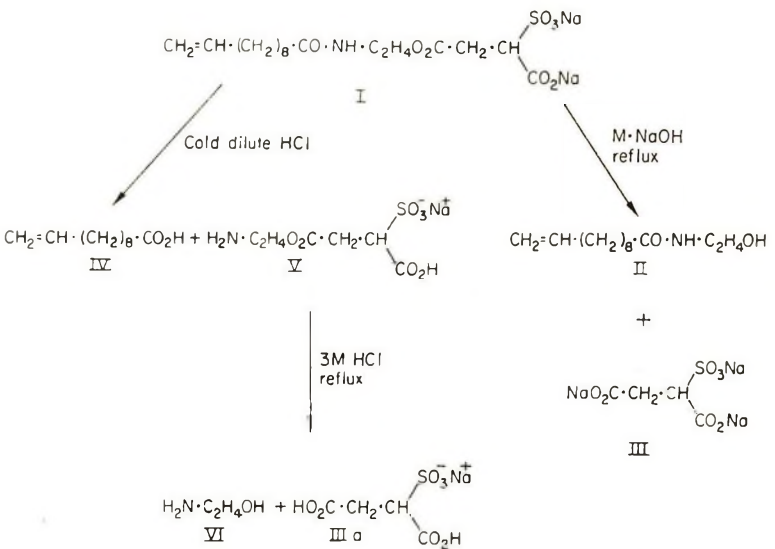


Figure 1. Routes of breakdown of DSUM by hydrolysis.

Storage test of shampoos

The second part of the investigation took the form of a storage test during which the colorimetric assay was used in conjunction with TLC screening. The stability of DSUM (1·0% w/w) in citrate buffered shampoo containing 17% triethanolamine lauryl sulphate was monitored as a function of apparent pH and of temperature. Control samples containing no DSUM were also monitored to provide 'blank' values. The DSUM assay results are presented in diagrammatic form in *Fig. 2* (*a*) and (*b*) and show clearly the dependance of stability on apparent pH. The loss of DSUM follows a first order rate equation. The concentration axes in *Fig. 2* (*a*) and (*b*) are logarithmic and the plots are therefore linear within the limits imposed by the assay procedure. At an apparent pH of 7 DSUM is rather unstable, 25% decomposing in 12 weeks at 20°, while at 37° 58% was lost over the same period. Confirmation of the validity of these results was obtained from the TLC screening test. A typical chromatogram is shown schematically in *Fig. 3*. The separated components are seen as coloured fluorescent zones. In addition to unidentified spots at R_F zero, 0·4–0·6 and 0·95 a clearly separated spot, having a pink

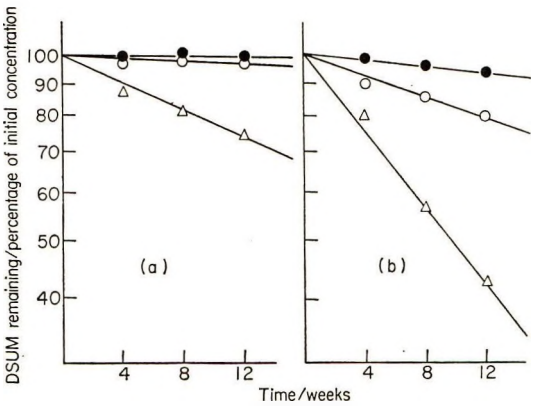


Figure 2. The loss of DSUM from shampoos at various pH values (a) at 20°C, (b) at 37°C. Key: ● pH 5·0; ○ pH 6·5; △pH 7·0.

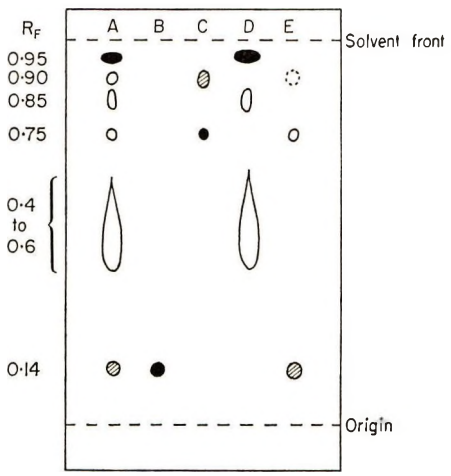


Figure 3. Schematic representation of developed TLC plate viewed under a UV lamp (365 nm). The extent of shading of the spots indicates approximate visual intensity. (A) Extract from shampoo containing DSUM and its breakdown products. (B) Solution of II—product of alkaline hydrolysis. (C) Solution of IV—product of acidic hydrolysis. (D) Extract from blank shampoo. (E) Extract from aqueous DSUM solution.

fluorescence, was seen at R_F 0·14. This corresponds in colour and mobility to II, undecylenic monoethanolamide, prepared from DSUM as described above. Trace amounts of the products of acidic hydrolysis were found in all samples including the original DSUM raw material, but only II was seen to increase in concentration on storage. Some approximate results obtained by TLC spot intensity comparisons as described above are shown in *Table I*.

From the chromatograms it was immediately evident that the shampoos at an apparent pH of 7 and stored at 37°C contained much more II than those at lower apparent pH. Furthermore the estimated quantities of II (*Table I*) corresponded quite closely with

Table I. Estimates of breakdown of DSUM from visual spot comparisons on TLC plates. Loss of DSUM (% of initial) by formation of II

	Period of storage at 37°	
pH of shampoo	8 *weeks*	12 *weeks*
5·0	< <25	< <25
6·0	< <25	< <25
7·0	~50	> 50

the loss of ester function as determined by colorimetry (*Fig. 2(b)*). These two observations are consistent with a hydrolysis reaction producing II and free sulphosuccinate, III, as shown in *Fig. 1*. At lower pH values this reaction does not occur and no other degradation products were observed to be produced.

It is interesting to note that the product of acidic hydrolysis, IV, was resolved into two separate components by TLC. No further work was done to identify the two components, but the hydrolysis product isolated could well have been a mixture of homologues.

Conclusions

The colorimetric assay procedure was shown to offer adequate specificity for use in stability studies of DSUM. The results of the storage test show that DSUM is sufficiently stable in the pH range 5 to 6·5 in shampoo formulations for commercial exploitation, but that at pH 7 and above significant loss occurs by hydrolysis with the formation of undecylenic monoethanolamide. The pH of such formulations should be carefully controlled to minimise the loss of DSUM. Loss of DSUM by formation of undecylenic monoalkanolamides may not however be entirely undesirable since these compounds are reported to have antimicrobial activity (5).

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Nail disorders caused by external influences

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Presented before the Society of Cosmetic Chemists of Great Britain on 6 November 1975

Synopsis

Damage to **nails** caused by physical or chemical trauma is outlined. A surprising number of cases are due to the patients themselves either by biting, fiddling with the nails, attempting to remove non-existent parasites and occasionally deliberately to obtain sympathy. Nail cosmetics are generally very well tolerated but deformities occur at times and are described. The wearing of footwear can also cause trouble. Chemical damage is rather uncommon but frequent contact with water or soap and water is responsible for much damage to nails.

Introduction

Many of the commoner nail disorders are due to intrinsic factors and this is especially true of psoriasis, eczema or dermatitis and impaired peripheral circulation. There are, however, many others which are due to trauma in its widest sense and the cause is easily overlooked if not suspected by patient or doctor. Fungal infections are due to invasion from without but are usually part of a more widespread infection and will not be discussed. Chronic paronychia, one of the commonest skin conditions of housewives will be described because it is believed that frequent contact with soap and water is the principal aetiological factor in this disorder.

This paper concentrates on the more subtle forms of trauma such as nail biting, fiddling with the nails, damage due to cosmetics and foot wear, accidental contamination with certain chemicals and the effects of soap and water on the nails and surrounding tissues Direct injury such as trapping of a finger in a car door can cause many forms of damage, temporary or permanent, to the nail but the cause is well known and therefore will not be discussed.

NAIL BITING

This is an extremely common habit and more than one member of a family often indulges in it. Generally the biting is at the tip of the nail resulting in a short ragged nail nibbled right back to the eponychium. Small spicules may be left which form into hang nails and occasionally secondary sepsis develops. One or more often many or all nails are bitten and occasionally one nail is spared for scratching! A common complication is the formation of periungual warts which may affect several fingers. A very similar appearance can be produced by paring down the nails with a razor blade. Many such cases were observed during a short visit to Nigeria some years ago.

Quite a different picture results if biting takes place further back on the nail. Biting may be mainly on the cuticle which becomes ragged and broken and a low grade paronychia is more common. Occasionally biting will actually injure the matrix resulting in a deformed nail easily mistaken for a fungal infection. This type of deformity occurs especially in young children watching an exciting or frightening television programme, and may be confined to one finger.

Recently a young boy was seen who managed to destroy all his finger nails by constant biting so that very little nail remained on any finger. As the patient also had psoriasis it was very easy to attribute the nail damage to psoriasis but the patient freely admitted to the habit. Another young lady admitted to being a nail biter but said one of her nails would not grow. On close questioning she admitted to having done more damage than usual 1 year previously whilst biting and subsequently she picked away tiny portions of nail as they formed. On examination there was almost no nail present but there seemed no reason why it should not grow normally. Fixed dressing was applied which was changed weekly and at the end of 3 months she had a normal nail (*Fig. 1 and 2*).

FIDDLING WITH THE NAILS

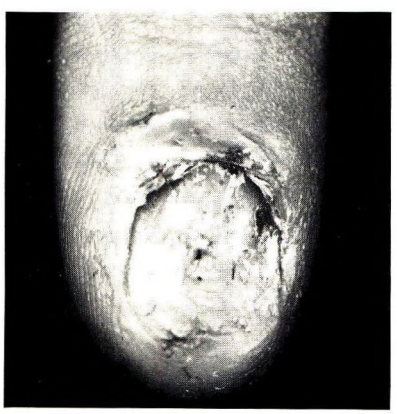
There are a number of other ways in which nails can be damaged by the patient himself. The first is a habit of fiddling with the nails. The patient picks at the cuticle subconsciously usually of the thumb nail with one of his other fingers on the same hand. This produces a rather characteristic deformity of a depression down the centre of the nail and ridges extending from it towards the edges of the nail. (*Fig. 3*). The patient freely admits to the habit but does not realize that it is the cause of the defect.

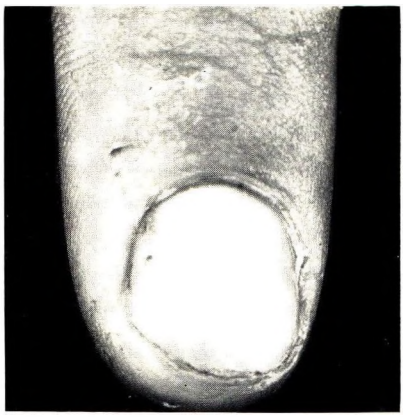
PARASITTOPHOBIA

This very distressing symptom may be confined to the nails and finger tips. The patient is convinced she is infested and this fear may be heightened by being told she has a fungal infection of the nails. The patient is constantly picking pieces off the nails and surrounding tissues and may bring a collection of them with her in a piece of tissue paper. It is almost impossible to convince these patients that they are not infested.

NAIL ARTEFACTS

Deliberate trauma in the form of an artefact is even less common. One child punctured the half moon area of her thumb nail and succeeded in producing infection which progressed to granulation tissue projecting through the hole in the nail. The bandaging which was needed to protect the thumb saved her from taking an exam which she feared she might fail. Another patient admitted deliberately inserting a nail file under her cuticles to produce subacute paronychia. She considered she was being imposed on by her daughter and son-in-law who had taken up residence in her house and expected her to do all the housework. Again the dressings which were required to cover the defects saved her from the housework. She even traumatized some of her toes. This rather gave the game away as chronic paronychia virtually never affects the toes. These patients may get much satisfaction from failures in treatment.





Figures 1 & 2. Nail destroyed by biting and picking off pieces; before and after 3 months occlusive dressings.

Figure 3. Deformity due to fiddling with the nail.

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NAIL COSMETICS

On the whole nail cosmetics are well tolerated and cause little trouble to the nails. One condition which although quite common is not well recognized, is the staining of the nails from nail varnish. Many patients have been observed with this condition but in the great majority it was an incidental finding, the patient having reported with some other skin or nail defect.

The staining is usually quite characteristic starting fairly near the cuticle and extending to the tip and getting progressively darker from base to tip. This indicates that the longer the varnish has been in contact with the nail, the more intense is the staining. The colour is almost always yellowish although the colour of the varnish varies widely. Often the patient is unable to incriminate any particular varnish as she may wear various colours. Calnan (1) traced staining in one patient to a pigment Transparent Yellow Lake 16901 in 1967.

The following investigation was carried out in connection with this type of staining to see if there was any common factor which might explain it. The names of five varnishes which had been the cause of complaint by their users was provided and the pigment composition of each was known. A 5% suspension of each of the dyes in each varnish was provided made up in an otherwise clear base. One of the named varnishes and each of the 5% solutions was tested on individual nails and left in place for one week and then removed. If there was no staining the varnish was reapplied and continued up to a total of 8 weeks. Staining occurred with the named varnish and with the following four colours:— Red 25777 (= D & C Red No. 7); Maroon 26095 (= D & C Red No. 34); Red 26094 (= D & C Red No. 6); Yellow 25776 (= FD & C Yellow No. 5 lake). The last named produced only slight coloration. One of these dyes was present in each of four of the named varnishes and appears to have been the worst offender—it was Red 26094. It seems almost certain that other pigments can leak out of the varnish base and stain the nail. Patients reporting this symptom only represent the tip of the iceberg, others remaining hidden below the varnish. The condition is of course quite harmless and grows out with the nail. Sensitive patients may however be very upset by it and many doctors including some dermatologists fail to recognize the cause of this complaint.

There are other causes of nail staining, nicotine being perhaps the most obvious. This tends to be more selective affecting two or three fingers only and is more intense than varnish staining. Henna hair dye will also stain the nails and is occasionally used for this purpose in some countries. Very occasionally long term tetracycline therapy will stain the nails and finally there is the yellow nail syndrome when all nails, fingers and toes take on a yellow colour. None of these conditions should be confused with staining from varnish.

Nail cosmetics cause remarkably little other damage. A few cases of onycholysis due to the use of nail hardeners containing formaldehyde are seen. The condition is usually limited to the distal one third of several nails and may be a sensitivity reaction. Very occasional cases may be due to a nickel sensitivity, the nickel being derived from metal pellets put in the varnish bottle to help keep the varnish liquid.

False nails if closely attached to the nail may damage the nail surface and more rarely may cause complete disruption of the nail. Cases have recently been seen of damage caused by nail improvers based on dental plate materials. It is probable that the materials sometimes cause a true sensitivity reaction but they may also act as primary irritants inducing local sepsis and possibly even nail loss. This type of reaction was recorded as long ago as 1957 (2) but seems to have been overlooked. It is apparent that nail cosmetics

must be free of sensitizing or irritant properties and must not cause complete occlusion of the surface interfering with the free exchange of moisture between the nail and the atmosphere. Overall, nail cosmetics are very safe as at present marketed.

Over-zealous manicure, pushing back the cuticles, may result in white streaks across several nails (leukonychia striata) (*Fig. 4*).

FOOTWEAR

A number of nail deformities may be attributed to footwear. Simply covering the feet encourages fungal infection, but this is an accepted feature of Western civilization and is unlikely to be abandoned. Open sandals or bare feet are much more sensible and are recommended for those living in hot countries.

Onychogryphosis is sometimes a developmental abnormality but often it starts with minor trauma and the nail thickening is increased as a result of repeated minor trauma from footwear. *Fig. 5* shows that nail hypertrophy can be the result of trauma as the damage here was the result of a single injury.

Shoe fashions change and with the change some differences in nail deformities are encountered. During the relatively short reign of the pointed 'winkle picker' shoes ingrowing nails in young men were very common. With the change to the platform shoe there has been an increase in complaints of shedding of great toe nails. The nail loss is usually preceded by subungual haemorrhage and affects one or both great toes (3). Sometimes the haemorrhage is insufficient to cause actual loss of the nail but haemorrhage under the nail can be very worrying unless the cause is recognized and corrected. Not infrequently we see onycholysis of a large part of one or both great toe nails as a lesser effect of the platform shoe. It seems probable that the platform shoe is too rigid and during walking causes repeated minor trauma to the nail. The 'peep-toe' style of platform shoe does not cause this trouble.

ACCIDENTAL CONTAMINATION WITH CHEMICALS

The nail matrix is really very well protected being covered by the two layers each of epidermis and dermis of the dorsal nail fold. It is rather more exposed distally where it is visible as the half moon and covered only by the nail plate. Substances acting from outside which interfere with the formation of the nail must reach the matrix either by penetrating deeply through the dorsal nail fold or by passing below or through the cuticle and extending along the surface of the nail (4) below the the dorsal nail fold. Damage of this sort has been seen from a weedkiller and from hydrofluoric acid. Paraquat and diquat are dipyridilium compounds which are marketed for home use as granules and are perfectly safe in this form. They are also available to farmers and foresters as a concentrated solution which has to be diluted before use. The makers give instructions that dilution must be done with great care and that protective gloves and face shields should be worn. If, in spite of this, a few drops of the concentrated liquid splash onto the hands it may deform the nails. The change is rather characteristic. A pale band appears across the nail just ahead of the cuticle (*Fig. 6*). This later becomes brownish and a gap appears between the cuticle and the proximal nail fold. This may progress to total loss of the nail.

Two patients have been seen whose nails have been damaged by hydrofluoric acid. The first was engaged in cleaning the outside of old buildings. For this purpose dilute

Figure 4. Leukonychia striata due to pushing back cuticles.

Figure 5. Nail hypertrophy following single injury.

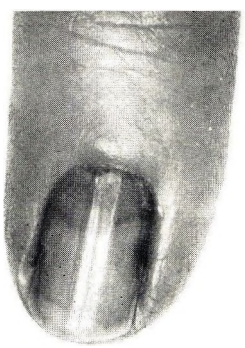

Figure 6. Nail dystrophy due to contamination with paraquat, pale white bard across nail.

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Figure 7. Chronic paronychia.

hydrofluoric acid is sprayed on the surface and then washed off with water. The operators wear protective gloves. On this occasion the glove leaked and the patient became aware of a burning sensation and on removing the glove found that four fingers were wet. He rinsed the hand but the next day he noted that the nails on the four fingers had all become separated from their beds. The damage in this case was not to the matrix. The second patient worked in a laboratory and got splashed with hydrofluoric acid when it was poured down the sink. It is possible that the fluid was more concentrated than in the other patient. In this case both thumb nails suffered damage to the matrix so that there was temporary interference with nail growth. With both paraquat and hydrofluoric acid the degree of contamination was quite minor and they must act as very potent chemicals interfering with keratinization.

EFFECTS OF WATER OR SOAP AND WATER

The normal nail plate probably holds about 15% of water vapour and takes up a good deal more when in direct contact with water. It becomes saturated at about 30% and the nail plate is then opalescent and quite soft. The excess is quickly lost to the atmosphere on removal from water and the nail returns to its normal state. Under very dry conditions the percentage of water in the nail falls below 15% and it then becomes brittle.

A number of things can happen if nails are constantly exposed to water and there is frequent alternate wetting and drying.

The first is loss of the cuticle and progress to chronic paronychia. The first organism to cause damaged encouraged by the moist conditions is often the staphylococcus. This probably helps to destroy the cuticle. Candida, usually Candida albicans, quickly takes over and is difficult to eradicate. Its presence leads to the bolstering of the dorsal nail fold (*Fig. 7*) which is a characteristic of this condition and it may also cause a small amount of pus formation. Gram negative organisms then appear, usually pseudomonas and proteus and these are responsible for the dark discoloration of the nail edge (*Fig. 7*). As a late development the whole nail organ may show shrinkage. The most important part of treatment is to keep the hands dry.

Instead of chronic paronychia or in addition to it the nail may become loosened from the nail bed. Many of the cases of so-called idiopathic onycholysis are probably due to soap and water. The warm moist space below the nail encourages infection and various organisms, fungal and bacterial, may be cultured from the subungual debris. There is often some discomfort and occasionally the nail may become dark green or black due to over growth of Pseudomonas aerugionosa. This may cause great anxiety to the patient. A curious feature of a nail which is loose from its bed is the fact that it grows faster than its neighbours and has to be cut more often.

The third apparent effect of water on the nails is splitting especially into layers. This is very common in housewives especially in winter. It is probably due to repeated wetting and drying causing softening and hardening and leading to loss of adhesion between the nail cells. There is some evidence that the nail is formed in layers and perhaps there is less adhesion between the layers than between individual cells.

Acknowledgments

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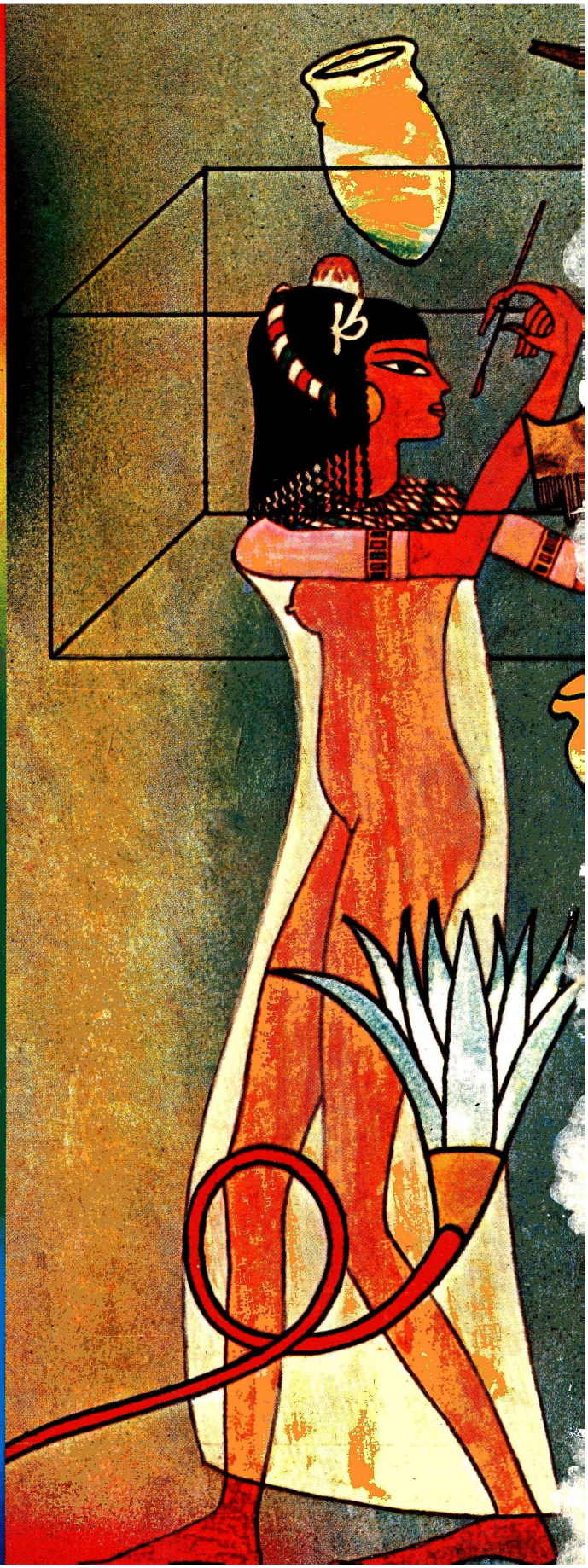
Cosmetic Colorants



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