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RELATIVE EFFICIENCY OF PIGMENT AND HORNY LAYER THICKNESS IN PROTECTING THE SKIN OF EUROPEANS AND AFRICANS AGAINST SOLAR ULTRAVIOLET RADIATION*

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The demonstration by Finsen (1900) that the skin of Europeans reacted to exposure to ultraviolet rays by producing pigment (tanning) and his deduction that melanin protected against these rays by absorbing them superficially appeared satisfactory until Guillaume (1926) and Miescher (1930) showed by studies on albinos and others with defective pigmentation that thickening of the stratum corneum was a much more important protective adaptation than pigmentation in white-skinned persons.

Pigment has been alleged to protect against visible rays (Bauwens, 1935; Goldsmith, 1936; Guillaume, 1926), against penetrating heat rays (Bauwens, 1935; Meyer & Kirchoff, 1932) and against ultraviolet rays (Finsen, 1900; Hill, 1934; Rollier, 1927).

From the point of view of thermoregulation at high environmental temperatures, a proven disadvantage of the Negro skin is that it reflects less, and therefore absorbs appreciably more (up to 36%) of solar radiation (Heer, 1952; Martin, 1930) than the white-skinned race. According to Aron (1911) this greater absorption of solar rays was an advantage because the temperature of the coloured skin was thereby more rapidly raised, stimulating the earlier onset of sweating. Since the relatively greater absorption of heat would, however, continue throughout subsequent exposure to the sun's radiation it would seem that a high price had been paid for a dubious initial advantage. The coloured skin does not reradiate more infrared radiation than the white (Hardy & Muschenheim, 1934); both skins behave virtually as black bodies for skin temperature radiation.

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Thus it is perhaps not surprising that Blum (1945) concluded that it was hard to demonstrate any advantage in the increased melanin content in coloured skins. Nevertheless, the undoubted relationship between geographical distribution of pigmentation and insolation (Fleure, 1945) required explanation as well as the observed higher threshold of the Negro skin to erythemogenic ultraviolet radiation (Hausser & Vahle, 1927; Miescher, 1932). It had also been shown (Thomson, 1951*a*) that when a West African Negro and a European were exposed to the same intensity of ultraviolet radiation, the resulting erythema was accompanied by reduction of sweating only in the white person, indicating that the Negro was less susceptible to this adverse effect of the radiation.

In view of the predominant role played by thickening of the stratum corneum in the development of protective adaptation in Europeans it was suggested (Blum, 1945) that the Negro's greater relative immunity might be explained if he possessed a thicker stratum corneum than the European. In attempting to answer these questions it was decided to investigate, first, the relative inherent thickness of the stratum corneum in Africans and Europeans and, secondly, the relative transmission of solar ultraviolet light through this layer in the two races.

METHOD

Horny layer was obtained for both thickness and transmission measurements by blistering with cantharides (B.P.C.) and, after removing the blister 'skin' with fine scissors, dissecting off the remains of the cellular layers with a fine camel-hair brush (Thomson & Sutarman, 1953). The specimens, which varied from 8 to 16 mm diameter, were mounted on glass slides and kept moist with distilled water.

Since high individual variability was expected it was thought better to concentrate on one body site and the hip was chosen, 1 in. behind the great trochanter, for the following reasons. Human skin reacts to most forms of injury, as well as ultraviolet radiation, by thickening of the horny layer and increased pigmentation. In this study of inherent qualities of this layer, therefore, these sources of variability had to be minimized as far as possible. The hip was normally covered by clothes in both races and therefore screened from solar ultraviolet rays. It lay outside the area of contact when sitting. In this way previous thickening from friction was minimized as well as discomfort after blistering.

Measurement of thickness

The ordinary methods of preparing stained sections were not adopted since they reduced the corneum to a wispy indefinite layer unsuitable for such measurement and would probably alter its thickness. The specimens were cut at right angles to the surface and the width of the cut surface was ascertained directly at 0·2 mm intervals using oblique illumination and linear eye-piece micrometer. The estimated accuracy was $\pm 7\frac{1}{2}\%$.

Transmission of ultraviolet radiation

The moist specimens of horny layer on quartz or filter glass were mounted against selochrome photographic plates so that the specimens were in close contact with the plates which were then exposed to filtered ultraviolet light from a Kromayer quartz mercury-vapour lamp. After developing the plates the densities beneath and around specimens were ascertained by photometers of photo-electric and integrating sphere types.

Transmission (T) was obtained from densitometric measurements of the photographic plates as follows. Where D_S and D_B are the densities at specimen and background respectively, I_S and I_B are the corresponding light intensities and the plate was developed to a contrast γ, where $\gamma = d(D)/d(\log I)$, then, assuming that the exposures were confined to the nearly linear part of the D-log I graph, we have $T = I_S/I_B$, where I = a constant multiplied by $e^{D/\gamma}$.
Hence $T^{\gamma} = e^{D_S}/e^{D_B}$.

RESULTS

Thickness of horny layer

Horny layer specimens were obtained from 22 European and 29 African subjects of whom 17 Europeans and 20 Africans were nursing orderlies and the remainder staff or laboratory workers. The Africans were mainly Ibos, but included men from most of the Nigerian tribes.

The terminal portions of pilo-sebaceous follicles and eccrine sweat gland ducts can always be clearly seen projecting from the under surface of such preparations of corneum (Thomson & Sutarman, 1953). Although the clean appearance of these rudiments in the present specimens indicated that all traces of epidermal cellular layers had been removed, one piece of blister skin was dissected over half of its surface, mounted in egg albumen, sectioned and

TABLE 1. Thickness of stratum corneum in Europeans and Africans; variation within individuals compared with that between individuals

	Source of variation	Sums of squares	Degrees of freedom	Mean square	Variance ratio
Europeans	Between persons	56·8967	21	2·7094	13·980***
	Within persons	32·1708	166	0·1938	
	Total		187		
Africans	Between persons	191·3343	28	6·8334	22·149***
	Within persons	117·5420	381	0·3085	
	Total		409		

*** Significant at 0·001% level.

Note. Analyses of variance were carried out for convenience in the arbitrary units (μ/4·255) in which the measurements were made.

stained in order to ascertain the completeness of separation. It was found in this way that only infrequent discrete, flat collections of cells remained on the dissected portion which may have been integral parts of the sweat gland ostia.

It was anticipated that in the hydrophilic stratum corneum the thickness would be affected by the humidity of the atmosphere and by the interval between removal and measurement. The rainy season in Nigeria ensured a high and relatively constant humidity (*c.* 28 millibars). It was found that specimens of corneum from three subjects showed a mean contraction of the order of 10% while adjusting to atmospheric humidity during the first 5 hr after removal and that thereafter thickness remained practically constant.

Analysis of variance (Table 1) shows significant variation between individuals in both Europeans and Africans so that, in view of the differences in numbers of observations contributing to individual means, the 't' test was subsequently carried out between individual means. The mean thickness of horny layer for Europeans was 9·51 μ and that for Africans was 11·13 μ. The difference between these means, 1·62 μ, was 1·929 times the standard error of the means (0·840 μ). For the associated degrees of freedom (49), $t = 2{\cdot}010$ at the 5 % level; the difference is therefore not significant.

In addition to the contraction due to drying described above, the method here used probably underestimates the true thickness because of stretching beyond its elastic limit of the roof of the blister during formation. If it be assumed that increase in area during blister formation measures reduction in thickness and that the blister had the form of a spherical cap it can be shown that percentage change in thickness $= 100\,h^2/d^2$, where h is the height and d the radius of the blister. The height and radius of the largest blister were *c.* 2 and 8 mm and those of the smallest blister were *c.* 1·5 and 4 mm, giving reductions in thickness of the order of 6 and 14 % respectively or a maximum difference between the two of 8 %. The size of these blisters was not recorded, but those of the Africans were on the whole larger than Europeans. Thus the difference between the two races, which almost reached the 5 % level of significance, was probably exaggerated.

Transmission of solar ultraviolet radiation

As soon as the specimens of African corneum had been exposed it was observed that pigment was undoubtedly present in this layer. The depth of colour varied from grey to black but was always darker than the more homogeneous European group.

Fig. 1 is a print of a photographic plate obtained by exposure to filtered ultraviolet radiation (3000–4000 Å) through twelve specimens of corneum (3 Europeans and 9 Africans). Skin grooves and pilo-sebaceous follicles can be seen in most specimens in this figure; these and the eccrine sweat gland ostia were clearly visible under low-power magnification throughout the entire surface of the actual specimens indicating complete separation from underlying layers. The mean transmission for 18 Europeans (64·24 %; S.D. = ± 9·19 %) was found to be 3·5 times greater than that for 7 Africans (18·48 %; S.D. = ± 7·22 %); the distributions did not overlap and the difference between the means was obviously significant (Fig. 2).

These plates were obtained in Nigeria within a few hours of blistering and almost certainly give the best estimate of transmission in the solar ultraviolet. The Chance's glass filter used, however, transmitted the strong mercury vapour line at 3650 Å in addition to the erythemogenic lines at 2950–3150 Å; moreover, the photographic emulsion was more sensitive to the ultraviolet radiation of longer wavelength at 3650 Å.

It is difficult to isolate the solar erythemogenic zone precisely but, after returning to England, a proportion of the specimens (2 Europeans and

5 Africans) was rephotographed by the same method using a chemical filter combination in place of Chance's glass. Two quartz cells were mounted in series in the light beam containing 1 cm each of the following solutions: (1) $NiSO_4.6H_2O$, 350 g/l.; $CoSO_4.1H_2O$, 100 g/l., (2) $CuSO_4.5H_2O$, 15 g/l. It was found by spectrographic measurement that this arrangement transmitted the erythemal rays but excluded the line at 3650 Å.

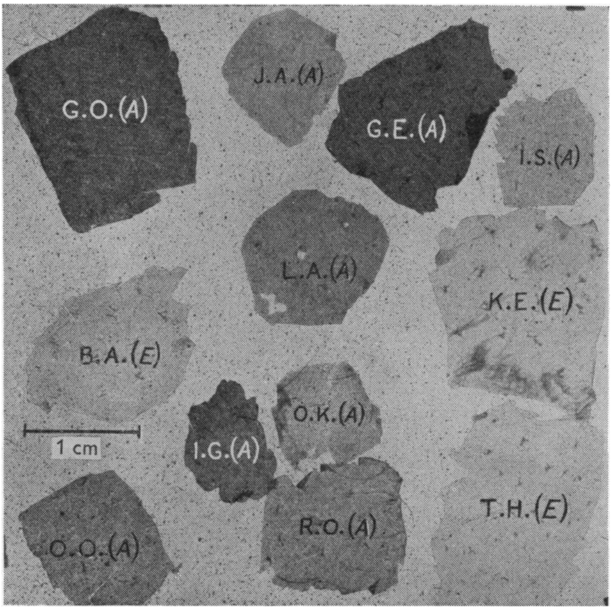

Fig. 1. Print of plate exposed to filtered ultraviolet radiation (3000–4000 Å) through twelve specimens of corneum (3 Europeans (*E*) and 9 Africans (*A*)).

Table 2 gives transmission data for the erythemal zone for 2 Europeans and 5 Africans. In view of the possible effects of the three and a half months' interval between removal and measurement, the transmission of total solar ultraviolet radiation obtained simultaneously, using the original Chance's glass filter, has also been inserted in Table 2 for comparison. The general increase in transmission at the 3000–4000 Å range may have been due to failure to reconstitute the specimens to their original humidity (Bachem & Reed, 1929). As would be expected, narrowing of the range from 3000–4000 to 2900–3400 Å has caused a general reduction in transmission. The gap is nevertheless still present between the two races, and, although the number of experimental subjects is small, there is evidence that the Europeans transmit appreciably more erythemal, as well as total solar ultraviolet radiation. This was confirmed by a study reported later in this paper of erythema production on living skin through these specimens.

TABLE 2. Transmission of erythemally active and total solar ultraviolet radiation through specimens of corneum 4 months after removal by blistering from the hip and, in one case, the arm

	Transmission (%)							
	Erythemogenic rays (2900–3400 Å)			Total solar ultraviolet rays (3000–4000 Å)			Mean erythemal	Mean total solar ultraviolet
Plate ...	12	13	14	9	10	11	—	—
Date ...	14. i.	15. i.	15. i.	14. i.	14. i.	15. i.	—	—
Initials of Europeans								
T.H.	59	48	49	81	71	60	52·0	70·7
J.E.	50	46	54	—	—	—	50·0	
T.H. (arm)	(16)	(21)	(25)	(51)	(43)	(28)	(21·0)	(41·0)
Initials of Africans								
P.A.	10	11	10	28	14	13	10·3	18·3
M.A.	19	22	29	53	50	29	23·3	44·0
F.R.	12	14	11	33	16	27	12·3	25·3
B.E.	25	28	28	49	49	39	27·0	45·7
W.M.	29	33	32	(45	30	30)*	31·3	38·4
				(50	34	41)		
				Mean Africans			20·84	34·34
				S.D. of the means			±9·18	±11·81

* Two measurements on a long specimen.

Relative contribution of pigment and corneum thickness to transmission

Although their thickness was known, the length of the path taken by light through these specimens could not be determined because of scattering and, under these circumstances, the absorption coefficient could not be validly used to separate the thickness factor from others responsible for variations in absorption. The statistical approach was therefore applied by plotting thickness against transmission in 7 Europeans and 18 Africans for which both measurements were available (Fig. 2). In view of the possibility of selection the difference between the mean corneum thickness for the two races in these smaller groups was again tested and gave $t = 1{\cdot}996\,\mu$ with difference between the means $= 2{\cdot}16\,\mu$ (S.E. $= \pm 1{\cdot}083\,\mu$). This t value was less than that required for significance at the 5 % level ($t = 2{\cdot}064$).

The correlation between thickness and transmission for Africans ($r = -0{\cdot}797$) was found to be very high and significant at the 0·1 % level; the regression of transmission on horny layer thickness, $y = 51{\cdot}807 - 3{\cdot}107x$ has been drawn in Fig. 2.

The relation between transmission and thickness is in fact exponential; transmission, for example, should be 100 % at zero thickness and at high thickness values it should approach, but not equal, zero. Nevertheless, it was certain that the line best fitting the present data could not be shown to differ significantly from a straight line, which appears to serve well in the range of thickness covered by this investigation.

It will be seen from Fig. 2 that although differences in corneum thickness account for part of the variability in transmission in Africans, they do not explain the marked difference between the two races.

The cause of the racial difference in transmission per unit thickness cannot be definitely assigned but the following evidence indicates that pigment was mainly responsible. The corneum of the albino African (S.A.), which resembled the European specimens visually with regard to absence of pigment, belonged to the European group in transmission per unit thickness. The percentage skin

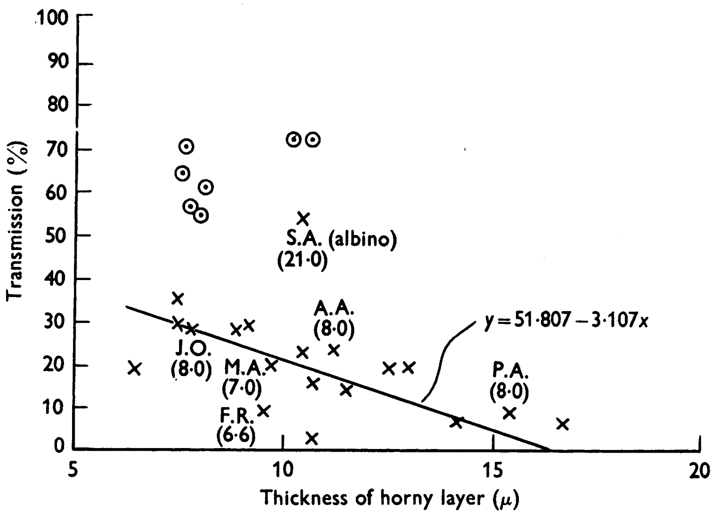

Fig. 2. Variation of transmission of solar ultraviolet light (3000–4000 Å) through the corneum against thickness of this layer. Europeans, ⊙; Africans, ×. Regression line for Africans only. The numbers in parentheses after initials are percentage reflectances for blue light on the forearm.

reflectance for six Africans including the albino, S.A. (Evans Reflectance Spectrophotometer, forearm, blue light) is given together with their initials in Fig. 2. The relation between reflectance and skin colour is complicated, and it is only in a pigment such as melanin, which absorbs fairly uniformly throughout the visible range, that reflectance measured in this way is inversely proportional to the darkness, and thus to the pigment content of the skin. The reflectance of the albino (21 %) is of the same order as Europeans (Edwards & Duntley, 1939), and more than twice as great as the remaining closely grouped Africans.

The numbers concerned are too small to conclude with certainty that the deviations of transmission from regression are caused by pigment content of the skin but it is of interest that the African with the lowest reflectance (F.R.) shows a transmission (9 %) which is less than half that expected on the basis of the thickness of his stratum corneum. The next lowest African (M.A.) is 3 % below expectation. The remaining three Africans with reflectance of 8 %

are on, or above, the regression line. These measurements are, therefore, consistent with the theory that differences between the two races in transmission per unit thickness, as well as scatter of Africans about the regression line, are caused by differences in pigmentation.

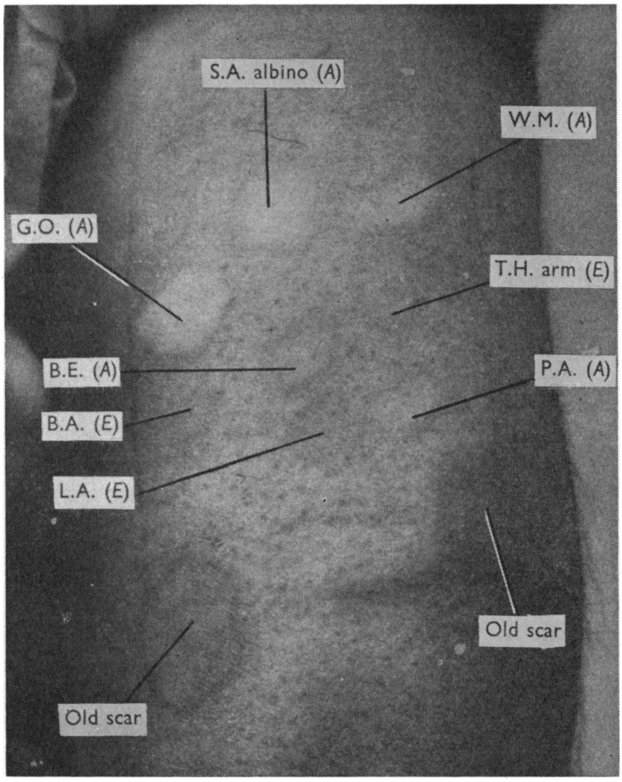

Fig. 3. Erythema produced by the sun on living skin through pieces of corneum; photograph, 3 days after exposure. After initials of subject, *E* = European and *A* = African.

Production of erythema on living skin through specimens of stratum corneum

Eight specimens of corneum, of which five were African and three European, were mounted in a moist state on living skin of the upper arm which was then exposed to the sun in Nigeria. Fig. 3 shows the appearance of this area 3 days after exposure.

This black and white photograph gives only an approximate indication of the colour contrast actually obtained. Darkness under specimens is, however, generally correlated with penetration of erythemal rays as evidenced by the degree of erythema, oedema and hyperaesthesia which developed after exposure.

All specimens protected the living skin to some extent against erythemogenic radiation. The two Europeans, B.A. and L.A., permitted greatest penetration of this radiation. The somewhat pigmented specimen from the arm of the European, T.H. (the only corneum sample not removed from the hip), and the African, B.E., were roughly intermediate between the Europeans and the dark-skinned Africans, G.O. and P.A. The protection afforded by the albino specimen, S.A., was greater than expected on the basis of transmission; this may have resulted from greater moisture content or departure from normal incidence of sunlight.

DISCUSSION

The complicating phenomena of scattering, reflexion and fluorescence must be considered in transmission studies through a non-homogeneous medium such as horny layer. From the viewpoint of racial comparison of the efficiency of the stratum corneum as a defence mechanism against solar radiation, however, it does not matter whether light is reflected, scattered or truly absorbed, provided measurement includes all transmitted light which reaches the underlying layers. In the present method scattered light was measured together with direct light since the horny layers were in contact with the photographic emulsion. Fluorescence, if present, might have caused an overestimate in transmission. This point was not specifically investigated here, but Bachem & Reed (1931) were unable to measure any fluorescence produced in human skin in a similar investigation.

Blister skin has frequently been used in the past for investigation of transmission of light through human skin (Bachem & Reed, 1931; Hardy & Muschenheim, 1934; Hasselbalch, 1911) and it is surprising that the possibility of obtaining clean sheets of stratum corneum in this way has not been previously realized. Immediately beneath this layer lies the stratum granulosum which is the seat of damage from ultraviolet radiation and, therefore, the mechanisms protecting against injury from these rays must be sought in the superficial horny layer.

Measurements reported above of the thickness of the stratum corneum on reasonably large samples of Africans and Europeans have not shown a significant difference between the two races. Variation in thickness between individual Africans was highly significant and, as indicated above, this partly accounts for variations in transmission between Africans as shown by the regression of transmission on thickness (Fig. 2). This regression also confirms that the observed difference between Europeans and Africans must be explained by some factor other than thickness of the corneum.

Evidence has been provided above that pigment may explain racial differences in transmission. Thus the stratum corneum of an African albino had a transmission per unit of thickness resembling that of the Europeans. Skin reflectance measurements indicated that the range of intensity of pigmentation was small among Africans and that a wide gulf existed in this respect between Africans and Europeans. These figures confirmed visual estimates of the relative blackness of the Africans' skins. Again the albino's reflectance placed him among the Europeans; the order of the reflectances of the remaining Africans was consistent with the theory that pigment accounted for deviations from regression of transmission on thickness.

Thus it would appear that skin pigmentation is mainly responsible for conferring relative immunity to solar ultraviolet radiation on the African. Evidence has been given that pigment protects against erythemal rays which are known to be capable of causing thermal type blister burns and interfering with thermoregulation (Thomson, 1951 *b*). In a wider sense the need for protection against this zone of radiation may account for the presence of pigment in the coloured races, its value for this purpose being such as to more than compensate for its disadvantage in absorbing more radiant energy from the sun.

SUMMARY

1. Specimens of stratum corneum obtained by blistering skin with cantharides have been used to compare the relative contributions of thickness and degree of pigmentation in protecting Europeans and Africans against solar ultraviolet light.

2. The mean thickness of the stratum corneum of the hip in European and African hospital orderlies and laboratory staff was found to be 9·5 and 11·1 μ respectively in the two races. The difference between means (1·6 μ) was not significant (S.E. = $\pm$ 0·84 μ).

3. The inherent transmission of solar ultraviolet radiation (3000–4000 Å) by the photographic method was on the average more than 3 times as great in Europeans (64 %) as in Africans (18 %); the distributions did not overlap.

4. The greater opacity of this layer in Africans was confirmed by exposing living skin to natural sunlight through specimens of stratum corneum from both races; the resulting erythema was stronger under European specimens.

5. A highly significant correlation was found between transmission of solar ultraviolet light and horny layer thickness in Africans. Nevertheless, regression of transmission on horny layer thickness confirmed that some factor or factors other than thickness of this layer must account for the differences in transmission between Africans and Europeans. Evidence that skin pigment was mainly responsible for the racial difference was provided by skin reflectance measurements, and by an albino African whose transmission lay in the European group.

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