OBSERVATIONS ON PRIMARY AND POSTMORTEM PIGMENTATION BY SUNLIGHT*

I. PRIMARY PIGMENTATION. ACTION SPECTRUM OF METHOXSALEN

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Primary pigmentation, which was discovered independently by Hauser[1] in 1938 and Henschke and Schulze[2] in 1938, is not a well-known phenomenon. It was also studied in detail in 1939 by Miescher and Minder.[3] Primary pigmentation occurs almost immediately after, or even during, exposure to sunlight, whereas the well-known secondary pigmentation (suntan) only occurs a few days after exposure.

The wavelengths responsible for primary pigmentation lie roughly between 300 and 460 mμ, while those for secondary pigmentation lie between 230 and 320 mμ. No ultraviolet rays from the sun shorter than 290 mμ reach the surface of the earth. Primary pigmentation can easily be produced in Coloured races after 10 to 30 minutes of exposure to sunlight.

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It is assumed that no new pigment is formed in primary pigmentation but that a simple oxidation of paler melanin to darker melanin takes place. The process is reversible; fading usually starts 30 to 60 minutes after exposure and disappears completely in 1 or more days.

The general occurrence of this phenomenon in coloured races was discovered accidentally by Kooij and Scott[4] in 1953 while experimenting with sunlight in a Coloured patient suffering from chronic porphyria.

More than 100 Bantu and Coloured subjects studied by them showed this phenomenon without a single exception. Although it could not be evoked in albino Bantu subjects it could be demonstrated in about one-third of White subjects exposed to sunlight, as it is found only when pigment is already present. Direct erythema often occurred simultaneously with the pigmentation, usually when filters transmitting wavelengths above 460 mμ were used.

With the aid of Ilford filters it was possible to show that the wavelengths between 300 and 460 mμ were responsible for the primary pigmentation. The slight increase of pigment in the basal layer described by Hamperl, Henschke and Schulze[5] and Miescher and Minder[3] was confirmed by frozen-section examinations made immediately after exposure.

Continued studies were done at Cape Town by one of us (R.K.) and at Bloemfontein by the other (F.P.S.) to establish the influence of different locally-applied substances on the phenomenon, and to investigate the occurrence of primary pigmentation after death and after excision of skin (see part II, to be published later).

MATERIAL AND METHODS

The experiments were carried out on a number of non-White subjects. Each substance tested was applied twice on the back of every subject — 2 hours before exposure to sunlight and also immediately before this exposure. Those substances applied 2 hours before were removed before exposure, but those applied immediately before the exposure were left on during the period of exposure.

The following substances were tested: 5% tannic acid in 95% ethyl alcohol, 5% chlorpromazine in 95% ethyl alcohol, 5% sulfanilamide ointment, anthisan ointment, 15% hydrogen peroxide in eucerin anhydricum, 10% ascorbic acid in eucerin anhydricum, 1% testosterone phenyl propionate in ethyl alcohol, 20% benoquin ointment, 2½% hydrocortisone ointment, ¼% oestrogens (premarin) in aqueous solution, ½% aqueous solution of copper sulphate, 1% aqueous solution of sodium fluoride, methoxsalen (8-methoxypsoralen) $^1/_{10,000}$, $^1/_{3,000}$, and $^1/_{1,000}$ in 95% ethyl alcohol, and 1% haematoporphyrin in ethyl alcohol.

The areas were exposed to sunlight for 20-minute periods between the hours of 2 p.m. and 3 p.m. during the months November to March. The results were read immediately after exposure and the areas were kept under close observation during the first hours and also for several days thereafter. The degree of erythema and pigmentation was estimated with the naked eye and marked +, ++, and +++. The exposed areas were covered with glass slides so as to cut out wavelengths below 320 mμ.

In some instances Schott filters were used. For details about the transmission (absorption spectra) of these filters see Table I.

TABLE I. THE ABSORPTION SPECTRA OF THE SCHOTT FILTERS USED*

Wavelengths in mμ	Filters							
	UG3	UG4T	UG5	UG11	WG1	GG3A	GG14	ARGIA
203	—	—	—	—	—	—	—	—
229	—	—	0·27	—	—	—	—	—
254	—	—	0·73	—	—	—	—	—
281	—	—	0·94	0·67	—	—	—	—
302	—	0·02	0·98	0·88	—	0·03	—	—
312	0·04	0·12	0·99	0·93	—	0·05	—	—
334	0·56	0·54	0·98	0·98	—	0·10	—	—
366	0·91	0·77	0·90	0·93	0·48	0·17	—	—
405	0·85	0·09	0·38	—	0·98	0·22	—	—
436	0·49	—	0·10	—	0·99	0·84	—	—
480	0·17	—	0·02	—	1·00	0·99	—	—
509	0·12	—	—	—	1·00	1·00	0·96	—
546	0·14	—	—	—	1·00	1·00	0·99	—
578	0·21	—	—	—	1·00	1·00	0·99	—
644	0·38	—	0·02	—	1·00	1·00	0·99	0·96
700	0·52	0·20	0·71	0·35	1·00	1·00	0·99	1·00
775	0·68	0·53	0·58	0·06	1·00	1·00	1·00	1·00
850	0·84	0·35	0·51	—	1·00	1·00	1·00	1·00
950	0·95	0·34	0·52	0·03	1·00	1·00	1·00	1·00
1050	0·98	0·29	0·53	0·08	1·00	1·00	1·00	1·00

All filters used were 1 mm. in thickness.

* Adapted from tables supplied by Schott and Gen., Jena Glassworks, Mainz, Germany.

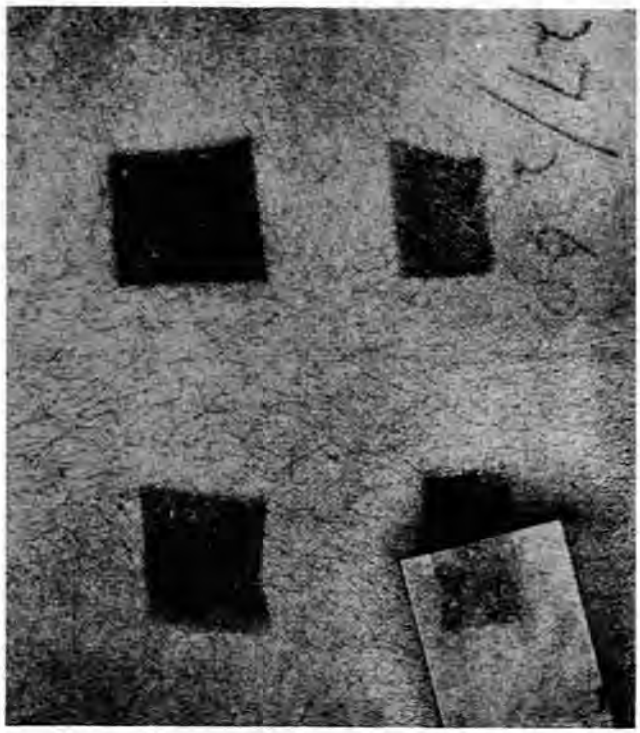

Fig. 1. Delayed erythema and pigmentation of areas treated with methoxsalen $^1/_{10,000}$ - $^1/_{1,000}$ following exposure for 20 minutes to sunlight which passed through glass. Photograph taken 3 days after exposure. Primary pigmentation had disappeared after 1 day.

RESULTS

The above substances did not exert any noticeable influence on primary pigmentation as compared with the controls. The duration and the degree of pigmentation was the same in both.

Primary pigmentation occurred in all the non-White subjects tested. It usually faded considerably after 30 minutes and sometimes it was hardly visible the next day. It could however often be observed several days after exposure. The duration of the primary pigmentation is probably due to the intensity of the irradiation and the ability of the individual to tan.

An interesting fact was observed in the exposed areas treated with methoxsalen. These areas showed a faint erythema on the day following the experimental exposure to sunlight. The primary pigmentation had disappeared by this time. The erythema gradually became stronger and was marked the next day, when slight pigmentation became

visible; this increased in intensity over the following days.

This delayed erythema followed by pigmentation also appeared when the methoxsalen-treated areas were covered with a glass slide, which cut out the 'sunburn rays' below 320 mμ (Fig. 1). No such reactions occurred in the areas treated with the other substances. With the aid of the absorption filters UG3, UG4T, WG1, and GG3A an attempt was made to determine the active wavelengths of this phenomenon (for details about the transmissions of these filters see Table I). The delayed erythema and pigmentation could only be evoked with the filters UG3 and UG4T and not with the filters WG1 and GG3A. This was shown on several occasions in 2 subjects.

From these results it can be concluded that the delayed erythema and pigmentation in the methoxsalen-treated areas are probably elicited by wavelengths near 334 mμ and below 366 mμ.

No delayed erythema occurred in areas not treated with methoxsalen and exposed to sunlight through the filters UG3, UG4T, UG5, UG11, WG1, GG3A, GG14A and RG1A. As could be expected primary pigmentation was only seen through the filters UG3, UG4T, UG5, UG11, WG1, GG3A but not with GG14A and RG1A.

DISCUSSION

None of the substances applied *in the concentration used* altered the primary pigmentation. It appeared at the same time and in the same degree as in the controls. In comparing degrees of primary pigmentation one must realize that slight alterations of the angle of incidence of the sun's rays may produce great changes in pigmentation. Tronnier *et al.*[6] showed that some of these substances (15% hydrogen peroxide and 10% ascorbic acid in eucerin anhydricum) activated the phenomenon of secondary erythema and pigmentation. It should be noted that the so-called 'sunburn-protecting' ointments, such as 5% tannic acid and 5% sulfanilamide ointment, with absorption spectra between 290 and 320 mμ, did not prevent primary pigmentation.

It is remarkable that chlorpromazine, haematoporphyrin, and methoxsalen with absorption spectra in long-wave ultraviolet light also did not alter the primary pigmentation. According to the Grotthus-Draper Law, radiation must be absorbed in order to bring about a reaction.

An interesting feature is the finding of a secondary or delayed erythema with pigmentation the day following exposure to sunlight in the areas treated with methoxsalen — this was not noticed when the other substances were used. It also occurred after the sunlight had passed through a glass slide which cuts out the ordinary sunburn-producing rays of between 290 and 320 mμ. From the experiments with these filters it is concluded that this 'sunburn reaction' is probably caused by ultraviolet wavelengths near 334 mμ and below 366 mμ. It is generally accepted that sunburn-reaction is evoked by wavelengths between 290 and 320 mμ, although long-wave ultraviolet light can also produce it; then, however, much more radiant energy is necessary.

Several observations in the literature suggest that the psoralens are able to potentiate ultraviolet radiation in long-wave ultraviolet light. Sidi and Bourgeois-Gavardin[7] and also Fitzpatrick[8] reported activity through window-glass. Pathak and Fitzpatrick[9] reported it with Wood's light, while Fowlks *et al.*[10] recently showed a photosensitizing action of furocoumarins, e.g. methoxsalen, in bacteria in the presence of long-wave ultraviolet light. The exact wavelengths causing the reaction are not mentioned in any of these publications and it is possible that a few wavelengths below 320 mμ still reached the skin. It is conceivable that the psoralens strongly increase sensitivity to the wavelengths of the sunburn spectrum and that even these few wavelengths below 320 mμ could evoke a sunburn reaction.

Our findings show that this is not the case, but that wavelengths near 334 mμ produce the effect. A few wavelengths below 320 mμ are however transmitted through our filters UG3 and UG4T. A monochromator or an interference filter is needed to exclude these minimal transmissions below 320 mμ with certainty.

Assuming that this 'methoxsalen sunburn reaction' is caused by long ultraviolet wavelengths near 334 mμ, then we are probably dealing with a shift of the wavelengths producing the sunburn reaction.

Not much is known about the nature of the shift by psoralens but the study of this phenomenon might explain light sensitivity reactions. It is possible that in light-sensitivity conditions substances are formed in the skin which cause a shift in the sunburn reaction similar to that evoked by the psoralens. It is also possible that these substances make the skin more sensitive to long-wave ultraviolet light so that a 'sunburn reaction' could be evoked by long-wave ultraviolet rays.

In agreement with this view are the following findings. Wiskemann and Wulf[11] found, in cases of polymorphic light eruption, a pathological reaction due to long-wave ultraviolet light (320 - 400 mμ). In cases of porphyria[12] they found an erythematous reaction to wavelengths above 320 mμ. Readings were made 7 and 24 hours after exposure. Magnus, Porter and Rimington[13] produced abnormal reactions in a patient with porphyria cutanea tarda with a monochromator, e.g. swelling, ecchymoses and even blistering, sometimes leading to secondary pigmentation, telangiectasia, and atrophy at wavelengths of 400 mμ. This point is at the region of maximal absorption, the Soret band, of most porphyrins. This strongly suggests that in this patient photosensitivity was initiated by porphyrins in the skin. The skin reactions of this patient to ultraviolet light in the normal sunburn region of the wavelengths (around 300 mμ) were normal. However, Magnus *et al.*[13], in the case they report, deal with an early or immediate erythema evoked by wavelengths of 400 mμ in contrast to secondary erythema, observed with methoxsalen and caused by long-wave ultraviolet light.

Magnus and Porter[14] observed this early erythema immediately after exposure to irradiation while the secondary or delayed erythemata develop within about 6 hours. With the aid of a monochromator they determined the wavelengths to be between 360 and 390 mμ, but not at 400 mμ. In pathological conditions other wavelengths evoked an early erythema.

Scott and Molhuysen van der Walle[15] reported increased

sensitivity to the long-wave direct erythema-producing rays (heat rays) in patients with lupus erythematosus; in patients with dermatitis solaris more sunburn reactions as well as direct erythema reactions to long rays (transmitted through a glass slide) were observed than were seen in normal persons.

Riemerschmid and Quin[16] have demonstrated a correspondence between the visible absorption spectra of eosin and the chlorophyll porphyrin, phyllo-erythrin, and the regions of the spectrum which are most effective in producing lesions of photosensitization in the skin of merino sheep in South Africa. The above observations are consistent with the conception that photosensitivity reactions can be produced by wavelengths above 320 mμ due to certain substances laid down in the skin. The practical conclusion from this is that in most of the above cases the common sun-protecting agents are not effective, because they usually prevent only the wavelengths between 290 - 320 mμ from reaching the skin.

Finally it should be mentioned that Wiskemann[17] and Wiskemann and Wulf[11] found that most patients with polymorphic light eruptions, in contrast to healthy people, did not show direct pigmentation at wavelengths of 300 - 350 mμ. In another article Wiskemann and Wisser[18] described the examination of 192 healthy individuals in Hamburg for the occurrence of direct pigmentation with the aid of a xenon arc lamp and filters. They reported that of 110 people between 7 and 45 years of age 98% showed primary pigmentation and of 82 people between 45 and 79 years of age 72% showed this phenomenon. It should be noted that the difference in incidence of primary pigmentation found by Wiskemann and Wisser[18] and by us can be explained chiefly by the fact that they used a xenon arc lamp and we used sunlight. Nevertheless the findings suggest a connection between absence of primary pigmentation and photosensitivity.

SUMMARY

1. Local application of various substances, e.g. tannic acid, sulfanilamide, hydrogen peroxide, ascorbic acid, benoquin, haematoporphyrin, hormones, and methoxsalen, did not influence the primary pigmentation which can generally be produced by sunlight in all non-White people.

2. Protective agents for sunburn could not prevent primary pigmentation.

3. Although methoxsalen had no effect on primary pigmentation, the methoxsalen-treated area showed a sunburn reaction by the following day. With the aid of filters it was shown that this reaction was caused by long ultraviolet rays near 334 mμ and below 366 mμ. This 'shift' of wavelengths producing a sunburn reaction by methoxsalen is discussed with regard to photosensitivity reactions.

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