OBSERVATIONS ON PRIMARY AND POSTMORTEM PIGMENTATION BY SUNLIGHT*

II. POSTMORTEM PIGMENTATION

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Pigmentation of excised and cadaver skin was studied by us because it resembles primary pigmentation in many respects. Meirowsky[1] discovered this phenomenon, which bears his name, in 1909. It is characterized by an increase in epidermal pigment when excised or cadaver skin is incubated at 37°C. or higher. Lignac[2] evoked a similar pigmentation by exposing cadaver skin to ultraviolet light (phenomenon of Lignac). Königstein[3] showed that postmortem pigmentation occurred even when the skin was kept in formalin or boiled in water, but no pigmentation took place when the skin was kept in saline or in cadmium-chloride solution. Lignac[2] noted that skin specimens kept in alcohol pigmented quicker than those in formalin. Neuberger,[4] Lignac[2] and others showed that postmortem pigmentation of the skin did not occur in an atmosphere free of oxygen. However, Sharlit *et al.*[5] reported that an external source of oxygen was unnecessary for the production of the Meirowsky phenomenon, because ionic oxygen from the cells could be made available throughout the epidermis. With cyanide and fluoride ions they could enhance the pigmentation process, whereas mercuric ions inhibited it.

Miescher and Minder[6] made an extensive study of primary and postmortem pigmentation and they concluded from their investigations that the processes are identical. In both there is oxygenation of already-formed melanin particles. According to them, melanin exists in different degrees of oxygen saturation. The size and the depth of colour of the particles are determined by the state of oxygenation; the less the oxygenation, the smaller the particle and the paler the colour. The process is reversible to a certain extent. Miescher and Minder[6] repeated the experiments of Lignac[2] by irradiating skin specimens, which were kept in alcohol or formalin, with the quartz lamp.

They could confirm his results and, in addition, by using

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filters preventing wavelengths below 320 mμ from acting, they showed that this postmortem pigmentation was evoked by long-wave ultraviolet light. This observation is another argument in support of the similarity of primary and postmortem pigmentation.

Although Hamperl, Henschke and Schulze[7] could not evoke postmortem pigmentation by irradiating cadaver skin, they could confirm the histological findings of Miescher and Minder.[6] They reported that, in primary pigmentation, the pigmentation takes place in the basal cells of the epidermis and not, as happens in secondary pigmentation (sunburn reaction), in the dendritic cells. Therefore, histologically, primary and secondary pigmentation also showed differences.

The purpose of our investigations was to evoke postmortem pigmentation by exposing excised and cadaver skin to sunlight and to determine the active wave-lengths. If postmortem pigmentation could be elicited by long-wave ultraviolet light, which produces primary pigmentation in the living skin, this would support the similarity of both processes. It would also enable us to carry out certain experiments in 'dead' skin, which are not feasible in living skin. For particulars on primary pigmentation reference can be made to Part I of this article.[8]

MATERIAL AND METHODS

1. *Cadaver Skin*

Pieces of cadaver skin from the abdomen or the thighs of a number of non-White subjects were exposed to sunlight for several hours. They were excised one or more days after death and used both untreated and after being kept in 95% alcohol or 4% formalin for one or more days. During the exposure the specimens were not moistened. Control specimens were kept in a dark cupboard. The specimens were covered with glass slides to prevent ultraviolet light below 320 mμ from acting. Other specimens were covered with cardboard and filters to determine the active wavelengths. After exposure to sunlight the specimens were kept under observation for several days. To prevent shrinkage the specimens were stretched with pins.

2. *Surgical Skin*

Long strips of skin, about 10 inches by ½ an inch, removed from patients undergoing thoracic operations, were cut in small blocks of about ½-inch square. These pieces of skin were treated by different methods to study the influence of these methods on pigmentation. Some skin specimens were stored in saline in an ice chest and used as controls.

The influence of sunlight was investigated immediately after excision, in dry skin, in skin submerged in ethyl alcohol 96%, and in saline.

The skin specimens were placed in Petri dishes covered with glass, and exposed to sunlight for about 4 hours. Control pieces covered with cardboard were in the same Petri dishes. The skin specimens used in these experiments were obtained from 6 Bantu patients.

Blocks of skin from at least 3 different individuals were submerged in solutions of various compounds or exposed to certain gases. No exposure to sunlight took place in these experiments. The specimens were kept at room temperature in a dark cupboard and the influence on pigmentation was noted after 24 hours, 48 hours, and 8 days. Specimens in saline, kept in an ice chest and at room temperature in a dark cupboard, were used as controls. No pigmentation takes place in skin specimens submerged in saline, kept either in an ice chest or at room temperature.

An anaerobic atmosphere was obtained with a Baird-Tactlock anaerobic jar. An oxygen atmosphere was created by allowing liquid oxygen to evaporate and then closing the bottle tightly. A carbon-dioxide atmosphere was created in the same way with solid carbon dioxide.

3. *Histological Investigations*

Frozen and paraffin sections were made of the specimens in which changes in pigmentation were noticed. The sections were examined both unstained and stained with haematoxylin and eosin and with silver nitrate, ½% in distilled water.

Skin specimens treated with copper salts were stained for copper with Mallory and Parkers' haematoxylin. Specimens treated with iron salts were stained with Prussian blue.

RESULTS

1. Cadaver Skin

It was not easy to evoke pigmentation in cadaver skin. Most of the specimens did not pigment at all after several hours of (up to 7 hours) exposure to sunlight, a few did after 2 - 6 hours. Skin from 7 out of 31 cadavers investigated showed postmortem pigmentation. Altogether, 51 skin specimens were exposed.

Pigmentation occurred in 3 of 15 untreated specimens, 6 of 26 alcohol specimens, and 1 of 10 formalin specimens.

Data concerning cases in which pigmentation was produced are shown in Table I. These results show that postmortem pigmentation can be evoked both by long-wave ultraviolet rays and by heat rays. In specimen I the pigmentation is

TABLE I. DATA CONCERNING SPECIMENS OF CADAVER SKIN WHICH SHOWED POSTMORTEM PIGMENTATION

		Untreated		*95% ethyl alcohol*		*4% formalin*	
No.	*Filters**	*Time (hours)*	*Pigmentation*	*Time (hours)*	*Pigmentation*	*Time (hours)*	*Pigmentation*
I	Glass	4	−	2	+	2	±
	Cardboard			2	−	2	−
III	Glass			2½	++	4	−
	Cardboard			2½	+	4	−
VII	Glass	2	++	7	±	6	−
	UG5	2½	−	7	±		
	GG14	2½	−	7	−		
VIII	Glass	2	+	7	±	6	−
	UG5	2	+	7	±		
	GG14	2	−	7	−		
X	Glass	5½	+				
	UG5	5½	−				
	GG14	5½	+				
XVI	Glass			4½	++	4½	−
XVII	Glass			4½	++	4¼	−

*For information about filters see Part I.[8]

probably caused by long-wave ultraviolet rays, although the negative results with cardboard do not exclude heat rays with certainty. A piece of copper, as used by Lignac in a few of his cases, would have been more suitable. However, in specimens VII and VIII the pigmentation can be ascribed to long-wave ultraviolet light, because only a few heat rays pass filter UG5.

In contradistinction to this, the postmortem pigmentation in specimens III and X must have been produced by heat rays, because filter GG14 cuts out wavelengths below 500 mμ. Although specimens XVI and XVII had been in alcohol for 2½ months, they still showed pigmentation after exposure to sunlight passing through glass.

The specimens were kept under close observation after the exposure was discontinued and an interesting feature was observed. In many specimens the pigmentation faded considerably in the first half hour after being taken out of the sun. Sometimes the pigmentation disappeared completely in a few days as happened in no. I; in another specimen (no. III) the pigmentation decreased during the first week of observation, and it became darker again thereafter. During this time the specimens were kept dry in a dark cupboard at room temperature.

2. Surgical Skin

A. Skin Specimens Exposed to Sunlight

Pigmentation was found in 4 of the 6 cases in which skin specimens in the dry state were exposed to sunlight. The pigmentation occurred after 2 - 4 hours of exposure. Pigmentation was more marked and became visible after half an hour

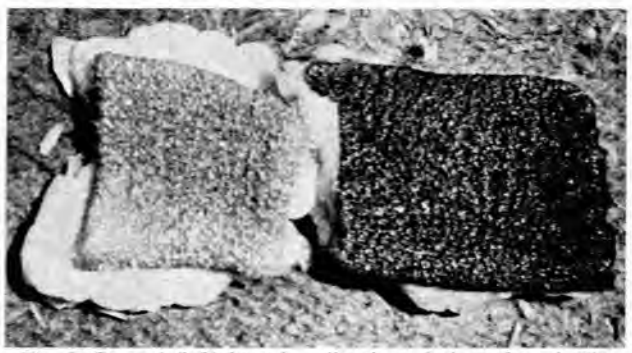

Fig. 1. Control (left) kept in saline in a dark cupboard. The other specimen, submerged in alcohol, was exposed for 4 hours to sunlight.

when the skin was submerged in alcohol during exposure to sunlight (Fig. 1). It took place through the thick glass cover of the Petri dish.

Skin specimens submerged in saline showed no pigmentation at all after 4 hours of exposure to direct sunlight. Skin specimens in saline, kept in an ice chest for 3 weeks, and thereafter submerged in ethyl alcohol, still showed pigmentation when exposed to sunlight.

The phenomenon of fading, as described in the cadaver skin, was also observed in surgical skin specimens.

B. *Skin Specimens Submerged in Various Substances Without Exposure to Sunlight*

The specimens were kept in a dark cupboard at room temperature. The results of these experiments are shown in Table II.

It was found that:

(*a*) Drying-out of the skin increased the pigmentation;

(*b*) pigmentation was prevented by keeping the specimens in saline or water;

(*c*) complete anaerobic atmosphere, obtained by means of a Baird-Tactlock anaerobic jar, prevented pigmentation;

(*d*) pigmentation was retarded by vitamin C and also when the specimen was kept in a carbon-dioxide atmosphere, as compared with controls which showed stronger pigmentation; and

(*e*) pigmentation was increased by alcohol, ether, oxygen, copper sulphate, zinc sulphate, ferrous sulphate, and silver nitrate. The pigmentation was also enhanced, but to a lesser degree, by sulphadiazine, 'anthisan', tannic acid, and potassium permanganate.

The following substances caused *depigmentation* of the skin:

(*a*) *Bleaching agents* like ammonia, chloresol and sodium hydroxide. In concentrated form the depigmentation was usually complete within half an hour. In diluted solutions the process was much slower. The depigmentation started from the sides of the skin specimen and the central part showed a reticulated depigmentation before it was completely depigmented (Fig. 2). This manner of depigmentation is probably the result of differences in the rate of diffusion;

Fig. 2. Reticulated depigmentation of a surgical skin specimen after 1 hour in 1% sodium hydroxide.

(*b*) *Acids.* Fluoric acid, 0·2%, and hydrochloric acid, 1%, caused cleaving of the skin; after 24 hours depigmentation was noticeable. The epidermis came off after a few days (see further under histology);

(*c*) Saturated bromine solution (in water) also caused cleaving and depigmentation like the acids, but after a few days repigmentation slowly took place, and

(*d*) After complete depigmentation with the bleaching agents, repigmentation took place when the specimen was submerged in ½% silver nitrate (see further under histology).

TABLE II. DEGREE OF PIGMENTATION AND DEPIGMENTATION OF SPECIMENS OF SURGICAL SKIN TREATED BY VARIOUS SUBSTANCES AFTER 24 HOURS* IN A DARK CUPBOARD AT ROOM TEMPERATURE

Pigmentation				*Depigmentation*
Nil	*Slight*	*Moderate*	*Extreme*	
Saline	Saline $+O_2$	Vitamin C, 10% in ethyl alcohol	Ethyl alcohol, 95%	Saturated bromine***
Vitamin C, 10%**	Anthisan, 1%***	Ethyl alcohol, 95%$+CO_2$	Ethyl alcohol $+O_2$	Fluoric acid, ½%***
Methoxsalen, 1%**	Tannic acid, 1%***	Benoquin†	Methoxsalen, 1% in 95% ethyl alcohol	Sulphuric acid, 1%***
Arovit, 20,000 units/ml.	Potassium permanganate, 1%***	Sulphadiazine sodium, 1%***	Silver nitrate, 1%***	Sodium hydroxide, 1%***
Cadmium chloride, 1%***		Copper sulphate, 1%***	Pure ether	Ammonia
Porphyrin, 1%***		Zinc sulphate, 1%***		Chloresol, 1%***
Chlorpromazine, 1%***		Ferrous sulphate, 1%***		
Testosterone, 5 mg./ml. in alcohol		Mercuric chloride, 1%***		

*The results after 48 hours and after 8 days were almost the same as these with the exception of bromine, in which specimen irregular repigmentation was noted after 8 days.

**Dissolved in saline.
***Dissolved in water.
†Cleaving took place, probably caused by the solvent, 5% isopropyl alcohol.

3. Histology

It was found that unstained sections were the most suitable for comparing changes in pigmentation. When pigmentation was noticed macroscopically, this was usually found histologically as well. A very marked increase of pigment was noted in the basal layer of skin specimens treated with copper sulphate, zinc sulphate, ferrous sulphate and sulphanilamide. There was also a slight increase of pigment in the epidermis and the papillary layer.

Skin specimens treated with copper and iron salts were stained for the presence of copper and iron respectively. A diffuse positive reaction was noted for copper in the specimens treated with a copper salt and for iron in those treated with iron salts. There was no change in the degree of pigmentation as compared with other staining methods.

The bleached skin showed no pigment when bleaching was complete, and an irregular pigmentation of the basal layer was found in the specimens with reticulated depigmentation.

Skin specimens treated with alkalis and acids showed a loosening of the epidermis just above the basal layer, while those treated with bromine showed loosening just below the basal layer of the epidermal junction. This has been noticed by others, including Rothman.[9]

DISCUSSION

1. *Cadaver Skin*

The findings show that postmortem pigmentation can be evoked by sunlight, although it happened in only 7 of the 31 cadavers investigated. There was no correlation between the occurrence of postmortem pigmentation and the particular disease which caused the death of the patient. Probably, previous exposure to sunlight and the time interval between death and excision of the skin specimens are of importance. The time necessary to evoke postmortem pigmentation varied from 2 to 7 hours, which is much longer than for living skin (see Part I[8]). This is probably owing to the fact that chemical processes are different in living and dead skin, particularly because of lack of circulation.

Postmortem pigmentation by sunlight was noted in untreated skin as well as in specimens kept in alcohol or formalin.

Usually the specimens had remained in alcohol or formalin for a day or two; however, specimens XVI and XVII had been kept in alcohol for 2½ months and thereafter still showed a marked pigmentation after exposure to sunlight. From the results of the experiments with exposure to sunlight through various filters, it can be concluded that postmortem pigmentation can be elicited by long-wave ultraviolet rays and by heat rays from the sun.

Fading of postmortem pigmentation soon after the discontinuation of the exposure to sunlight has not been described in the available literature. It is of practical importance for the photographing of the results and also for histological investigations. These procedures have to be done immediately after discontinuation of the irradiation.

The fact that postmortem pigmentation can be evoked by long-wave ultraviolet rays, and the occurrence of the fading phenomenon after discontinuation of the irradiation, support the view that postmortem pigmentation and primary pigmentation are similar processes. We therefore felt justified in carrying out certain experiments in cadaver and surgical skin, not possible in the living skin, which might give some insight into the process of primary pigmentation.

2. *Surgical Skin*

Postmortem pigmentation by sunlight occurred more often in excised surgical skin than in cadaver skin. This can probably be ascribed to the fact that in the surgical specimens only a short time had elapsed between 'death' of the skin and exposure to sunlight.

The pigmentation occurred quicker and was more marked with skin specimens in alcohol, while skin specimens in saline did not show pigmentation at all. An explanation for this behaviour is difficult to find. Possibly alcohol acts by extracting water from the skin and this drying-out accelerates the pigmentation. The phenomenon of fading of the pigmentation was also noted in surgical skin specimens.

From the results of the influence of various substances at room temperature on surgical skin specimens without exposure to sunlight, as shown in Table II, it may be concluded that oxygen is necessary for postmortem pigmentation. Since it is assumed that oxygen is also necessary for the occurrence of primary pigmentation, this finding supports the view that postmortem pigmentation and primary pigmentation are similar processes.

The increase in pigmentation by metal salts, especially in the basal layer of the epidermis, is striking. In contrast to the statement of Sharlit,[5] that mercuric ions inhibit postmortem pigmentation, we found an increase of pigmentation with mercuric chloride. Findlay[10] also found an increase of postmortem pigmentation with mercuric compounds.

It should be noted that the solvent for the testing of the various compounds is of great importance. Since skin specimens, submerged in alcohol only, show a strong pigmentation already, wrong conclusions may be drawn from the results of experiments using substances dissolved in alcohol. For instance, the results obtained with methoxsalen in water and alcohol differ; they are negative in water and positive in alcohol. Postmortem pigmentation does not occur in specimens in water or saline, therefore positive results with substances dissolved in these solvents are valid.

The bleaching by various compounds is also of interest. However, the interpretation of the influence of the various substances on pigmentation and depigmentation at the present stage of our knowledge is very difficult. The aim of these experiments was to collect data for a better understanding of the phenomenon of postmortem pigmentation, and consequently also of primary pigmentation. More data are necessary, and the investigations are being continued along various lines; the results will be reported in a separate article.

So far, findings in cadaver skin as well as in surgical skin are in support of the view that primary and postmortem pigmentation are similar processes.

SUMMARY

1. It was found with the aid of filters that postmortem pigmentation could be evoked by the long-wave ultraviolet rays of the sun and that this pigmentation was dependent on the presence of oxygen. Fading of postmortem pigmentation, just as in primary pigmentation in the living skin, was observed and has not been described before. These findings are in support of the view that primary and postmortem pigmentation are similar processes.

2. The time necessary to produce postmortem pigmentation by sunlight varied from 2 to 7 hours, which is much longer than the time necessary to elicit primary pigmentation in the

living skin. It is not easy to evoke postmortem pigmentation in the cadaver skin; it only occurred in the skin of 7 out of 31 cadavers investigated. It can be elicited more easily in surgical skin specimens, while submersion in alcohol accelerates the pigmentation.

3. The influence of many substances on surgical skin specimens, *without* exposure to sunlight, was investigated. Postmortem pigmentation does not occur in skin specimens submerged in water or saline, while alcohol promotes the pigmentation. Therefore, the solvent used is of great importance in the testing of compounds regarding their ability to produce postmortem pigmentation. Metal salts enhanced the postmortem pigmentation considerably; on the other hand methoxsalen and haematoporphyrin had no influence.

Bleaching by various compounds was also observed. The findings regarding postmortem pigmentation were discussed.

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