COMPARISON OF SKIN COLOR WITH MELANIN CONTENT*


The study of skin color has recently undergone some developments. In 1949 the senior author published a chart of eight individual skin colors ranging from full black to white, which can be used in recording the skin color of persons of mixed descent (1). In making use of this chart, certain modifications have been found necessary, and a new genetic theory of skin color has been developed (2). As a different approach to the subject, spectrophotometer reflectance curves of various human skin colors have been published, including those of the original chart (3). In a further effort to analyze the nature of the differences between various skin colors, it was considered desirable to make a histologic study of the skin of different individuals from biopsy specimens.

TABLE I

<table>
<thead>
<tr>
<th>COLOR CHART DETERMINATION</th>
<th>LETTER ON PHIAL</th>
<th>DECREASING ORDER OF PIGMENTATION AS DETERMINED FROM SKIN SECTIONS</th>
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<tbody>
<tr>
<td>F2A2</td>
<td>A</td>
<td>No. 1 Fig. 1</td>
</tr>
<tr>
<td>F1A2</td>
<td>G</td>
<td>No. 2 Fig. 2</td>
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<tr>
<td>F3A3</td>
<td>D</td>
<td>No. 3 Fig. 3</td>
</tr>
<tr>
<td>F5A5</td>
<td>C</td>
<td>No. 4 Fig. 4</td>
</tr>
<tr>
<td>F4-5A5</td>
<td>E</td>
<td>No. 5 Fig. 5</td>
</tr>
<tr>
<td>F7A6</td>
<td>F</td>
<td>No. 6 Fig. 6</td>
</tr>
<tr>
<td>F8A8</td>
<td>B</td>
<td>No. 7 Fig. 7</td>
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</table>

For this purpose a series of teen-age Negro boys was taken to the Massachusetts General Hospital and a small biopsy specimen of skin from the upper arm, over the triceps muscle, was removed from each, the senior author's arm serving as control. For this service we are indebted to the authorities of the Hospital and particularly to Dr. John E. LeDonne. We are also indebted to Dr. E. M. Hartl for help in selecting the boys and bringing them to the hospital. The skin specimens were each placed in a phial of 10% formalin and given a letter, the order of letters being quite different from that of depth of external skin color. They were then sent to the junior author, who made the histologic studies, and independently arranged them in the order of relative amounts of melanin encountered in the skin sections. A previous study of melanin development in the Negro fetus had particularly fitted him for this work (4).

The order in which the specimens were placed on the basis of histologic examinations of unstained sections coincided essentially with the order of phenotypic skin color, as seen in Table I. This shows that the microscopic closely corresponded with the macroscopic differences, and that they were sufficiently con-

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spicuous to be classified on either basis. In the Table, the skin colors are in decreasing order of pigmentation from 1 to 7. No full black was available. Nos. 7 and 4 of the color chart were not represented in the series of arm colors, but 4 appears to differ from 5 in tone rather than in degree of pigmentation (see Gates 1953) and 7 corresponds in color with a brunette of the white race.

In the Table, F refers to face color, A to upper arm color. The arm color in each case was determined at the point from which the biopsy specimen was taken immediately afterwards. Upper arm color is uniform, but face color is not always uniform. It may differ from arm color, and the highlights also may vary. Thus in specimen G in the second line of the Table, the face was one shade darker than the arm (see color chart in Gates 1949), whereas in line one (specimen A) both face and arm were recorded as 2. Although both fall into 2 of the color chart, it will be seen from Figs. 1 and 2 that the skin section of G contains less melanin than that of A. The slightly darker face of G is probably a result of tanning.

Again, the arm color of E fell in the same position (5) on the color chart as C, but comparison of Figs. 4 and 5 shows that the latter contains a little less melanin. Again, the face of E was a little darker, probably through tanning. Thus, as might be expected, smaller differences can be determined under the microscope than by the naked eye. In so far as each color in the chart may be considered to represent a certain combination of genes for skin color, there will also be a certain range of accompanying uninherited variation. Specimen B, a white skin (Fig. 7),
Fig. 2. Unstained section of biopsy-specimen G. There is less melanin in the basal layer of the epidermis than in the preceding section. The amount and distribution of melanin in the stratum spinosum is approximately the same. The dermis contains isolated melanophores. Their scant number could not have appreciably influenced the overall skin color.

Fig. 3. Unstained section of biopsy-specimen D. Both the basal layer and the stratum spinosum contain less melanin as compared with previous sections. Zones of heavier concentration of melanin in the basal layer alternate with lighter areas.
FIG. 4. Unstained section of biopsy-specimen C. The irregularity in the deposits of melanin within the basal layer is more striking and there are fewer supranuclear caps in the stratum spinosum.

FIG. 5. Unstained section of biopsy-specimen E. The melanin content of the basal cell layer is further decreased and there are only traces of the pigment in the stratum spinosum.
shows markedly less pigment than F (Fig. 6), a light-colored skin. These are the obvious conclusions from a first inspection of the sections, and of Figs. 1 to 7.

Fig. 6. Unstained section of biopsy-specimen F. This lightly colored epidermis contains some melanin throughout the basal layer but only scattered cells appear dark. Some of these cells may be melanocytes. There is practically no melanin in the stratum spinosum.

Fig. 7. Unstained section of biopsy-specimen B. (light brunette white race). A few basal cells contain traces of melanin.

Edwards and Duntley (5) have made an interesting color analysis of the human skin in white and colored races by spectrophotometry. Their instrument recorded objective measurements of the light reflectance at each wave length of
the visible spectrum. In a subsequent report they extended their readings of reflected values into the ultraviolet range of the spectrum (6). The total reflected light has the absorption characteristics of the pigments in all layers of the skin. In addition, it is influenced by the turbidity of the deeper layers of the skin which produces a scattering optical effect and adds a blue component to the total reflected light. By comparing the skin reflectance spectra with those of solutions of known substances, Edwards and Duntley were able to determine that skin color is determined by five pigments: melanin, melanoid (a derivative of melanin), oxyhemoglobin, reduced hemoglobin and carotene.

Of particular interest is their observation that melanin and melanoid "are alone responsible for variable skin color in the so-called dark races." This is in full agreement with our own histologic findings in unstained sections from biopsies of a graded series of pigmented skins. It may therefore be considered well established, and confirmed, that it is the amount of melanin in the epidermis which is solely responsible for the differences in racial skin color. Other findings by Edwards and Duntley will be considered in the discussion of our own observations.

**HISTOLOGIC FINDINGS**

The skin biopsy-specimens were fixed in 10 per cent formalin and labelled with a code letter unknown to the histologist as to its significance within the graded series of skin color established by the senior author. Gross examination of the 5 mm punch-specimens, however, revealed obvious differences in the degree of pigmentation. The biopsies were received at intervals and, though processed independently, treated as much as possible in an identical manner. Paraffin sections of 10 microns thickness were made. Of each specimen a number of sections were mounted unstained, others stained either with hematoxylin-eosin or with Bodian's reduced silver method.

For the purpose of comparing the amounts and distribution of melanin the unstained series of sections gave the best results. Selected sections of this series were photographed at a 350 × magnification. A neutral density filter was used for each exposure (½ sec.) and the negatives were developed together in a single rack and tank. The printing of the positives, likewise, was done under controlled similar conditions. They are reproduced in the illustrations of this report.

Additional features of skin structure may be considered as possible factors secondarily influencing skin color: a) the thickness of the stratum corneum, b) the depth of the pigmented layer, c) the size and color of the melanin granules and d) the histologic nature of the underlying dermis. Since all the biopsies had been obtained from comparable skin areas (arm) of the individuals, the epidermis was of strikingly similar thickness in all studied sections. The same holds true for the histologic nature of the connective tissue in the dermis. Evidence for these similarities will be readily recognized by a comparison of the photomicrographs of the unstained sections.

The thickness of the stratum corneum was practically alike in all sections. Due

*We appreciate the technical assistance of Mrs. Eunice Mahon (histologic preparations) and of Mr. Lawrence A. Toriello (photomicrographs).
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to a degree of desquamation and minor artefacts of fixation and embedding it was
difficult to make accurate measurements of that layer. As would be expected,
considering the skin areas from which the biopsies were obtained, it was thin and
averaged 10–12 microns in depth. The total thickness of the epidermis varied
between 75 and 100 microns. In the darker skin specimens the stratum corneum
contained scattered melanin granules and probably some melanoid substances.
In comparison with the intensity of melanin deposits in the deeper layers of the
epidermis, any influence by the stratum corneum on skin color in the compared
areas can be only an insignificant factor. Edwards and Duntley (5) have shown
that the melanoid content is low in regions of the epidermis where the stratum
corneum is thin. Carotene, likewise, was demonstrable in the epidermis only
where the stratum corneum is thick. Edwards and Duntley believed that the
lipins in the stratum corneum have an affinity for carotene which thereby becomes
stored in that layer, as well as in the fat of the subcutis. They emphasized, how-
ever, that the amounts of this pigment are not significant in contributing to the
skin color of dark races. Carotenes are isomerises of a large class of hydrocarbons,
the terpenes. It might be of secondary interest that carotene as a lipochrome is
not necessarily identical with the carotene that is the precursor of Vitamin A.

The depth of the pigmented layer varied in the skin specimens. In all cases
significant melanin deposits occurred only within the epidermis. The basal layer
(stratum germinativum) contained the most and there was a decreasing amount
in the stratum spinosum from the darkest to the lightest skin samples. In some
sections of the darker skins a few scattered macrophages of the dermis contained
melanin. These cells evidently had phagocyted some melanin that normally
stems from the epidermis with the tissue fluids. The amount of these dermal
melanin deposits were entirely too small to be of any significance in the skin color
of the specimens.

The size of the melanin granules in the epidermal pigment layers is remarka-
ibly homogeneous. Melanin appears essentially as a fine “dust” within the epider-
mal cells, in contradistinction to that contained in dermal macrophages where it
is clumped and the granules are of irregular size. In the human epidermis the
melanin granules measure 0.1 micron. Melanin granules are light brown and give
a black effect only through their dense accumulation in the deeper layers of the
epidermis. This report is not concerned with melanogenesis per se. Recent
investigations have made it clear, however, that the basal cells do not produce
melanin but receive it secondarily from highly specialized cells, the melanocytes,
or dendritic cells, which transfer the pigment to the ordinary epidermal cells.
Such potential melanin-producing dendrites exist alike in the so-called white
and colored epidermis. The main genetic factor determining “white” skin resides
in the presence of SH groups (7, 8) which inhibit the effects of the enzyme tyro-
sinase upon tyrosine in the production of melanin. Any agent that eliminates the
inhibitory action of the sulphhydryl groups, e.g. actinic rays, will result in melanin
production in the white skin (9).

The histologic nature of the connective tissue in the dermis was essentially
alike and quite comparable in the skin samples studied. The presence of an occa-
sional melanophore was pointed out in a preceding paragraph. The nature of this skin layer can have no effect on the color of the skin areas under consideration. In particular, there were no noticeable differences in the vascularization in this tissue. Edwards and Duntley (5) have demonstrated characteristic absorption bands in solutions of oxyhemoglobin and of reduced hemoglobin. From the presence of these bands in the light reflected from living skin areas they were able to determine the relative amounts of arterial and venous blood of the skin. Their spectrophotometric method revealed and confirmed that certain skin areas were particularly well supplied either with arterial or with venous blood. These differences affect the skin reflection of light. In the arm areas of our biopsies these conditions evidently were alike and any significant influence of vascularization on skin color can be ruled out.

The biopsies secured for this study were not all of equal depth. Before embedding in paraffin they were trimmed, however, to represent only the cutis. Within the latter, melanin alone is the determining color factor.

The detailed analysis of the relative amounts and distribution of melanin in our skin specimens enabled us to establish a graded series, from the darkest to the lightest skin samples, which corresponded very closely to the order of skin colors postulated by the senior author. Differences were only of a minor nature. Fig. 1 illustrates the histology of an unstained section of the skin sample containing the greatest amount of melanin in the epidermis. Melanin is heavily concentrated in the basal layer of the stratum mucosum. The characteristic supranuclear caps of these pigment deposits are clearly visible. They are not confined to the basal layer but similar, though fainter, accumulations of melanin may be recognized throughout the stratum spinosum. Some scattered melanin granules are also present within the stratum corneum. The dermis is clear of all melanin.

Fig. 2 represents the histology of the second darkest skin sample. When compared with Fig. 1 the smaller amount of melanin, particularly within the basal layer of the epidermis, will be readily noticed. However, the stratum spinosum appears to contain an equal amount of melanin deposits as that of the darkest skin specimen. There are also some traces of melanin in the stratum corneum. The dermis is comparable to that of Fig. 1 with the exception that it contains a few melanophores which, in their number and amounts of melanin carried, could not have affected the overall skin color or light reflectance.

The next lighter biopsy is illustrated in Fig. 3. Supranuclear caps of melanin accumulations in many basal cells are still very prominent. However, there are zones of considerably lighter melanin deposits in the same layer. The melanin contents of the cells in the stratum spinosum are less and appear as fainter "caps." There is practically no melanin in the stratum corneum nor in the dermis.

The histologic aspects of a still lighter skin are shown in Fig. 4. It might appear that the amounts of melanin are not appreciably less than in the preceding specimen. The evaluation of the relative degree of pigmentation naturally was not based on an examination of a single field. Even for an approximate estimation of the amounts of melanin present, the study of many segments of the epidermis is necessary. With decreasing amounts of epidermal melanin a marked irregularity
of its deposits becomes characteristic. There appears a degree of "patchiness," similar to the condition in freckles. Fig. 4, therefore, reveals zones of fairly heavy melanin content and others of definitely less pigment. This irregularity in melanin deposits makes it particularly difficult to place a given section within an order of increasing or decreasing amounts of melanin. The areas of heavier melanization appear to be related to the presence, within the epidermis, of particularly active melanoblasts which become vectors for melanin distribution in neighboring epithelial cells. The same effects were observed by the junior author in biopsies of his own skin after ultraviolet light exposure.

Throughout the sections of specimen C (Fig. 4) there was less pigment in the cells of the stratum spinosum than in sections of darker skins. Such cells contain relatively greater amounts of melanin only in zones overlying heavily pigmented basal cells (and presumably active melanocytes).

Fig. 5 illustrates the histology of skin specimen E. The irregularity in the distribution of melanin is marked and there is a smaller amount than shown in the preceding figure. Melanin deposits in the stratum spinosum likewise are scant and there is none in the stratum corneum. Histologically there appeared to be less difference in the relative amounts of melanin between the specimens C and E (Figs. 4 and 5) than between others of this series. Therefore, an approximate estimation of the respective degrees of pigmentation was difficult. This appears to be partly due to the manner of distribution of the melanin present in the sections.

There was, however, a considerable difference in the amounts of melanin between specimens E and F. The histology of the latter is illustrated in Fig. 6. Here, the amount of melanin is scant also in the basal layer of the epidermis. Only an occasional basal cell appears "capped" by melanin. One gains the impression that some of these clusters of melanin granules are contained within the cell-bodies of melanoblasts. There is practically no melanin in the stratum spinosum.

Fig. 7, skin specimen B, shows the least amount of melanin. Traces of pigment can be recognized in some basal cells. Unknown to the histologist, this specimen was a biopsy of the senior author's own skin. As indicated in the first part of this report it represents the unstained skin features of a light brunette of the white race.

**SUMMARY**

Skin specimens (biopsies) of various degrees of melanization and therefore of depths of skin color were studied histologically in order to compare the relative amounts and the distribution of melanin. The histologic evaluation and the classification of the specimens, in a decreasing order of amounts of melanin present, was made independently and unprejudiced by the macroscopic appearance and order of skin color. A color chart of the various depths of skin color had previously been published. The order of sequence, based on histologic examination of the variably pigmented skin specimens corresponds to that of the macroscopically determined phenotypic skin color. The differences in the graded series of skin colors...
color are sufficiently marked to be classified on either basis. This confirms that depths of skin color depend on the amount of melanin present in the epidermis, and especially in the stratum germinativum and spinosum. When less melanin is present (e.g. #3, 4 and 5) there is more variation both in amount and distribution within the epidermis. In practice, the phenotypes of #3, 4 and 5 are therefore combined into one genotype. The sparse amounts of melanin within melano-phores of the dermis do not influence skin color of colored races.

BIBLIOGRAPHY