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Quantitative Analysis of Skin Color from Melanin Content of Superficial Skin Cells

The color of human skin is determined by many factors. Blood supply, epidermal thickness, and pigments, both intrinsic and extrinsic, are responsible for the many variations we see. An important factor is the amount of the intrinsic pigment melanin, which absorbs and scatters ultraviolet radiation. In recent studies of the morphology of surface skin cells and their melanin content, we noticed a clear correlation between gross skin color and the average number of melanin granules per cell. This suggested that a quantitative analysis of melanin granules in skin cells from microscopic skin specimens, such as those present on clothing, could help to determine the skin color of the individual from which the cells came. This paper describes the methods of cell collection and staining followed and summarizes the results of the quantitative studies.

Anatomy of the Skin

A brief review of skin structure is presented to aid in understanding the basis of our cytological techniques. Skin is composed of three layers: an outer epidermis; an intermediate dermis containing blood vessels, nerves, glandular appendages, and hair follicles in a stroma of mostly noncellular collagenous material; and an inner, thick pad of fatty subcutaneous tissue [*1*]. The epidermis itself is a stratified tissue varying from 30 to 500 μm in thickness (palms and soles) composed of two main sections, the stratum malpighii, or germinativum (the living epidermis), and the stratum corneum, or horny layer (nonliving cell structures) (Fig. 1). The strautm malpighii is further subdivided into a basal layer, a prickle layer, and the stratum granulosum. This stratification is simply a reflection of the various stages through which basal cells pass in their gradual conversion into the horny material of the stratum corneum.

The stratum malpighii contains two distinct cell types of different lineage—keratinocytes and melanocytes. Keratinocytes, or malpighian cells, are keratin-synthesizing cells that comprise 95 percent of the epidermis. In addition to keratin, these cells contain melanin that has been produced by the melanocytes and transferred from them by a process called apocopation [*2*]. Melanocytes, or melanoblasts, are the only cells capable of synthesizing the principle pigment of the epidermis, melanin. The number of melanocytes varies from site to site on the human body but is independent of both race and sex [*3*]. Differences of skin color among individuals are determined not by the number of melanocytes but by the differences in the activity of the melanin producing process. The process is under the influence of genetic factors, hormones, inflammation within the skin, and ultraviolet radiation.

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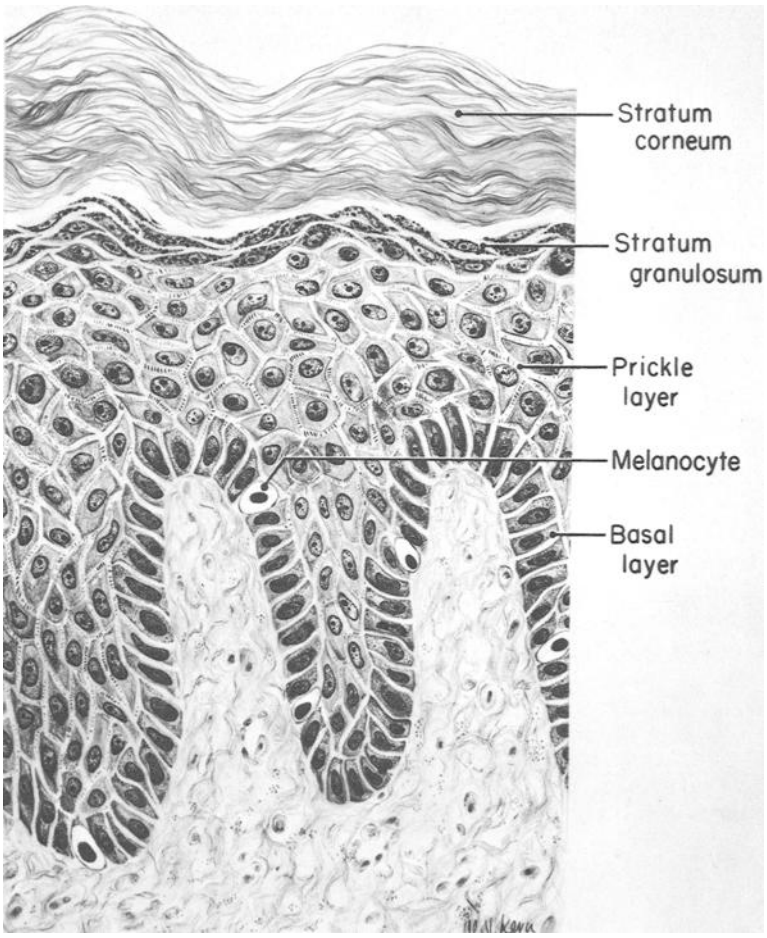

FIG. 1—*The epidermis.* (*Reprinted from Pillsbury et al* [17].)

The stratum corneum is the outermost section of the epidermis and consists of layers of dead keratinized, or horny, cells. These cells are flat, polygonal plates about 40 μm wide and less than 1 μm thick (Fig. 2). They contain water, melanin pigment, and a complex substance called keratin. It is the study of these horny cells, which are replaced from below while the surface cells are exfoliated, and their melanin granules which has led to a technique for the identification of skin fragments.

Investigational Studies

Methods of Cell Removal

In the routine procedure of exfoliative cytology (as used in gynecology and other medical fields for early cancer detection), specimens are taken directly from the area to be investigated either with an applicator or by aspiration. Such methods proved to be unfeasible in this study, particularly when human horny cells had to be removed from inanimate surfaces where few cells were present. The following techniques were found useful in our previous studies and, consequently, were adapted for this investigation.

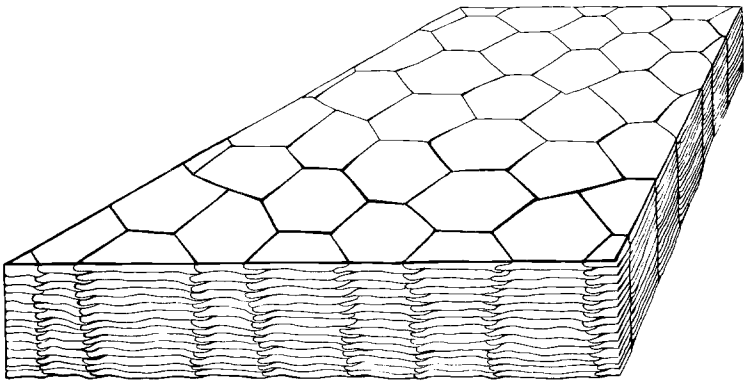

FIG. 2—*Schematic representation of cells of the stratum corneum. Average width, 40 μm; average thickness, 1 μm.*

Preparation of Slides

Adhesive slides—Goldschmidt and Kligman [*4*] recently developed an easy method for obtaining horny cells so that their large, flat surfaces are exposed for study. One drop of a nondrying, pressure-sensitive liquid adhesive mass is placed in the center of a microscope slide with a glass rod. Another slide is pressed down over the first, causing the mass to spread out into a thin film and resulting in two slides whose central areas are coated with adhesive. Then, the adhesive coated surface of one of the slides is briefly pressed to the surface being studied with a rocking motion. If applied directly to human skin, the slide provides a prepared specimen consisting of a monolayer of thousands of cells. The technique is markedly superior to scrapings and smears, which often result in slides that are unsuitable for cytologic examination owing to clumping and overlapping of cells.

The following two pressure-sensitive adhesive masses were found useful for preparation of adhesive slides: (1) long tacky 3M adhesive EC 791 and (2) permanently tacky synthetic rubber resin (Bondmaster P 554).[2] Slides coated with these adhesive masses can be prepared in advance and remain tacky for many weeks. If these two adhesives are not available, a simple adhesive mass can be prepared by diluting balsam with carbon tetrachloride or acetone; however, its tackiness is of short duration and slides must be used immediately after preparation.

Adhesive slides with double-coated tape—If regular adhesive slides cannot be prepared, readily available commercial double-coated tapes can be used, even though the results are not always as satisfactory as with adhesive slides. A 2-cm-long piece of ½-in.-wide cellophane tape coated on both sides with an adhesive mass (Scotch Brand double-coated tape, 3M Co., or Sears double-stick tape, Sears, Roebuck and Co.) is applied to the center of a microscopic glass slide. The slide is then applied to the surface and processed in a manner similar to that followed with an adhesive slide.

Under special circumstances a piece of tape may be first applied directly to the surface to be examined and then put down on a glass slide with the specimen side up. In this case, care must be taken to avoid fingerprints on the back side over the center of the tape.

[2] Samples of long tacky 3M adhesive EC 791 were supplied by the Minnesota Mining and Manufacturing Co., Adhesives, Coatings, and Sealers Div., St. Paul, Minn. Samples of permanently tacky synthetic rubber resin (Bondmaster P 554) were supplied by Pittsburgh Plate Glass Co., Adhesives Products Div., Bloomfield, N.J.

Horny cells from the examiner's fingers at the undersurface of the tape may confuse the unexperienced if the wrong surface of the tape is focused during microscopic examination.

Removal of Horny Cells from Inanimate Objects—Adhesive slides were touched to articles of clothing, door knobs, wooden clubs, and telephones to see if horny cells could be obtained. It was found that a sufficient number of cells was obtained from clothing that had been in direct contact with the skin, such as cotton underwear, shirt collars and sleeves, towels, and hatbands, but not from wooden objects or materials with a smooth surface.

Removal of Horny Cells from Skin Fragments—Several skin biopsies, 4 mm in diameter by 3 mm deep, were taken from volunteers to simulate conditions where only skin fragments are available for study. The adhesive slide method was employed every 3 to 4 days and good results were obtained for periods up to 2 to 3 weeks, at which time the skin fragments had become too dehydrated to be manipulated satisfactorily. It must be emphasized, however, that under normal conditions isolated human horny cells (in contrast to macroscopic fragments of hydrated skin which contain dermis and subcutaneous elements) do not deteriorate for many months and can be stained at any time.

Removal of Horny Cells from Human Subjects—Adhesive slides were applied to the extensor surface of the forearms of 25 subjects; Negroes, Caucasians, and Mongoloids. In addition, several subungual scrapings were spread onto slides. The scrapings were made both before and after subjects scratched the forearms of other subjects of different degrees of pigmentation.

Quantitative Determination of Melanosomes (Melanin Granules) in Human Horny Cells

In routine transverse sections melanin granules seem to be absent from the horny layer. Because of the extreme thinness of the sections analyzed, electron micrographs also often fail to show them. However, they do appear in surprising abundance throughout the horny cell when it is viewed as a flat, polygonal plate by the adhesive slide technique [*4*,*5*] or by other methods [*6*].

Fixation—Fixation is not regularly required in the cytologic study of normal horny cells. The transformation of a living, soft basal cell into a dead, hardened horny cell is an effective form of fixation. In any event, the routine fixatives, such as formalin and 95 percent alcohol, are acceptable. Ether and chloroform are to be avoided since they often dissolve the adhesive mass.

Staining—Staining of the melanin granules is achieved with either Masson's ammoniacal silver nitrate or Gomori's methenamine silver procedure [7]. The large horny cells, usually 40 μm in diameter, can be easily discerned under high dry magnification ($\times$400). Under oil immersion ($\times$900) the number of melanin granules can be easily counted or estimated.

Counting—Counts of melanin granules were made of 25 random cells and the arithmetic average was obtained. In some horny cells large clusters of granules were noticed, particularly in the center of the cells. Since exact counting was impossible in these cases, estimates were made of the approximate number of granules which occupied the ova of the cluster (Fig. 3).

Results

Cell Removal

Microscopic Specimens from Inanimate Objects—The largest number of cells could be removed from inanimate objects and skin fragments with the adhesive slide technique. Double-coated tape also gave satisfactory results and had the advantage of easy availability in laboratories which do not specialize in cutaneous exfoliative cytology. Poor

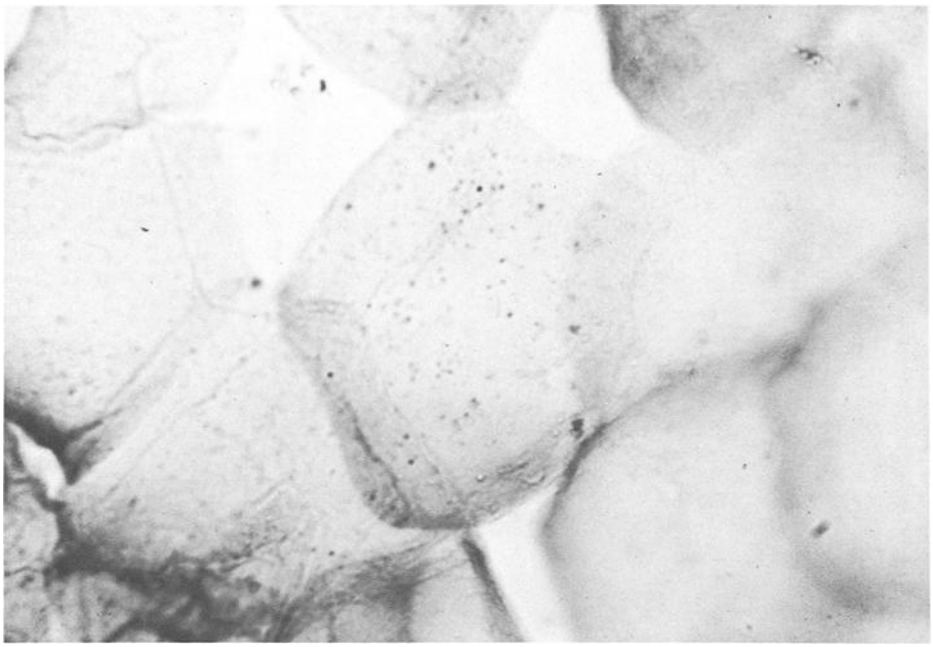

FIG. 3—*Melanin granules in horny cells of dark-skinned Caucasian* (×650).

results were achieved with scrapings that required subsequent smearing on slides prior to staining.

Skin Fragments—The removal of horny cells from small skin fragments was successful even when the fragment had been separated from the subject for as long as three weeks. At longer intervals, study was hindered only if the specimen had become sufficiently dehydrated to make physical manipulation difficult; the horny cells of interest are preserved for long periods of time. Successful examination requires only that the adhesive slide be applied to the predetermined surface area of the skin specimen with moderate pressure.

Human Subjects—Use of adhesive slides on forearm akin consistently produced monolayers of horny cells that were easily studied after staining (Figs. 3 and 4). Subungual scrapings and smears produced clumping and irregularly oriented cells that were difficult to study. No reliable information could be obtained by this method.

Correlation of Number of Melanosomes and Skin Color

The results of the investigation are summarized in Table 1. In addition to the obvious differences in the number of melanin granules, it was noted that cells from Negroes frequently showed central clustering of granules. The phenomenon was not found in Caucasians and occurred only occasionally in Mongoloids.

Discussion

Of the many factors responsible for variability in skin color, melanin content is perhaps the most important. Our quantitative studies show correlations between the number of melanosomes (melanin granules) and skin color. Other authors have investigated qualitative differences of melanosomes among different races using the electron microscope.

TABLE 1—*Correlation of skin color with number of melanosomes and amount of central clustering.*

Race	Melanin Granules per Cell		Central Clumping
	Average Number	Range	
Dark Negro	520	450–600	60%–95%
Light Negro	195	150–250	10%–30%
Dark Mongoloid	450	250–500	5%–20%
Light Mongoloid	205	150–300	rare
Dark Caucasian	67	25–125	none
Dark Caucasian (tanned)	195	100–230	none
Light Caucasian	6	2–12	none
Light Caucasian (tanned)	16	5–32	none

Szabo et al [*8*] found that, in the malpighian cells of the living epidermis, melanosomes in both Caucasians and Mongoloids are clustered in small groups surrounded by membranes, whereas the melanosomes of Negroes are singly dispersed. In the horny cells of the outer dead epidermis, however, the melanosomes of all races appear dispersed. Szabo et al also noticed that the individual melanosomes in Negroes are longer and wider than those in other races.

In our studies with the light microscope, several characteristics were apparent in the horny cells of Negroes which distinguished them from the cells of other races. In Negroes, in addition to the markedly increased number of melanosomes, the individual granules appeared larger and the melanosomes tended to cluster centrally within the cell. The granule size is possibly a genetically determined phenomenon. The central clustering is related to the fact that in the nucleated malpighian cell melanosomes cluster about the

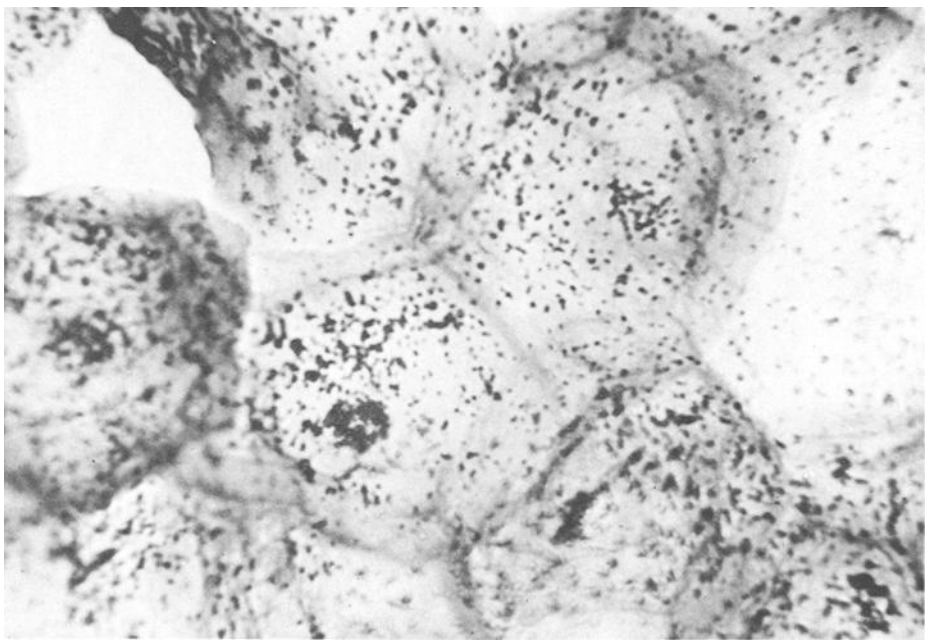

FIG. 4—*Melanin granules in horny cells of dark Negro* (×650). *Note central clustering of melanin granules.*

centrally located nucleus presumably to shield it from damaging ultraviolet radiation. Once the malpighian cell is transformed into a nonnucleated horny cell, the melanosomes still retain their original central orientation. Except for the central grouping, the melanosomes in the remainder of the cell are fairly uniformly dispersed (Figs. 3 and 4). This clustering is in part dependent on the relative number of melanosomes, appearing only when they are numerous (greater than 200). Another factor may be the ability of the granules to adhere to one another. The central clustering was not seen in any Caucasians and in few Mongoloids, despite relatively high melanosome counts in the latter.

The most easily discernible difference among horny cells was the abundance of melanosomes in Negroes and their scarcity in Caucasians (see Table 1). While Mongoloids are intermediate, their numbers more nearly approximated those seen in Negroes than in Caucasians. However, the individual granules in Mongoloids are noticeably smaller than those in Negroes, a point for differentiation.

Examination of exfoliated horny cells stained for melanin, then, provides a means for identifying race and degree of pigmentation of the person from which the cells were obtained. It is clear that in some cases, for example, the dark Negro or the fair-skinned Caucasian, determinations are easy. However, when melanosome counts are intermediate (100 to 400), judgments must be based on other factors. Frequency of central clustering is more suggestive of the Negro race. Small, but numerous melanosomes (>200) suggest the Mongoloid race. These factors are not always reliable and it is suggested that the examiner become familiar with preparations from known persons before unknown slides are studied.

The described technique allows determination between dark and light skin color of unknown persons from minute skin specimens whose color cannot be determined with the naked eye alone or where doubt exists that the examined specimen is even human skin. Probably of even greater practical importance is the fact that it is possible to determine the skin color of unknown persons from cells collected from certain rough-surfaced objects with which they have had prolonged contact. Cells could be detached with ease from clothing (undershirts, shirt collars, sleeves), scarves, hatbands, and towels. Not surprisingly, cells were less easily obtained from smooth objects (doorknobs, telephones, etc.). Furthermore, one would not expect much information from cells obtained from the palms since this area is only lightly pigmented in all races.

Examination of subungual scrapings after scratching skin of different color showed cells with different melanin content. The method is admittedly poor because of the technical difficulty in obtaining horny cells in a good orientation for study.

Summary

A new method is described for studying trace amounts of human skin in order to help identify race and degree of pigmentation. The easily performed technique involves the use of adhesive slides, a stain for melanin, and a light microscope. It requires only small skin fragments or, in fact, merely horny layer cells which can be collected from articles of clothing that have been in contact with the subject's skin. Positive conclusions are feasible in cases involving dark Negroes or fair-skinned Caucasians, but results are less certain in cases of Mongoloids, light Negroes, and deeply tanned Caucasians.

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