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An Estimate of the Melanocyte Mass in Humans

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The size of the melanocyte system in humans was estimated as though all active melanocytes in the body were assembled in a single compact organ. Our estimates indicate that the epidermal melanocytes constitute the dominant part of the "melanocyte organ." In an adult human not recently exposed to sunlight, the functionally active epidermal melanocytes form a tissue 1.0–1.5 cm^3. Other melanocytes, such as those in the mucous membranes, the follicles, and the eyes, constitute only a small proportion of the total melanocyte cell mass.

During the last decade it has become possible to identify several metabolic products originating from melanocytes [1–3]. Generally these substances have been considered as inactive "side-products" of the pigment synthesis, but they may well have biologic effects outside the immediate vicinity of the synthesizing melanocyte. The recent finding, that repeated UV irradiation of a restricted skin area induces delayed melanocyte proliferation in shielded skin areas, may be an example of such remote control via circulating factors [4]. An analogous phenomenon could be the delayed increase in serum concentrations of 5-S-cysteinyldopa following PUVA (psoralen + UVA) treatment of a limited skin area in humans [5].

With this background it seemed interesting to obtain some idea of the total size of the active melanocyte system. This is difficult to achieve intuitively, because the melanocytes are dispersed as individual cells. We have tried to estimate the size of the system as though all pigment-producing melanocytes in the body were assembled in a single compact organ. This approach should allow more concrete comparison with other organs and might also give an indication of the size of melanoma metastasis that can be detected by measurements of biochemical markers in the urine of patients with melanoma [6,7]. We concentrated our calculations on the epidermal melanocyte population, because a rough estimate indicates that the epidermal and follicular melanocytes constitute the overwhelming proportion of the pigment-forming melanocytes in the body [8–11].

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MATERIALS AND METHODS

Epidermal Melanocyte Population Density

The number of epidermal melanocytes per mm^2 was estimated in dopa-incubated split-skin preparations [12,13]. A total of 20 skin biopsies were taken from the buttock, arm, back, thigh, face, and genitals of 18 male donors aged 25–46 (mean 31) years. The donors had not been exposed to tanning sunlight for 5 months prior to obtaining the biopsies. In each preparation melanocyte counts were made over 10 square ocular fields using objectives 25× or 40× [12].

The proportion of the skin surface covered by melanocytes was estimated in the same preparation by a random sampling procedure, using a 40× objective and an ocular plate containing 25 randomly distributed spots arranged in a circle (G 52, Chalkley Point Array, Leitz). With this plate the number of spots superimposed on melanocyte bodies was counted over 10 randomly selected fields in each preparation. The proportion of positive points gives a direct measure of the relative skin area covered by melanocyte cell bodies.

To estimate the overall change in size of the split-skin preparation, we compared the diameters of the mounted split-skin specimens with the diameter of the biopsy punch (3.0 mm). The mean diameter of the mounted specimens was 3.2 mm (2.9–3.4 mm), indicating that slight overall swelling of the preparation had taken place.

The Size of the Epidermal Melanocyte

The typical epidermal melanocyte is roughly spindle-shaped, with the long axis parallel to the basal lamina of the epidermis. We estimated the mean volume of the cell by measuring 3 axes: the length and breadth in the plane parallel to the surface, and the height at right angles to this plane. The two first-named measures were obtained from dopa-incubated split-skin preparations. The height was measured in the skin sections taken from the same specimens. These specimens were not dopa-incubated, but immediately fixed in 3% glutaraldehyde, embedded in Epon, and 3-μm sections were cut. An ocular micrometer was used for all measurements. The mean size of the melanocytes from

each donor was calculated from the formula for the volume of an ellipsoid, viz. $\frac{4}{3} \pi \times r_1 r_2 r_3$.

Follicular Melanocytes

The numbers of melanocytes in the follicular epithelium and the bulb of the scalp hair were counted in 28 anagen follicles from 3 people with warm-blond hair and aged 3, 35, and 60 years. The specimens were dopa-incubated and embedded in JB-4 plastic (Sorvall), and 3-μm serial sections were cut. To avoid double counts every third section was first studied, and the intervening sections were then screened for any missed cells. For the hair bulb melanocytes the longest axis was measured together with the maximum diameter at right angles. The third diameter of the cell body was taken as the mean of the two measured diameters, and the volume was calculated as for epidermal melanocytes.

RESULTS

Epidermal Melanocytes

Two procedures were used to assess the size of the epidermal melanocyte system.

Method I is based on estimates of the epidermal melanocyte population density, measurements of the melanocyte cell volume, and calculation of the area of the skin surface. Extensive counts of the melanocyte population density in the epidermis of different parts of the body have been published by Szabó [12], but he gives no figures for the overall change in size of the preparation during the histologic procedure or for the volume of the individual melanocytes. We therefore repeated the measurements in split-skin preparations from a few specimens. The results are summarized in Fig 1, with correction for changes in size of the individual biopsy specimens during the histologic procedure. In most cases there was a small increase (mean 12%) in the area of the mounted preparation, which may explain why our corrected figures are slightly higher than those of Szabó [12]. Apart from this, the density pattern was similar in both studies, with high counts in skin from face and genitals and low counts from arm and trunk. Considering the relative size of the different skin regions, a weighted mean value for the melanocyte population density would be 1000–1500 melanocytes/mm^2 (Fig 1).

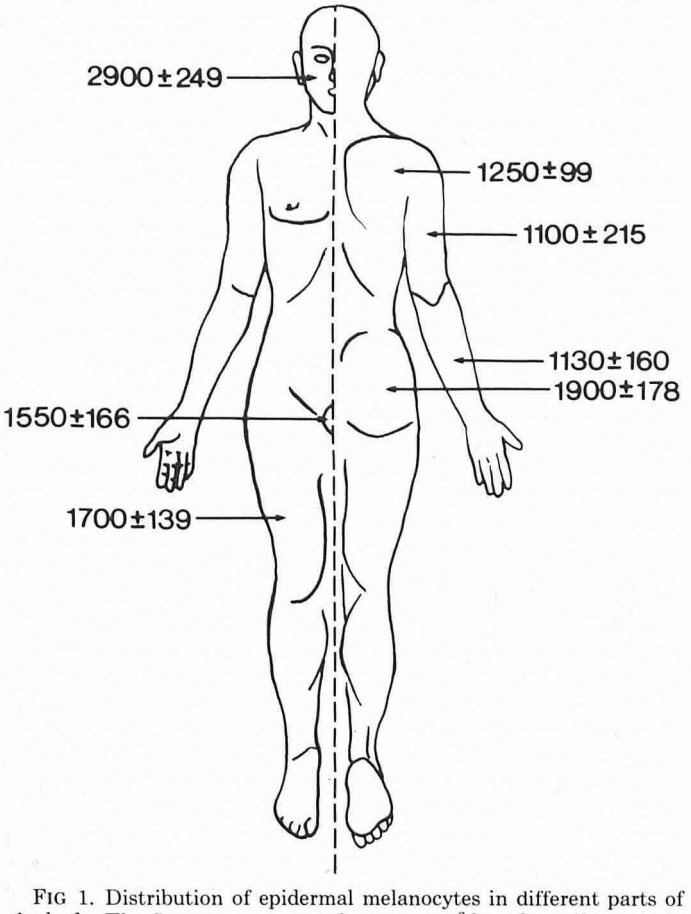

FIG 1. Distribution of epidermal melanocytes in different parts of the body. The figures are mean values per mm^2 based on all counts in 10 fields (0.16 mm^2 each, × 25) from 1–5 biopsy specimens ± SEM.

The cell body of most melanocytes is round to spindle-shaped, or roughly ellipsoid. To calculate the volume, we measured the length and breadth of 125 typical melanocytes in split-skin preparations, and the height of 55 cells in Epon sections prepared from the same skin (see *Materials and Methods*). The mean axes were: 12.5 ± 0.3 μm (SEM), breadth 7.9 ± 0.2 μm (SEM), height 10.4 ± 0.4 μm (SEM). These figures give a mean volume of 537 μm^3 for the cell body of the epidermal melanocyte. The figures are given without compensation for possible swelling or shrinkage of individual cells during the histologic procedure (see *Discussion*). The dendrites seem to add little to the total volume of the cells. Their size was estimated for 20 melanocytes in the split skin. The cells had 2–6 primary dendrites with a mean assembled length of 41 ± 2 μm (SEM). Assuming a mean diameter of 1 μm, the total dendritic volume will be 32 μm^3. This figure may be somewhat underestimated, since the vertical extension of the dendrites was difficult to measure. Therefore, it seems reasonable to approximate the mean melanocyte volume at 600 μm^3. A melanocyte density of 1000–1500 cells/mm^2 and a cell volume of 600 μm^3 gives a total melanocyte volume of 6×10^5 to 9×10^5 μm^3/mm^2. With a body surface of 1.8 m^2 for an adult male [14,15], the total epidermal melanocyte volume will be 1.1–1.6 cm^3. A comparable value of 1.2 cm^3 is obtained if Szabó's figure for the total epidermal melanocyte population of a 24-year-old donor is used (2×10^9 cells) [12].

In Method II we used a random-spot pattern to estimate the proportion of the skin surface occupied by melanocytes (see *Materials and Methods*). The same split-skin preparations were used, but this method was tried because it is simpler and faster than actual cell counts and is also less sensitive to swelling or shrinkage of the preparation. The method gives percentage values closely correlating with the melanocyte population density, as can be seen in Fig 2 in which the proportion of the skin surface covered by melanocytes is plotted against the cell density in the same preparation. Values between 9–14% were found for most specimens from large skin areas such as the trunk and extremities, whereas most higher values originated from genital and facial skin. For an average adult the projected area of epidermal melanocytes will thus be 0.16–0.25 m^2. Since the height of the cells was 10 μm in the sectioned material, the total melanocyte volume, corrected for the ellipsoid form of the cell, will be 1.1–1.6 cm^3. These values tally with those obtained by method I, which is reassuring, because only the height of the melanocytes was used as a common measure in the two calculations.

Melanocytes of the Hair Follicle

We suspected that the relatively large melanocyte of the hair bulb would contribute significantly to the total melanocyte volume (cf. [16]). We therefore counted the melanocytes in anagen follicles in 3 specimens from the scalp. A few melanocytes were found in the follicular epithelium but most melanocytes were situated along the upper border of the dermal papillae. These melanocytes contained large numbers of melanosomes, and the same was true also of the surrounding cells. Despite dopa incubation and the thin plastic sections, it was therefore sometimes difficult to differentiate between melanocytes and other cells of the bulb. This may explain in part the large variation in the number of melanocytes per follicle (range 26–94), but is of little importance for the conclusions (see

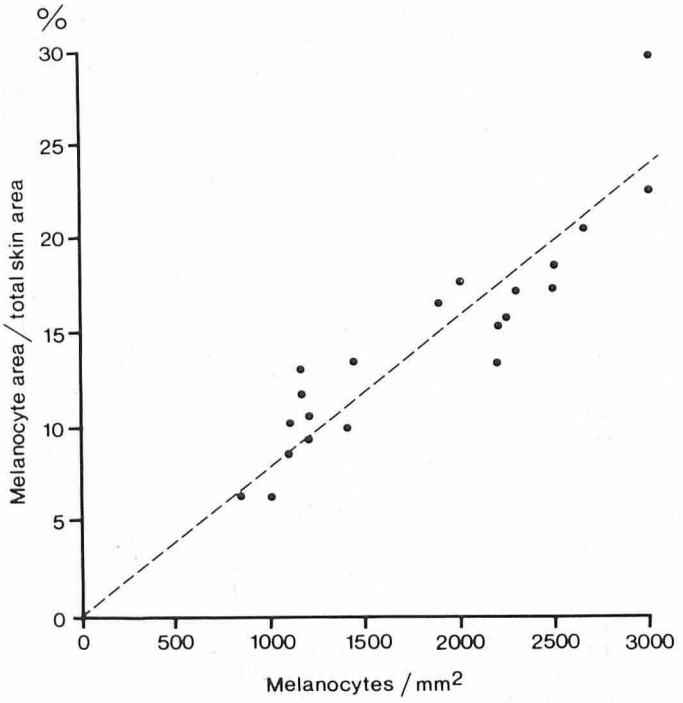

FIG 2. Relation between cell counts and random-dot estimates of the epidermal melanocyte population. The proportion of skin surface occupied by melanocytes is plotted against the population density in the same biopsy specimen.

below). The overall mean number of melanocytes per follicle in these specimens was 54 ± 4 (SEM).

The follicular melanocytes were larger but similar in shape to the epidermal ones. Only two diameters could be measured in our sections, and their mean values were 14.5 μm ± 0.3 (SEM) and 12.6 μm ± 0.2 (SEM) for 20 cells. The third diameter was taken to be 13.6 μm, the mean of the other two axes. This gave a cell volume of 1300 μm^3. If 90% of the 100,000 scalp hairs [17] are anagen hairs [18], this gives a total follicular melanocyte volume of 0.006 cm^3 for the scalp. Since the density of hair follicles is much lower for most of the body, and only a minor fraction of these follicles are in anagen phase, it is clear that follicular melanocytes will add, at most, a few percentages to the epidermal melanocyte volume. This is less than the uncertainty level in the calculation of the epidermal melanocyte volume. The contribution of follicular melanocytes may therefore be disregarded.

DISCUSSION

Our results indicate that the active epidermal melanocytes of an average-sized Swedish man form a tissue mass of 1.1–1.6 cm^3, if assembled in a compact organ. This calculation involves several approximations. The first problem concerns the validity of our estimates of cell density and volume. It is well known that tissue samples may change considerably in size during histologic procedures, and such changes may affect both cell density counts and measurements of volume. To compensate for this, the overall change in size of the specimens during the preparation was measured, and the population density was corrected accordingly. Nevertheless, it is difficult to estimate the total melanocyte population from biopsy specimens, because the cell density varies considerably among different regions of the body and also among individuals. The high-density regions, viz. face and genitals, constitute only small parts of the skin surface however, and it is probable that the true weighted mean value for the melanocyte population density is somewhere between 1000–1500 cells/mm^2, the values used in the calculations. We stress that these figures refer to the enzymatically active melanocytes in the skin (cf. [19]).

The cell volume estimate is more critical. Firstly, the cell body was regarded as an ellipsoid, which probably gives a slight overestimation of the cell size. A survey of a large number of melanocytes convinced us that the ellipsoid formula represents the best approximation of the melanocyte pericaryon. Secondly, there was a small increase in size of most split-skin preparations used for cell measurements. There is no easy way to determine whether the size of the individual melanocyte changes in proportion to the overall change in the specimens. As the melanocytes showed no apparent signs of shrinkage or detachment from surrounding cells, it is possible that they too were somewhat enlarged. We did not correct our measurements for this possible enlargement, since it apparently compensates for another source of error. The two cell axes measured in the split-skin preparations were in fact the projection of the length and breadth toward a plane parallel to the skin surface. Since the long axis of the melanocytes usually lies parallel to the undulating basal lamina, this projected length represents a small underestimate, roughly of the same size as the possible enlargement of the cells during the histologic preparation.

The dimensions of the melanocytes measured by us are smaller than those given by Clark and Bretton [20]. Unfortunately, they do not explain how they arrived at their values for the melanocyte axes. For several reasons we do not believe we have underestimated melanocyte axes by as much as 20%, as would be required in order to equate our measurements with those of Clark and Bretton. There was no significant difference in the longest axis obtained in split-skin preparations or in the sectioned material prepared by a gentler histologic procedure (see *Materials and Methods*). For comparison we also measured 20 melanocytes in earlier electron microscopic material from the densely populated foreskin [21]. The mean volume of these cell bodies was 460 μm^3, i.e., somewhat smaller than in the present study. Furthermore, the close agreement between the estimated size of the melanocyte system, whether obtained by cell counts or by largely independent random-dot procedure, indicates that there were no major systematic errors in our calculation.

Despite the problems of estimating the size of the melanocyte system, the uncertainty should not be overemphasized. In fact, the errors in our calculation seem to be small compared to possible changes in the melanocyte system due to external factors. For example, repeated exposure to sunlight may increase the active epidermal melanocyte population by a factor of 2–3 [22,23]. Of course, the size of the system will also differ among individuals of different sizes; for instance, the skin surface increases about 8 times from birth to adulthood [14]. Even among adults there is a difference of 30% between the skin area of a man at the upper 66% percentile level and a woman at the lower 66% percentile level [14]. There is also a small gradual decrease in the melanocyte population density with age [23]. It should therefore be noted that our calculation is based on figures for skin of young Swedish men of average size.

In addition to the epidermis, melanocytes can be found in the mucous membranes, the eyes, and occasionally also in other organs of the body. There is little quantitative data on extraepidermal melanocytes, but a rough calculation suggests that they contribute only marginally to the total size of the "melanocyte organ" [8–11]. The melanocyte population density in the nasal and oral epithelium seems to be comparable to that in the epidermis [12] while only few melanocytes are found in the vaginal mucosa [10]. The total surface area of these mucous membranes is only a few percentages of the body surface. Therefore this population contributes little to the total melanocyte volume. A similar argument applies to the melanocytes of the eyes. Even though these cells form a dense cell layer, owing to the relatively small size of the eyeballs their total volume is less than 1% of the epidermal melanocyte volume. The occasional extraepidermal melanocytes in other organs seem to be even less important from a metabolic point of view,

because in most cases their pigment production is very low or absent [10,11]. This argument does not apply to the hair bulb melanocytes, which can produce melanin at an even higher rate than the epidermal melanocytes. Nevertheless, our calculation indicates that the total cell mass of the follicular melanocytes is at most a few percentages of the value for the epidermal melanocytes. Accordingly, the metabolic rate of the follicular melanocyte system would have to be very high in order to influence significantly the serum levels of melanocyte metabolites. Numerous and/or large nevi may contribute substantially to the size of the "melanocyte organ," a fact which also must be considered in metabolic studies of the melanocyte system.

The present study was motivated in part by our interest in the possibility of diagnosing early melanoma metastases by measuring biochemical markers [6,7]. The most useful metabolite in this respect to date has been 5-S-cysteinyldopa. After several years of experience we now feel we can suspect spreading of the melanoma when the urinary excretion of 5-S-cysteinyldopa is 1.5–2 times greater than the mean value for normal persons [7]. Our present calculation of the size of the "melanocyte organ" indicates that doubling of the excretion level may occur with a melanoma metastasis about 1 cm^3 in size. More metabolically active melanoma metastases may be detected even earlier.

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