THE CIRCULATION OF MELANIN—ITS CLINICAL AND PHYSIOLOGICAL SIGNIFICANCE

REVIEW OF LITERATURE ON LEUCOCYTIC MELANIN TRANSPORT

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When Robin[1] first used the term melanin in 1893 to describe a group of pigments ranging from yellow to black,[2] neither its chemical composition nor its cellular origin were known. In retrospect, it can now be seen that the *circulation* of the pigment was actually the first described, but in terms of existing knowledge at that time, the transporting cells were considered to be melanin-producing cells and pigment synthesis was thought to be derived from haemoglobin degradation. When in the late 1930s the origin of melanocytes could be traced, by elegant methods, to the neural crest in amphibians,[3] birds[4] and mammals[5] and the biochemical studies, strengthened by Bloch's dopa-reaction,[6] confirmed its sole production by melanocytes, a cell-type *sui-generis*, interest in the circulation of melanin soon disappeared. At the time of publication of our first report suggesting a transport of melanin[7] the only direct reference to be found to a possible circulation of melanin was the study by Jacobsen and Klinck[8] in 1934. Recent developments in the field of pigmentation research have now enhanced the importance of the concept of a circulation of melanin by the demonstration of its free radical properties,[9-11] its relationship to mitochondria,[12-15] and the realization that pigmentation in the substantia nigra and locus coeruleus may be related to extrapyramidal function.[16,17] The circulation of melanin should also be considered in relation to the pigmentation which follows on long-term phenothiazine therapy.[18-20]

Not yet fully explored is the possibility that racial differences in disease incidence may be related to differences in pigment biology and its control.[21]

HISTORICAL DEVELOPMENT OF THE CONCEPT

One of the earliest studies relating melanin production to cellular function, was that of Aeby[22] in 1885, who suggested that wandering cells (leucocytes) phagocytosed erythrocytes and transmitted the resulting pigment to epidermal cells as nutritive material. This view was shared by Meyerson,[23] and by List[24] in 1890. The latter, in demonstrating a dense accumulation of pigment-containing cells around blood-vessels coursing through the dermis in various melanoses and melanodermas, interpreted these findings as leucocytes collecting pigment from blood-vessels en route to the epidermis. His evidence for transformation of haemoglobin to melanin derives from studies by Langhans on the resorption of extravasated blood in tissues and its degradation in macrophages. Rabl[25] in 1896 regarded pigment cells as modified connective tissue cells, while epidermal chromatophores were still considered to be leucocytes. When Schuberg[26] in 1903 described pigmented leucocytes in the epidermis and Schmidt[27] (1920) and Elias[28] (1931) described the phagocytosis of pigment from epidermal cells by leucocytes, they, for the first time, clearly indicated that they thought the leucocytes to be unrelated to the pigment-producing cells of the epidermis.

Another early study referring to melanophagocytosis was that of Metchnikoff,[29] who described phagocytes engulfing pigment from human hair. On this evidence he believed it possible to turn grey overnight. The branched phagocyte may equally well have been a melanocyte donating pigment to the hair.[102]

At an approximately synchronous time with these tissue observations on melanophagocytosis, haematologists reported melanin-containing leucocytes in the blood of various animals. Freidsohn,[30] in 1910, reported pigmented leucocytes as forming 0·7% of the leucocytes in cardiac blood of adult *Bufo esculentia*, while Jordan reported[31,32] and illustrated in colour[33] such pigmented leucocytes. He advanced evidence that a source of pigment is contained in the resorbing yolk of the egg mass of female toads in winter.[32] This yolk mass was invaded by capillaries and connective tissue and a large number of cells resembling large lymphocytes, representing chiefly migratory elements, begin to function as monocytes ingesting the free pigment granules, and as pigmented leucocytes they enter the circulation. Their ultimate fate is largely to disintegrate within the spleen.[32] This first description of a concept of circulating melanin was only preceded by List's description in 1890 indicating that the brown pigment in trout embryos was transported by leucocytes to nearby tissues.[24] Due to the fact that melanocytes were not recognized as such, one is left in doubt as to whether or not he was describing melanocyte migration or actual leucocyte-transport. This difficulty is illustrated by the modern description by Colle-Vandevelde[34] of star-shaped melanocytes migrating over the extra-embryonic differentiating blood-cells in teleost (*Blennius gattoruginae*) embryos. Freidsohn[30] interpreted his findings as evidence for the development of granulocytes from lymphocytes, while Jordan[31-33] erred in ascribing melanin production to leucocytes when he refers to Prenant's view that the pigment was a degradation product of haemoglobin.

Alder and Huber[35] in their early review discussed pigmented leucocytes under the heading of 'Melaninzelle'. Emphasizing this as a rare cell-type, they quote Kiyono and Nakanoin to the effect that these cells represent blood histiocytes (monocytes) with ingested pigment. Among other studies reporting circulating leucocytes with ingested pigment are those of Wood,[36] reporting such leucocytes in the blood of the gecko (*Tarentola mauritania*) and Wertzberg[37] in the blood of the chameleon (*Chameleo dilepis dilepis*).

Lewis,[38] in a study on the origin of phagocytic cells in the lung of the frog, utilized the transparency of the distended amphibian lung for *in vivo* observations, and reports on the ingestion of carbon particles. She identified the pigment in these cells as melanin. 'The reptile and amphibian, also some fish, differ from the higher forms in that there is present in the blood-stream an occasional

pigmented leucocyte. This cell is about the size of a mononuclear leucocyte and contains melanin granules. There may be several masses of them, almost completely filling the cytoplasm of the cell. These cells may be readily differentiated from cells carrying carbon . . .'[38]

Pigmented leucocytes were reported twice in the blood of man in the past century—in 1929 by Liebman[39] in a patient with malignant melanoma and more recently (1961) by Goodall *et al.*[40] The latter differentiated the pigment from lipofuscin.

After Jordan's brief suggestion, the first definitive delineation of the concept of melanin circulation was made by Jacobsen and Klinck[8] in 1934. They were apparently unaware of the studies on pigmented leucocytes in the peripheral blood and favoured a transport in colloidal form as melanin 'has rarely if ever been found in leucocytes'.

THE CONCEPT IN PERSPECTIVE

The concept that melanin circulates through the body, has received very little attention. Thus in Rothman's textbook on the physiology of the skin[41] (Chapter by Lorincz) this is mentioned in passing to the effect that the main loss of melanin is in desquamated epidermis, but that some melanin may be circulating. It is obvious that the amount of melanin lost by desquamation should balance the amount produced to maintain a steady pigmentary state. In hyperpigmented individuals who, however, have the same number of melanocytes as lighter individuals,[42] (and this applies to racial pigmentation as well),[43] either greater desquamation should occur to maintain a steady state in the face of increased activity of these cells, or the balance of melanin produced should circulate internally. Since the studies referred to above either studied highly pigmented lower animals or Whites with melanoses, it would suggest that the circulation of melanin becomes quantitatively more important in those cases where there is a high rate of melanin production. This also confirms, in a wider sense than originally intended, Lerner's law of pigmentation:[44] 'skin areas normally hyperpigmented are more subjected to change either in the direction of further hyperpigmentation or depigmentation'. This may be interpreted that the normally hyperpigmented areas are physiologically and kinetically more active than normally lesser pigmented areas. The circulation of melanin should therefore be studied in hyperpigmented individuals.

Since we are concerned with human pigmentation only, further discussion is directed only to available human studies. The circulation of melanin in amphibians, reptiles, and especially invertebrates, could easily be demonstrated. The evolutionary relationship is briefly appended here to illustrate the generalized occurrence of this biological mechanism:

Melanocytes were demonstrated in a skin fragment of 150 million-years-old fossil *ichtyosaur*.[45] In sponges, coelenterates and flatworms, free wandering cells thought to be analogous to the vertebrate macrophage are found in the absence of a vascular system.[46] Phylogenetically therefore the circulation of melanin would appear to be older than the circulation of blood. 'Blood'-cell types, distributed through the echinoderm-phylum, included the following cell-type: 'cells with spherules which may be colourless, red, green, yellow or brown'. Liebman[47] classifies the latter under trephocytes—in distinction from lymphoïdocytes which phagocytose—and eliminates noxious material. He states that these cells:[47] 'frequently contain coloured material which is assumed to have some relation to respiration. So far this has only been established for echinochrome. The pigment of trephocytes is often directly or indirectly responsible for the animal's colouration.' As discussed elsewhere[48] the pigmented leucocytes in peripheral blood of amphibians and reptiles were recently confirmed and deposition of melanin in liver, spleen, lung and myocardium, approximately in this order of intensity, could be confirmed[48] as well.

EVIDENCE FOR HUMAN CIRCULATION OF MELANIN

In a study of the inflammatory reaction in Bantu and Cape Coloured persons the acquisition of melanin by leucocytes could be studied, and especially striking was the acquisition by lymphocytes even before starting their subsequent hypertrophy to macrophages.[7,49,50] Subsequently we have been able to present a line-diagram for melanin-labelling in relation to cell-type,[50] to study the formation of LE cells in such a response,[51] and from the melanophagocytosis could deduce that lesser-lobed neutrophils are apparently phagocytically more active than the more segmented neutrophils. This suggests a progressively decreasing phagocytic ability of neutrophils in relation to time, so that, at the stage of mononuclear invasion, this function is apparently passed on to these cells and macrophages.

Lymphocytes have been shown to re-enter the circulation.[104] They constitute 1 - 4% of the cells in the normal epidermis of rats[52] and man.[53] The melanin-containing lymphocytes would thus map the route of re-entry and should also be found in the peripheral blood.

The route of re-entry via lymphatics has for long been traced by the melanin-deposition in lymph nodes in lipomelanic reticulosis,[54] and recently the finding of melanin deposits in lymph nodes of normal Bantu has been mentioned[54] and studied.[55] This is quite common (Table I).

On this evidence pigment-containing leucocytes were searched for in the peripheral blood of normal Bantu males and Bantu patients without melanoma. In leucocyte-concentrates such pigment-containing mononuclear cells could be demonstrated and the pigment identified as melanin.[48] This finding,[48] apart from confirming the circulation of melanin, also confirms the re-entry of lymphocytes,[104] after a sojourn in the tissues.

Further support for the concept that melanin circulates in the human, has been presented in the findings of Ishizaki and Belter[56] that melanin deposition occurs in the placenta, irrespective of race, but more frequently in those women who had a history of skin injury during pregnancy.

The finding of melanin deposits in bone marrow[57-59] in malignant melanoma may similarly be related to a circulation of melanin, as well as the finding of melanin in sweat glands,[60] lacrimal glands,[61] and in the labyrinth of the ear.[62]

Melanokinetics

According to modern concept, melanin-production is the prerogative of melanocytes, a cell-type *sui-generis*.[63]

TABLE I. AVAILABLE QUANTITATIVE DATA ON MELANOKINETICS IN WHITES AND BANTU

A. *Circulatory data.*		
Lymph nodes	*Whites*[103]	*Bantu*[55]
Inguinal	26%	85%
Cervical	10%	44·5%
Axillary	20%	90%
Abdominal	—	4%
(Note higher melanin content in skin-draining glands as compared to abdominal glands.)		
Placenta	*Whites*[56]	*Negro*[56]
Melanin deposits	$\frac{21}{61}=33\%$	$\frac{64}{149}=43\%$
In cases with chronic skin lesions during pregnancy:	$\frac{7}{8}=88\%$[56]	$\frac{18}{21}=86\%$[56]
Authors remark that relationship to iron and calcium deposits 'suggests more than casual relationship'.		
B. *Melanin-containing leucocytes.*	*Whites*	*Bantu*
1. *Inflammatory reaction*		
(*a*) Normal individuals	Inconspicuous[7]	Marked[7, 50]
(*b*) In phenothiazine melanosis	Marked[99]	—
2. *In peripheral blood*	(i) Not demonstrated in normals[48]	(i) Occasional mononuclears demonstrated in *normal* subjects[48]
	(ii) Twice past century in melanoma[39, 40]	—
	(iii) Demonstrated in phenothiazine melanosis[99]	—
C. *Reticulo-endothelial activity data:*		
Gammaglobulin*	0·8 ± 0·24 G/100 ml.[105] 0·93 ± 0·114 G/100 ml.[106]	1·4 ± 0·30 G/100 ml.[105] 1·57 ± 0·34G/100 ml.[106]
D. *Adrenocortical activity data:*†		
17-ketogenic steroids	14·7 ± 3·3 mg./24 hr.[95]	6·8 ± 2·6 mg./24 hr.[95]
Inverted neutrophil-lymphocyte ratio	9%[67]	54%[67]
17-OHCS, postoperative increase		Ca. 30% less than in Whites,[97] studied under similar operative procedures by same authors.

*Reviewed by Schür[51]
†More extensively reviewed by Wassermann[65]

In his studies on pigment distribution and melanocytes in normal human skin, Ikeda[64] has found very little or no extracellular pigment in the stratum Malpighii, but there was less pigment within melanocytes than in other cells. His finding was summarized in an abstract as follows:

Intracellular melanin pigment in stratum Malpighii >> dermal melanin pigment >> melanin pigment in melanocytes > extracellular melanin in stratum Malpighii → O.

He considered the amount of melanin in the skin to be better correlated with the *activity* of pigment formation of melanocytes and the *ability* of uptake and retention of melanin by Malphighian cells than with the count of melanocytes. This view is supported by Szabo's demonstration that the number of melanocytes is not significantly different between Whites, Negroes and albinos.[42, 43]

It corresponds with Mason's view that melanocytes are unicellular glands secreting melanin which is transferred by 'cytocrine activity' to nearby cells.[65] Such a cytocrine transport of melanin granules occurs between leucocytes as well.[7]

Considering a steady state of pigmentation melanin production, transcellular migration of granules and the removal of melanin should be in balance. If production exceeds removal, darkening should occur. If retention in melanophages exceeds removal, hyperpigmentation would occur locally, but even in the face of a high production excessive removal may cause depigmentation, e.g. vitiliginous areas in Addison's pigmentation.[44] It can thus be seen that areas normally hyperpigmented may change more readily in either direction depending on whether or not removal by melanin transport is correspondingly hyper-, normo- or hypoactive.

Most environmental factors influencing leucocyte counts operate through changes in the adrenocortical activity.[66] We were able to confirm, under controlled conditions of diet, altitude and socio-economic status in a malaria-free area, the higher lymphocyte count of the Bantu compared to Whites.[67] A review of the literature indicates lesser adrenocortical activity in the Bantu as the most likely factor involved.[68] This finding has also been substantiated in studies on American Negroes,[69] Australian Aborigines[70] and Indians.[71]

In hyperthyroidism, Addison's disease and cachexia, hyper- or hypopigmentation occurs with an increase in circulating lymphocytes. Increased ACTH excretion has been demonstrated in hyperthyroidism with hyperpigmentation[72] and also in Addison's disease[73] in which pigmentation may be the first and only manifestation of the disease.[74]

Recently McGuinness[76] was able to show that conditions where a high pituitary output of ACTH can be expected are associated with increased blood levels of MSH, while Dahlberg[75] suggested urinary MSH determination as an index of pituitary function. Therefore melanocyte activity, peripheral leucocyte count and reticulo-endothelial activity[77, 78] may be regulated by anterior pituitary activity. Evidence for greater RES activity in the Bantu may be found in the increased phagocytic ability for malaria pigment,[79] the hypertrophic lymph nodes[80] (which often contain melanin[55]) in normal Bantu, and the increased γ-globulin in the serum (reviewed by Schür[51]).

This view has been presented diagrammatically in Fig. 1 showing simultaneous pituitary control of RES function, leucocyte counts and melanin production. While qualitatively similar in both White and darker races, the quantitative setting of this system may be regulated by adrenocortical sensitivity to ACTH. This may be genetically determined at the enzyme-level where phosphorylase requires activation by 3'5'AMP generated in the hexose-monophosphate shunt, for cortisol synthesis.[82]

Available quantitative data on melanokinetics are summarized in Table I.

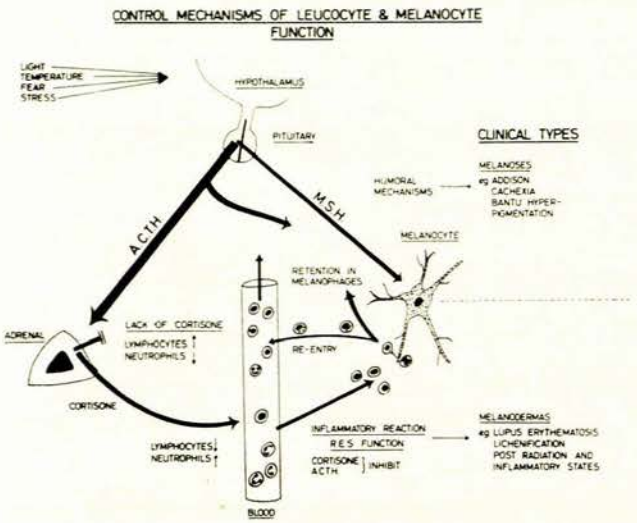

Fig. 1. Diagrammatic presentation of interrelationship between pituitary and adrenocortical control of leucocyte pattern, reticulo-endothelial and melanocyte function. Feedback-inhibition of ACTH and MSH secretion[76] by cortisol is not shown.

The Circulating Granule

Studies on the melanin granule and its ontogeny and properties show a potent structure. The melanin granule exhibits 3 stages in its intracellular development.[12] Stage I consists of the biosynthesis of polypeptides on the ribosomes of the melanocyte and its possible condensation with phospholipids in the Golgi vesicles. In stage II protyrosinase is arranged in structural form on intermediate vesicles and hence on premelanosomes. At this stage the metabolic block in albinism is present.[12] The final stage III consists of the melanization of melanosomes by tyrosinase and ultimately, after full melanization, no tyrosinase is detectable on the melanosome.[12] According to the studies of Woods *et al.*[13] the melanosomes may be identical with mitochondria since the enzyme systems detectable in these bodies are similar to those of mitochondria. Then the earlier stages of melanosome development[12] would indicate the ontogeny of mitochondria.[13] Genetic studies have indicated that the three components of melanin granules are apparently regulated by genetic loci on three different chromosomes,[15] and this could explain the intricate relationship of pigmentation defects with other and associated genetic defects. The RNA-rich granule which circulates, is thus endowed with great potentialities due to its unique structural and enzymatic characteristics.

Of more immediate importance to clinical studies would be the work on melanin which has defined its free radical properties.[9-11,17] These are very similar to properties found in mitochondria.[16] Metal-binding is its most important characteristic and up to the present, zinc,[83] copper,[83,84] iron,[84] manganese,[85] titanium,[86] cobalt,[86] nickle[86] and molybdenum[86] have been localized in one type or another of melanin granules. Strong electron spin resonance has been observed in melanin granules and in this they differ from mitochondria, as in the case of melanin granules this is due to the semiquinone structure of the pigment, while in the case of mitochondria this is due to copper in the cytochrome oxidase and iron in the DPNH dehydrogenase and flavin semiquinones.[16]

Free radicals in some melanin granules increase during irradiation with photons.[87,88] These properties were considered at length by Cotzias *et al.*[16] who present the few, but very significant, records relating melanogenesis to the extrapyramidal diseases. Depigmentation of the substantia nigra and locus coeruleus are important findings in Parkinsonism and phenylketonuria, both conditions being apparently rare in highly pigmented races.[16]

Melanin is the pigment responsible for the pigmentation of these areas, as identified by histological and histochemical appearance.[16] They also tend to disappear concomitantly with the skin melanin in phenylketonuria.[16] Where this melanin comes from is not established. Though, in consideration of the neural crest origin of melanocytes, their presence in the brain would not be unexpected, melanocytes in the human brain have apparently not been demonstrated. Melanogenesis outside melanocytes has not been shown to occur *in vivo*. A circulation of preformed melanin cannot at present be either definitely associated with or excluded from being at least a contributory source of this pigmentation.

It is known that the Bantu have a higher serum iron-binding capacity[89] and a high incidence of haemosiderosis[90] and circulating melanin should perhaps be considered in this regard. In haemochromatosis the skin pigmentation is due to melanin deposition.[91] In vitiligo and jaundice, vitiliginous areas do not become yellow,[44] a phenomenon which may be related to the free radical property of melanin. Bone formation in pigmented naevi has been reported,[92] and the relationship of melanin deposits to iron and calcium in the placenta in the case of dermatopathic melanosis of the placenta has been considered to have a more than casual relationship.[56] With the demonstration of a circulation of melanin, which has free radical properties giving it a strong affinity for metal-binding, these associations justify further investigation.

Pigmentation with Chlorpromazine

During the past year several reports were published within a few months of each other on patients on prolonged high dosage of chlorpromazine (500 - 3,000 mg. daily) who developed a slate-grey pigmentation. Visual impairment has been reported in some of these cases.[18-20]

Pathological studies showed extensive deposits of pigment (exhibiting the physical and histochemical properties of melanin) in dermal macrophages, throughout the RES and in the parenchymal cells of internal organs. Increased

dermal melanocyte activity could be demonstrated by dopa-tyrosinase reaction.[19]

Greiner and Nicolson[19] discuss (i) the nature of the pigment, (ii) the mechanism of pigment production, and (iii) the effect this may have on tissue function, but the crucial point as to how the melanin reaches the RES and other organs is not considered, possibly due to the limited interest in the circulation of melanin. Reference is made to the tendency for iron to favour deposition of melanin, quoting Hedinger[93] and Heilmeyer's[94] evidence of an influence of iron in oxidative processes, but the physical property of melanin, which favours metal-binding by free radicals, is not considered. On the other hand, Hays, Lyle and Wheeler,[15] not visualizing extra pigment in the epidermis, but collections around dermal blood-vessels, follow the line of argument of early investigators that the 'unlikely possibility' exists that some other pigment may be involved which reaches the skin through the blood.

The considerations on MSH- and melatonin-influence on pigmentation appear to concern the pigment production in these cases and are fully discussed,[18-20] but the unusual sites of deposition, which indicate a hyperactive transport of melanin, are not considered, probably due to the slight attention which such a circulation has received in the literature.

Very recently, a report has appeared[99] on 78 White Canadian patients on high and prolonged dosage of phenothiazines, which confirmed our original observations in Bantu and Cape Coloured patients.[7] According to a personal communication by Nicolson to Satanove,[99] pigmented leucocytes could be found in the buffy coat of the blood from these patients, and Satanove considers the transport of melanin at length in this condition in Whites.[99]

CLINICAL CONDITIONS IN WHICH A CIRCULATION OF MELANIN APPEARS TO BE OF SIGNIFICANCE

The circulation of melanin depends on leucocytic function, reticulo-endothelial activity and anterior pituitary control. The differences in disease incidence in the racial groups of Africa may be an indication of a quantitatively different milieu interieur which is visible in a differently set melanokinetic-homeostat.

This is reflected in differences in leucocyte count[67] and -activity,[79] higher gammaglobulin levels[81] and lesser 17-ketosteroid and 17-hydroxycorticoid excretion as well as a lesser postoperative increase of these fractions in Bantu compared to Whites.[95-98]

The higher incidence of haemosiderosis in the Bantu should be considered in relation to the metal-binding activity of melanin, as well as the apparently lesser incidence of Parkinsonism and phenylketonuria[16] in the Bantu.

Likewise, as the transport mechanism is qualitatively similar in all races, it may be inferred that the darker the skin pigmentation the greater the likelihood that the disease incidence in such Whites would approximate that of the higher pigmented races, for this would indicate increased RES activity, decreased adrenocortical function and more active melanokinetics.

Melanin-containing histiocytes have been reported in subacute bacterial endocarditis, which may be of aetiological significance in the increase in skin pigmentation in this condition.[100]

The unexplained hyperpigmentation in rheumatoid arthritis, hepatic cirrhosis and steathorrhoea may, through the effect of nutrition on adrenocortical activity,[101] or the alteration by stress of pituitary-adrenocortical function,[76] change the activity-level of melanokinetics.

These suggestions need further investigation and still require experimental proof.

SUMMARY

A brief review of the literature on the recently confirmed human circulation of melanin lends importance to its role in metal metabolism and neurophysiology, which is not readily appreciated from the quantitatively small amount which circulates in Whites. This aspect becomes of more significance in darker individuals, where, according to our concept of melanokinetics, melanin circulation becomes quantitatively more, and plays a correspondingly more significant role.

The transport of melanin and its handling by the reticulo-endothelial system appears to be under anterior pituitary control, which, simultaneously, influences pigment production.

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