MELANOTIC TUMORS OF THE SKIN

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ARMED FORCES INSTITUTE OF PATHOLOGY

ATLAS OF TUMOR PATHOLOGY

Section 1—Fascicle 3

MELANOTIC TUMORS OF THE SKIN

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MELANOTIC TUMORS OF THE SKIN

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MELANOTIC TUMORS OF THE SKIN

INTRODUCTION

Cutaneous tumors of many kinds contain melanin pigment, and in the broad sense, all of these could be classified as melanotic tumors. Occasionally this meaning is implied; for example, a pigmented tumor of the epidermis, commonly called seborrheic keratosis, has been classified as a benign melanoma (Harvey et al.). However, the term melanotic tumor usually has stricter usage—it designates tumors composed of cells that have the characteristic function of pigment formation, even though this function may be dormant or lost under certain circumstances. Tumors of this group are the true melanotic tumors and are the principal subjects of discussion in this fascicle. The remaining tumors are considered as incidentally pigmented; the pigment, whether in interstitial tissues or within tumor cells, is thought to be contributed by non-neoplastic melanocytes. Distinction of these two groups is important in general biology as well as in human pathology, because pigmentation may be an incidental property of tumors of many different animals and may accompany inflammation and reparative reactions. It must be pointed out, however, that these two general groups cannot always be sharply separated. If we accept the neural origin of pigment cells and recognize that pigmented nevi have neuroid components, we cannot clearly distinguish true melanotic tumors from incidentally pigmented neuromas.

NOMENCLATURE

At the Third Conference on Biology of Normal and Atypical Pigment Cell Growth in 1951 sponsored by the New York Zoological Society, an informal group including one of us (H.Z.L.) convened in an attempt to adopt uniform nomenclature for pigmented cells (Gordon). Recommendations of this group were endorsed by the Subcommittee on Oncology of the Committee on Pathology of the National Research Council for use in the Atlas of Tumor Pathology. We have slightly enlarged on this terminology by suggesting

that the word melanophage be retained and that the term incidentally pigmented cells be added as follows:

Types of Cells	Biologic Terminology	Medical Terminology	Recommended Terminology
"Contractile" cells*	Melanophore	.....	Melanophore
Pigment cells proper	Melanophore	Melanoblast	Melanocyte**
Immature pigment cells	Melanoblast	.....	Melanoblast
Pigmented macrophages	Melanophage	Melanophore	Melanophage (Macrophage)
Incidentally pigmented cells	.....	Melanophore***	Pigmented basal cells, neurons, tumor cells

*In lower animals, e.g. lizards, some pigment cells are capable of projecting pigment into the dendritic cytoplasmic processes of the cells and, conversely, of returning it to the cell bodies; this activity produces color change.

**Frequently subclassified according to normal anatomic distribution, such as epidermal, dermal, hair matrix, meningeal, neural, choroidal, and mucosal. Melanocytes may be tyrosinase active or inactive, or dopa-oxidase active or inactive, according to their physiologic status at a given time.

***Term used by Masson (1948).

The commonest abnormal cells of melanotic tumors in man are nevus cells. It is thought that they are derived from normal melanocytes or progenitors thereof and hence are atypical melanocytes. This assumption is the basis of the general classification used in this fascicle. However, whether or not this interpretation is valid, it is practical to retain the term nevus cell because such cells are characteristic and differ in many respects from normal melanocytes. For instance, with maturation they lose the property of pigment formation, and then in structure, arrangement, and certain histochemical properties, they tend to resemble cells of nervous tissue.

There is no generally accepted nomenclature for the various types of melanotic tumors, not only because disagreement on histogenesis continues but also because old, often self-conflicting terminologies have become firmly entrenched by daily usage; one such term is nevus. Although it would be impractical to discard the word nevus, its use must be clarified (see Fascicle 2, "Tumors of the Skin"). The term in the broadest sense includes localized malformations of many types of tissues—epithelial, fibrous, vascular, or pigmentary; the malformations may be represented either by an excess growth or by a deficiency. The term in a stricter sense applies only to localized pigmentary abnormalities. In the strictest sense, that which is used in this fascicle, the term nevus designates benign tumors composed of characteristic

cells known as nevus cells. It is not used as a term intended to distinguish malformation from neoplasm because the distinction is vague and impractical.

CLASSIFICATION

For purposes of general classification, it is suggested that hyperplasia and/or hyperactivity of melanocytes be called melanosis; that the word melanocytosis specifically be used to stress hyperplasia in the sense of an increased number of melanocytes; and that neoplasms be called either melanocytic tumors (Shaffer) or melanocytomas. Melanoma would be a simpler designation for melanocytic tumor, but unfortunately it usually has a connotation of malignancy and, loosely used, it could lead to disastrous misinterpretation.

The principal categories, then, which will be considered in the fascicle are:

Melanosis
- Epidermal
- Dermal

Melanocytic Tumors (Principally of Epidermal Origin)
- Benign (Pigmented or nevocytic nevus)
- Malignant (Malignant melanoma)

Melanocytic Tumors (Principally of Dermal Origin)
- Benign (Blue nevus or dermal melanocytoma)
- Malignant (Malignant blue nevus or malignant dermal melanocytoma)

In the fascicle, in deference to common usage and unless otherwise modified, the term malignant melanoma will designate malignant tumors of epidermal melanocytes; it must be recognized, however, that the term is not restrictive and includes the rare malignant blue nevus. The term nevocarcinoma will not be used because it stresses the questionable theory of epithelial origin for the common malignant melanoma of human beings. The old term melanosarcoma likewise is misleading; melanocytes are not mesodermal in origin. Dermal melanocytic tumors (blue nevi) are not to be confused with dermal nevi or intradermal nevi, terms often used in the United States of America to designate the ordinary pigmented nevus that has evolved beyond the junctional stage.

More precise terminology is especially needed in comparative pathology, because it is sometimes erroneously assumed that all melanotic tumors in all animals are similar. For example, some of the so-called malignant melanomas of animals are actually dermal melanocytomas more akin to the human blue nevus than the usual human malignant melanoma. However, there are occasional malignant epidermal melanocytomas in animals. Fortner and Allen (1958) described a tumor of hamsters which evolved in a manner similar to that of malignant melanoma in a human being. Some

so-called melanomas of animals are probably incidentally pigmented mesodermal or epithelial tumors (Miescher, 1933). As far as we know, a close counterpart of the common pigmented nevus of human beings is not found in lower animals, even though Fortner and Allen (1959) observed similar junctional changes in the epidermis of hamsters.

HISTOGENESIS OF NORMAL AND NEOPLASTIC PIGMENT CELLS

Theories of connective tissue origin and endothelial origin have been advanced in past years; it is now accepted, however, with the rarest exception (Goldberg), that normal and neoplastic melanocytes are ectodermal in origin (Becker, 1954, 1st reference; Miescher, 1933; Voss). Granting this, there is still some controversy as to the exact ectodermal tissue involved.

The theory which has the strongest supporting evidence specifies the ectodermal source as the neural crest. This theory is largely based on experimental embryology (Rawles) and may be outlined as follows: (1) that the embryonic origin of cells destined to become melanocytes is the neural crest; (2) that embryonic cells (melanoblasts) indistinguishable from other primitive cells migrate to ultimate destinations, such as epidermis, hair matrix, dermis, choroid, leptomeninges, olfactory and auditory mucosae, and in certain animals perivascular and pericelomic tissues; (3) that upon reaching and penetrating the ultimate tissue site, melanoblasts differentiate into recognizable melanocytes; (4) that the melanocytes are modified by, adapted to, and become symbiotic with the tissue into which they migrate (Pinkus et al.). The theory thus designates melanocytes as neural in origin and as histogenetically independent of tissues in which the mature cells lie.

Actual migration of melanoblasts from the neural crest has not been demonstrated. So far, the only evidence of migration in embryonal life is limited to migration within the skin itself. This was presented by Zimmermann and Becker. They observed by reduced silver technics that pigment cells first appear in human dermis as rounded dendrite-free cells and only later are melanocytes seen in their usual site, the dermoepidermal junction. We question whether we must assume that the element which migrates from the neural crest is necessarily the melanoblast; perhaps it is neural tissue which subsequently and secondarily differentiates into melanoblasts, or it may be some other neural element destined to regulate metabolism, growth, or differentiation of melanoblasts already in the sites which become pigmented.

A different concept is that melanocytes are not a histogenetically independent strain of cells but arise from epithelial or neural tissues indigenous to a given site. Thus it was proposed that normal epidermal melanocytes differentiate from basal cells and may be temporary modifications of basal cells to fulfill the function of pigment formation (Bloch). Neoplastic cells of the common pigmented nevus and the malignant melanoma were thought to

have similar origins (Unna, 1893, 1896, 2nd reference; Bloch; Dawson; Allen, 1949; Allen and Spitz). It is true that during the earliest period of development the cells of a common pigmented nevus or a malignant melanoma are at the boundaries of epithelium and connective tissue intimately associated with epithelium (Unna, 1893, 1896, 2nd reference; Masson, 1921; Dawson; Allen, 1949; Miescher, 1927, 1933; Lund and Stobbe) and resemble epithelial cells to a certain extent. However, they lack tonofibrils and bridges. Furthermore, evidence of transition of epithelium to melanocytes or nevus cells is based exclusively on the examination of fixed tissue sections and must necessarily remain speculative.

Another proposal is that neoplastic pigment cells arise from neural tissues indigenous to the skin. This proposal is based on the neuroid histologic properties of pigmented nevi. It was advanced by Masson (1926, 1951) who suggested dual origin for nevus cells: (1) from a primitive neuroectodermal element within the epidermis akin to the cells of Ranvier, and (2) from schwannian sheaths of dermal nerves, the combination of the two imitating a grotesque neurotactile organ. It is also of speculative interest that certain nevi, especially those of antenatal origin, are associated with hyperplasias of hair follicles which are also tactile organs.

Evidence concerning the origin of normal pigment cells can be summarized as follows: normal melanocytes are ectodermal in origin; an element arising in and migrating from the neural crest is essential to pigmentation of the skin and other tissues; the migrating element is thought to be the melanoblast, although this is yet to be conclusively demonstrated; it is probable that from embryonic life onward melanocytes are independent strains of cells.

Neoplastic pigment cells are probably derived from melanocytes or from immediate antecedents thereof. The possibility that they can arise from postembryonic neural tissue or even epithelial tissue appears unlikely but is not excluded. The neuroid histologic properties of nevus cells may be explained as (1) a development from primitive or dedifferentiated ectodermal cells in the skin, having both pigmentary and neuroid properties (melanoblasts may have unsuspected pluripotentialities), or (2) a simultaneous organoid overgrowth of pigmentary, neurotactile, and pilary tissues. We do not believe that the neural resemblance is specious and merely due to atrophy and fibrosis as has been suggested by others.

HISTOLOGY AND PHYSIOLOGY OF PIGMENT CELLS

Epidermal melanocytes are distributed among cells of the basal layer and can be recognized in routine preparations because of clear cytoplasm which is free of tonofibrils and prickles (fig. 1). The numerous dendritic processes of these cells are best seen by observing the epidermis in the horizontal plane. In ordinary vertical sections, dendrites usually are not evi-

dent unless there is hyperactivity, although they can be accentuated by silver (fig. 2) or dopa technics. The dendrites are true cytoplasmic processes and not merely granules of pigment free in intercellular spaces. This is shown by staining nonpigmented melanocytes by supravital (Billingham and Medawar, 1953; Szabó, 1959), gold (Billingham, 1948; Becker et al., 1952), or osmic iodide technics (Mishima and Miller-Milinska), by tissue culture of dendritic cells (Grand and Cameron; Hu), by electron microscopy (Clark and Hibbs), and by observations of partially digested epidermis (Billingham, 1949). Occasionally the dendrites have been seen to extend into underlying connective tissue; occasionally, too, a cell body seems to be isolated in a dermal papilla; however, Masson (1948) considered these as artifacts of direction of the section. It is possible that epidermal melanocytes may migrate into, or at least grow into, adjacent connective tissue for short distances. This may account for Ito's finding of dopa-oxidase activity in the dermal pigmented cells of Riehl's melanosis (war melanosis; see Epidermal Melanosis).

Clear cells with an affinity for gold are sometimes found in the upper part of the prickle cell layer and have been named Langerhans' cells, after their original observer. Masson (1948) considered these as shed, degenerating melanocytes. In certain hyperplasias, e.g. nevoid lentigo, melanocytes may be present in upper layers of the epidermis (Szabó, 1959).

The discussion of melanocytes has been principally concerned with those of the epidermis. Melanocytes are also related to the external root sheaths of follicles, sebaceous glands, and probably sweat ducts. Their probable role in neoplasia will be discussed (see Pigmented Nevus and Blue Nevus). The melanocytes of hair matrix basically resemble those of the epidermis. Their role in neoplasia is uncertain (see Blue Nevus).

Basal cells may be pigmented, and their melanin usually appears as a distinct supranuclear cap (fig. 1). Bloch, using frozen sections of unfixed tissues in pioneer studies of dopa reactions, thought that the basal cells contained dopa-oxidase and were therefore active in melanogenesis. In more precise technics which employed fixation, dopa-oxidase activity was limited to melanocytes; therefore it appears that the findings of Bloch were artifacts of diffusion (Becker et al., 1935; Masson, 1948). In studies of unfixed, separated epidermis, Staricco and Pinkus were able to demonstrate preformed melanin in basal cells; but melanin elicited by dopa reactions, which was distinguished by a black color, was limited to melanocytes. Tyrosinase activity has not been demonstrated in basal cells (Becker et al., 1952).

If melanin is formed by melanocytes alone, as could be inferred from this evidence, it must somehow be transferred to the basal cells. Masson (1948) thought of the epidermal melanocyte as an amboceptor receiving substrate from the dermis orming melanin, and inoculating it into the epidermal cells—a "cytocrine" function. In a similar manner, pigment possibly may be transferred to dermal connective tissue cells. Grand described clasma-

tocytosis, i.e. a narrowing and pinching-off of pigmented cytoplasmic processes, in cultures of neoplastic melanocytes; perhaps this is related to the "cytocrine" activity. According to Billingham (1948), dendrites of melanocytes terminate in epinuclear pigment caps of basal cells and, in some instances, appear to be applied to the outside of basal cells. The transfer of melanin from pilar melanocytes to cortical cells of hair is described by Birbeck and Barnicot. In certain inflammatory conditions, although melanocytes are actively forming pigment, transfer to epidermal cells appears to be blocked (Pinkus et al.), and the pigment is taken up by macrophages.

Masson (1948) observed xanthoma lipids, paraffin, and iron pigment in melanocytes under pathologic conditions; this suggests functions other than pigment formation. Perhaps epidermal cells partly receive and eliminate metabolites by means of the amboceptor melanocyte.

Macrophages containing melanin (melanophages) differ from melanocytes in that they have rounded contours, contain coarser and more irregular pigment granules, lack extended or branched cytoplasmic processes, and lack dopa-oxidase activity (Miescher, 1922). Pigment within the macrophages may come from melanocytes (Masson, 1948) by direct transfer or from the phagocytosis of free granules or remnants of degenerated pigment cells. Bloch also suggested that melanin may go into solution and be absorbed by macrophages. Identification of melanophages is often made solely because of their position within connective tissue; thus true dermal melanocytes may not be correctly identified. Melanophages appear in various dermatoses (fig. 5) and in and around pigmented tumors. They are related to hair papillae during active growth and at the onset of graying of the hair (Bloch).

MELANOCYTES (ACTIVE AND INACTIVE IN MELANIN FORMATION)

Skin color is determined by the amount of melanin formed by melanocytes and retained in the epidermal cells, not by the number of melanocytes present. Even in albino skin, melanocytes are present but are inactive and nonpigmented.

It is often assumed that melanocytes constantly show tyrosinase or dopa-oxidase activity, but this is not true. Such enzymic activity is present only during melanin formation to meet the physiologic demand for pigment in a given site. For example, melanin formation in the eye occurs only in embryonic and early postnatal development, and dopa-oxidase activity is present only during this time (Miescher, 1933); tyrosinase activity follows a similar pattern (Miyamoto and Fitzpatrick). In the skin, since continual melanin formation replaces that lost by epidermal shedding, it could be expected that enzymic activity is constantly present; this is true of the dopa reaction, but tyrosinase activity in sections of normal human skin could not be demonstrated until melanocytes were stimulated by exposure to ultraviolet irradiation (Fitzpatrick et al., 1950; Lerner and Fitzpatrick, 1950; figs. 3, 4). With a

different technic, however, Szabó (1957) demonstrated tyrosinase activity without using preliminary irradiation. Tyrosinase activity and dopa-oxidase activity in hair bulbs have been found to parallel the cycles of hair growth (Fitzpatrick et al., 1958).

Similarly, neoplastic melanocytes may be active or inactive and may parallel actual pigment formation in the tumor; pigment formation ceases in mature and differentiated nevus cells. In malignant melanomas, dopa-oxidase activity may be focally distributed or may not be present (Miescher, 1933); Fitzpatrick and Kukita (1959) found that tyrosinase activity was almost constantly present. In contrast, Greenstein and Algire, working with mouse melanomas, noted strikingly reduced dopa-oxidase and tyrosinase activity in amelanotic melanomas.

Activity of epidermal melanocytes is regulated by a variety of factors, some of which are normal and some pathologic (Lerner and Fitzpatrick, 1953; Blum; Jeghers). Melanin production is stimulated by radiant energy such as ultraviolet light, roentgen rays, and heat; in some instances, photosensitizing agents, heavy metals, and inflammation may provoke melanization. Sulfhydryl groups are normal inhibitory elements, probably because they bind copper which is important in tyrosinase activity. Hydroquinone, as found in the industrial preparation monobenzyl ether of hydroquinone, inhibits tyrosinase and causes depigmentation (Lorincz, 1950).

A melanin stimulating hormone (MSH) which is formed by the pituitary has been described by Lerner and associates. Hydrocortisone inhibits the release of MSH, and adrenalin and noradrenalin can reduce or prevent the action of MSH on the melanocyte. ACTH stimulates pigmentation, although possible contamination with MSH has been suggested as the mechanism of action. A hormone (melatonin) which diminishes pigmentation of amphibians also has been recovered from the pineal gland (Lerner and Case). Further data are needed concerning its importance in human physiology. Effects of pregnancy, vitamins, and hormones on pigmentation will be discussed (see Epidermal Melanosis). The human male tends to have greater melanin pigmentation than the female and a lesser degree of carotene pigment in the skin (Edwards and Duntley).

Melanin, specifically the brown-black eumelanin, forms intracellularly from tyrosine by the specific action of tyrosinase (Raper; Becker, 1930; Hogeboom and Adams; Fitzpatrick et al., 1950; Lerner and Fitzpatrick, 1950; figs. 3, 4). This process involves step by step oxidation through a series of intermediate indoles and quinones. Dopa is an intermediate product of this process and can be converted to an artificial form of melanin by dopa-oxidase and other oxidases (Hesselbach et al.). Oxidases of granulocytic leukocytes also have this property of conversion but differ from tyrosinase and dopa-oxidase in that they are not inactivated by cyanide. It is not clear whether tyrosinase can elicit the entire series of reactions from tyrosine to melanin

or requires the aid of other enzymes. The final step in melanin formation is a complex polymerization, with formation of a variety of melanins which vary in structure and color. Natural melanins are combined with protein (Lerner and Case). Probable chemical differences between eumelanin (brown-black) and pheomelanin (yellow-red) are discussed by Fitzpatrick and associates (1958) and by Lerner and Case. Tryptophane may be the substrate of pheomelanin.

Pigment may be seen as granules or rods, the former being usual in the epidermis. It is thought by many that pigment and tyrosinase enzymes are associated with mitochondria (Woods et al.; DuBuy et al.), but more recent observations suggest that the melanin particle is an entity apart (Dalton and Felix; Barnicot and Birbeck; Fitzpatrick et al., 1961). The intimate structure of the melanin-producing centers (melanosomes) has been described by Fitzpatrick and associates (1961). Meirowsky and Freeman believe that melanin arises from chromatin or chromatin derivatives, but this has not been substantiated.

Melanin darkens by oxidation when aided by light of great intensity or heat (Blum). Therefore, the seasonal appearance of freckles is a darkening of the melanin as well as actual melanin formation (Felsher et al.).

Melanin finally becomes degraded to melanoid and disappears as the cells which contain it migrate from the basal layer and are shed. The degraded melanin may be demonstrated by silver reactions when it may not be evident in ordinary preparations (fig. 2). The mechanism of this degradation is not understood (Masson, 1948; Bloch; Edwards and Duntley).

HISTOLOGIC METHODS FOR STUDYING PIGMENT CELLS

Studies of melanotic tumors may be aided by the histologic technics outlined in textbooks, particularly those for iron reactions and reticulum and trichrome stains. They also may be aided by special technics: Polarized light distinguishes melanin from birefringent pigments (Troxell), and darkfield examination identifies silver in argyria.

More specialized technics deserve further comment: Dopa-oxidase activity, originally studied by frozen section of unfixed tissue, is more precise after brief fixation (Becker et al., 1935; Laidlaw and Blackberg); Radaeli offered refinements of this technic; Staricco and Pinkus described a technic with separated epidermis.

Tyrosinase activity can be demonstrated by immersing pigment-forming tissue in an appropriate solution of L-tyrosine (Fitzpatrick et al., 1950; Lerner and Fitzpatrick, 1950; Fitzpatrick; figs. 3, 4). If the tissues are already heavily pigmented, reaction may be disguised; because of this, an autoradiographic technic using C^{14} labeled tyrosine has been developed (Fitzpatrick and Kukita, 1956; Fitzpatrick et al., 1958). The technic of Szabó (1955) em-