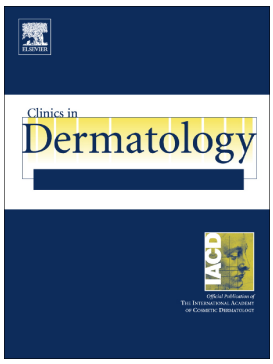

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The physiology of melanin deposition in health and disease

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Abstract

Eumelanin is the major pigment responsible for human skin color. This black/brown pigment is localized in membrane bound organelles, melanosomes, found in specialized cells, melanocytes, in the basal layer of the epidermis. This review highlights the steps involved in melanogenesis in the epidermis and the disorders in skin pigmentation that occur when specific steps critical for this process are defective. Melanosomes, which contain tyrosinase, a major enzyme involved in melanin synthesis, develop through a series of steps in the melanocyte. They are donated from the melanocyte dendrites to the surrounding keratinocytes in the epidermis. In the keratinocytes, the melanosomes are found singly or packaged into groups, and as the keratinocytes move upward in the epidermis, the melanosomes start to degrade. This sequence of events is critical for melanin pigmentation in the skin and can be influenced by genetic, hormonal, and environmental factors, which all play a role in levels of melanization of the epidermis. The effects these factors have on skin pigmentation can be due to different underlying mechanisms involved in the melanization process leading to either hypopigmentary or hyperpigmentary disorders. These disorders highlight the importance of mechanistic studies on the specific steps involved in the melanization process.

Introduction

Pigmentation of the skin depends on the synthesis and deposition of the pigment melanin in the epidermis by melanocytes. Melanin, a complex biopolymer, is formed in specialized membrane bound organelles, melanosomes, present in melanocytes.[1-6] Melanocytes are derived from precursor cells, melanoblasts, which develop in the neural crest during early embryonic development.[1,3,6] The melanoblasts subsequently migrate from the neural crest in the developing organism to the skin, the hair bulb, the eyes and the leptomenages where they further differentiate into melanocytes (Figure 1).[1-3,6] In the skin, the melanoblasts are localized to the dermis where they differentiate into melanocytes during the 10^{th} to 12^{th} week of development.[1,3,6] The melanocytes then secondarily migrate to the epidermis and localize at the dermal/epidermal junction by the 12^{th} to 14^{th} week of development (Figure 1).[1,3,6] The migration of melanoblasts from the neuronal crest to the dermis of the skin, their differentiation into melanocytes and then their movement to the epidermis are critical factors in the determination of visible pigmentation in the skin.[1,4,6] Also critical for this process is the functional interaction of the melanocytes with the surrounding keratinocytes in the epidermis and the transfer of melanin from the melanocytes to the keratinocytes (Figure 1).[1-4,6] Deficiencies or irregularities in any one of these critical steps can lead to a number of different pigmentary disorders which can be the result of decreased or increased melanization of the epidermis. Under certain circumstances, pigmentary change may result from the presence of melanin in the dermis. This review highlights these important physiological processes.

The Physiology of the Melanocyte

Melanocytes in the basal layer of the epidermis, though they are solely responsible for production of all the melanin found in the epidermis, comprise less than 1% of the cells present in the epidermis.[5,7] Among the cells in the epidermis, the melanocytes are highly unique in that they not only produce a membrane bound organelle containing melanin, the melanosome, but they are also involved in the transfer of this organelle to the surrounding keratinocytes, which results in the deposition of melanin in the epidermis.[4-6,8-10] The functional interaction between the melanocytes and keratinocytes is of major importance in skin pigmentation. Each melanocyte is surrounded by approximately 36 keratinocytes which form what has been termed the epidermal melanin unit; this is the fundamental integrated multicellular unit that is responsible for melanin pigmentation in the skin.[10,11] Within this unit, the tips of the dendrites from the polydendritic melanocytes make contact with the surrounding keratinocytes (Figure 2).[4,9,10] E-cadherin, which is a member of a family of transmembrane proteins mediating cell-cell adhesion, is expressed on the cell surfaces of both melanocytes and keratinocytes where it functions as a major cell adhesion molecule between these cells.[12] This interaction is unlike that present between individual keratinocytes, which make contact with each other via desmosomes.[13]

Of the two main types of melanin present in the skin and hair of humans, eumelanin (brown/black melanin) and pheomelanin (yellow/red melanin), eumelanin is the major melanin present in melanosomes in melanocytes in the skin.[1,2,4,6,11,13-15] Depending on the skin phototype (I-VI based on the response to UV exposure), pheomelanin may also be present.[1,2,6,15,16] The biogenesis of melanosomes is a

multistep process which involves unique sorting and trafficking pathways.[1,2] The initial steps involve the synthesis of tyrosinase in the rough endoplasmic reticulum, where it is packaged into vesicles and transported to the Golgi network.[1,2,6,17] In the Golgi, further sorting and processing is carried out and vesicles containing tyrosinase and enzymes involved in formation of melanin bud off of the Golgi. These vesicles develop into the first of the four stages involved in the maturation of the melanosome (Figure 1).[1,2,6,11,17] Stage I melanosomes are round spherical vesicles which contain tyrosinase, tyrosinase-related proteins, and an amorphous matrix (Figure 1). [1,2,6,11] The exact pathway for formation of these stage I melanosomes is not completely understood. Stage I melanosomes develop into stage II melanosomes as they elongate and a pigment cell specific protein, PMEL, a main component of stage II melanosomes, organizes into parallel, proteinaceous fibrils which are mediated by amyloid-related interactions (Figure 1).[1,2,6,11] In stage III melanosomes, melanin synthesis is initiated by tyrosinase via the oxidation of L-tyrosine to dopa (3,4-dihydroxyphenylalanine) and dopa to dopaquinone.[1,2,18,19] These steps are common to synthesis of both eumelanin and pheomelanin after which these two biosynthetic pathways diverge.[1,2]

Eumelanin is deposited on the fibrils in stage III melanosomes (Figure 1).[1,2,6,11] In stage IV melanosomes, melanin deposition in the melanosome is completed and occludes the internal structure of the melanosome (Figures 1 and 3).[1,2,6,11] During these stages in melanosome development, additional proteins involved in this process are imported into the melanosomes.[1,2,5,6,17,20] These stages of development apply mainly to melanosomes in which eumelanin is synthesized.[6] There are slight differences in maturation of melanosomes containing pheomelanin, mainly in the spherical nature of

the mature melanosome and the lack of an internal fibrillar structure.[6] Production of melanin in melanocytes in the epidermis can be assessed by examination of tyrosinase activity in the melanocytes using histochemical analyses (Figure 2).[21,22]

As the melanosomes mature, they are transported by motor proteins (kinesins) along microtubules from the perinuclear area to the tips of the melanocyte dendrites (Figure 1).[4,17,23] Rab27a, a GTPase on the melanosome surface, recruits the adaptor protein melanophilin, which in turn recruits the actin motor protein, myosin-Va to the melanosome.[17,24-27] Myosin-Va then associates with the peripherial actin network in the tip of the melanocyte dendrites dispersing the melanosomes (Figure 1).[17,24-28] This dynamic interaction between myosin-Va and the melanosome via melanophilin, enables the association of melanosomes with actin filaments in the tips of the melanocyte dendrites.[24-26] Critical to visible pigmentation in the skin is the transfer of these melanosomes from the melanocyte dendrites to the surrounding keratinocytes (Figure 1). If this process is interrupted or not completed, melanin will not be deposited into the keratinocytes, and the skin will have very much diminished color; hence, the uniquiness of the human skin pigmentary system.

Deposition of Melanin in the Epidermis

Exactly how melanin is deposited into the keratinocytes in human skin is still not completely understood. Both the melanocytes and the keratinocytes actively participate in this process. There are a number of theories and models regarding how this occurs (Figure 4).[4,29,30] Insights into this process have been gained from both light and electron microscopic studies of human skin as well as studies in which melanocytes and

keratinocytes have been co-cultured and this process examined *in vitro.*[8,31-41] In one model, known as the *cytophagocytosis or dendritic phagocytosis model*, the tip of the melanocyte dendrite makes contact with the keratinocyte and is pinched off and taken up by cytophagocytosis by the keratinocyte (Figure 4A).[9,29,30] This model is supported by studies on co-culture of melanocytes and keratinocytes in which this process was examined by both time-lapse video or digital microscopy[8,32-36] and electron microscopy.[33-36] In the co-culture studies, the melanocyte dendrites have been shown to make contact with the keratinocytes (Figure 5).[33-36] According to this model, melanosome transfer occurs when the tip of the melanocyte dendrite is pinched off and taken up by phagocytosis by the keratinocyte (Figures 6 and 7). This process is aided by a band of actin filaments in the cytoplasm of the melanocyte just beneath the cell membrane (Figure 8).[33,35]

Phagocytosis of the tip of the melanocyte dendrite by the keratinocyte is aided by a cortical band of actin filaments beneath the keratinocyte cell membrane (Figure 9).[33,35] Breakdown of these bands of actin filaments in both the melanocytes and keratinocytes, by an agent such as cytochalasin, leads to loss of melanosome donation from the melanocytes to the keratinocytes.[33-35] This indicates that these cellular bands of actin filaments are needed for this autophagocytotic process to occur in these cells in culture.[33,35] In an extension of this model, known as the *filopodia-phagocytosis model*, filopodia, which are narrow extensions of the cellular membrane, extend from the melanocyte and make contact with a keratinocyte.[10,29,30] Melanosomes are transported along the filopodia, which is engulfed by the keratinocyte, and phagocytosis takes place with the melanosomes transferred to the keratinocytes.[37]

In a second model, the *membrane fusion or tunneling nanotube model*, the tip of the melanocyte membrane fuses with the keratinocyte membrane and a tunneling nanotube or channel forms and serves as a conduit for transfer of melanosomes from the melanocyte to the keratinocyte (Figure 4B).[9,29,30,38] These nanotubes contain actin which is thought to be important in the transport process.[30,38]

In a third model, the *shedding-phagocytosis model*, melanosomes are shed in membrane bound vesicles from melanocytes.[9,29,30] These vesicles then either fuse with the keratinocyte cell membrane, and melanosomes are transferred to the keratinocytes by phagocytosis or these vesicles are present extracellularly and then secondarily internalized by the keratinocyte via phagocytosis (Figure 4C).[9,29,30,39,40]

A fourth model for melanosome transfer, the *exocytosis/endocytosis model*, proposes a mechanism in which individual melanosomes are exocytosed by the melanocyte dendrite and endocytosed by the keratinocytes (Fig. 4D).[9,29,30] This model is supported by electron microscopic studies of human skin samples and studies from co-cultures of melanocytes and keratinocytes.[41]

As a result, a number of different mechanisms for transfer of melanosomes from melanocytes to keratinocytes have been proposed which provide explanations on how melanin present in melanocytes is deposited in keratinocytes in the epidermis. There are studies which support each of these models. Deposition of melanin in the epidermis is necessary and critical for the coloration seen in the skin. Whether one or more of the mechanisms proposed is utilized in human skin pigmentation and whether a particular set of circumstances could dictate the specific mechanism taking place is not known. This is an exceedingly interesting area that needs to be investigated further.

Once the melanosomes have been transferred to the keratinocytes, they localize in the keratinocytes either singly or in groups surrounded by a membrane (Figure 10).[11,42-45] This is under genetic control (as will be discussed below). As the keratinocytes differentiate and move upwards in the epidermis through the stratum spinosum and stratum granulosum to the stratum corneum they transport the melanosomes with them which results in melanin being present in keratinocytes throughout the epidermis. During this process, the melanosomes start to degrade.[11,44-46] The degree to which this degradation occurs affects the level of melanin pigmentation in the skin.

The level of melanization of the skin (i.e., visible pigmentation in the skin) is related to a number of critical biologic processes (Figure 1):

(1) migration of melanoblasts from the neural crest to the skin

(2) differentiation of melanoblasts into melanocytes in the dermis

(3) movement of melanocytes from the dermis to the basal layer of the epidermis

(4) production of melanosomes in the melanocytes

(5) melanization of melanosomes in the melanocytes

(6) transport of the melanosomes to the tips of the melanocyte dendrites

(7) transfer of melanosomes from the melanocytes to the keratinocytes

(8) degradation of melanosomes within the keratinocytes

Disturbances in any one of these processes can lead to increased or decreased melanin pigmentation in the skin, as will be discussed below.

Variations in Melanization of the Skin

The levels and uniformity of melanization of normal human skin can vary over a wide range and these variations can be due to genetic, hormonal or environmental factors. The intrinsic or constitutive coloration of the skin is mainly under genetic and hormonal control; more transient changes in levels of skin pigmentation (facultative skin pigmentation) are largely influenced by environmental factors such as ultraviolet radiation (UVR) or by some type of physiological regulation.[5,6,47-49] The amount of melanin present in the keratinocytes is a significant factor in determination of skin pigmentation and is one of the main determinants of differences in skin color between individuals;[11] however, the differences in intrinsic skin pigmentation from dark to light that are observed among the various racial/ethnic populations are not due to differences in the number of melanocytes present in the epidermis.

The number of melanocytes in the basal layer of the epidermis, in any given area of the body, is roughly similar in Caucasian skin, when compared to African/American skin or Asian skin.[50-53] These differences in melanin pigmentation and the functional activities of the epidermal melanin units are under genetic control. Three of the major determinants in skin pigmentation include:

1. the nature of the deposition of melanosomes within the keratinocytes
2. the degree of melanization of the melanosomes
3. the distribution and degradation of melanosomes in the keratinocytes.[11,42-46]

In Caucasian skin, the melanosomes in the keratinocytes are predominantly arranged in groups of two to eight and surrounded by a membrane (Figure 10A).[11,42-45] In African/American skin, single melanosomes are predominantly present in the keratinocytes rather than in groups surrounded by a membrane, as they are in Caucasian skin (Figure 10C).[11,42-45] The melanosomes are also larger in size, and there are more Stage IV melanosomes; thus there is more melanin in these melanosomes.[11,42-46] In Asian skin, the distribution of melanosomes in the keratinocytes is intermediate between African/American and Caucasian skin. The melanosomes are arranged in groups in the keratinocytes (Figure 10B) and are also individually distributed within the keratinocytes.[11,42-46] Within each group of melanosomes, there may be fewer melanosomes than in Caucasian skin, but the melanosomes are larger in size.[11,42-46]

Another factor important in skin pigmentation is the degree to which the melanosomes are degraded in the keratinocytes as they undergo terminal differentiation and migrate upwards in the epidermis to the stratum corneum. In the epidermis of Caucasian skin, the melanosomes are generally completely degraded and are absent from the corneocytes in the stratum corneum.[11,44-46] In African/American skin, there is less degradation of the melanosomes in the keratinocytes as they migrate upwards through the epidermis to the stratum corneum.[11,44-46] In Asian skin, the degree of degradation of melanosomes in the stratum corneum is intermediate between that found in African/American or Caucasian skin and individual melanosomes rather than clusters of melanosomes are more prominent in these terminally differentiated cells.[11,44-46]

The major factors contributing to degree of melanization of the skin of individuals of different racial/ethnic backgrounds are:

- the nature of the deposition of melanosomes in the keratinocytes (e.g., singly or in groups surrounded by a membrane)
- the size of the melanosomes in the keratinocytes
- the level of melanization of the melanosomes
- the degree of degradation of melanosomes in the upper layers of the epidermis.

In individuals with dark constitutive skin pigmentation, the melanosomes in the epidermal keratinocytes are larger and contain more melanin, they are individually distributed in the keratinocytes and show less degradation in the upper layers of the epidermis and the stratum corneum than in individuals with light constitutive skin pigmentation.

Levels of pigmentation (melanization) in the skin can also be under hormonal control. One of the most important positive regulators of melanogenesis is the melanocortin receptor, in particular melanocortin type 1 receptor (MC1R), which regulates the quantity and quality of melanins produced.[15,54-56] This receptor on the melanocyte cell surface is stimulated by the melanocortins, MSH (melanocyte stimulating hormone) and ACTH (adrenocorticotropic hormone), which up-regulate the expression and function of MC1R.[15,54-56] In skin, α-MSH is the predominant biologically active form of MSH which can stimulate melanocyte activity; it has a heptapeptide core which is responsible for its melanogenic activity.[1,2,5,15] It is produced by both keratinocytes and melanocytes and acts to exclusively enhance the synthesis of eumelanin.[6,57-60]

When MSH binds to melanocortin receptors present on the melanocyte, it activates adenyl cyclase, which leads to increased synthesis of cyclic AMP (cyclic adenosine monophosphate) (cAMP).[1,2,4,6,61] The increased levels of cAMP modulate the expression of several melanocyte specific genes, including the gene coding for tyrosinase, leading to increased tyrosinase activity and stimulation of melanogenesis.[1,2,4,6,61] This leads to increased production of melanosomes, increased deposition of melanin in the melanosomes, and increased transfer of melanosomes from the melanocytes to the keratinocytes[2,6,61] . In support of this, increased transfer of melanosomes from melanocytes to keratinocytes has been observed, using time-lapse video-microscopy, light microscopy and electron microscopy, in co-cultures of mammalian melanocytes and keratinocytes which have been treated with cAMP or MSH.[34,62] MSH can additionally increase the number of melanocytes in the epidermis.[1,2,5,38,51] ACTH, which has the same heptapeptide core present in MSH and which is involved in melanogenic activity, has a similar effect on melanogenesis.[6,61] Thus stimulation of the MC1R on melanocytes in the epidermis by MSH as well as ACTH can upregulate melanogenesis and lead to enhanced darkening of the skin**.**

Environmental factors can have a significant effect on melanization of the skin. Ultraviolet radiation (UV), by both UVA and UVB, is one of the most important external factors influencing human skin pigmentation. It can lead to both immediate darkening of the skin, mainly by UVA, and to longer term persistent pigmentation of the skin, mainly by UVB.[5,6,14,47,48] The immediate darkening effects by UVA are due mainly to oxidation of existing melanin or melanin precursors and redistribution of the melanosomes in the

keratinocytes from a perinuclear localization to a more general dispersement throughout the cell leading to more visible pigmentation.[6,14,47]

Persistent pigment darkening of the skin and the longer term effects involve activation of melanocyte function.[5,6,14,47,48] UV radiation exposure leads to increased expression of the transcription factor MITF (microphthalmia-associated transcription factor), a transcriptional regulator of melanocyte function, which has a number of protein targets, an important one of which is tyrosinase.[5,6,47] Activation of tyrosinase and some of these other melanogenic proteins (i.e., tyrosinase-related protein 1; DOPAchrome tautomerase) leads eventually to increases in melanin synthesis and content in the melanosomes and over an extended of time leads to increases in melanocyte density in the epidermis[1,2,5,6,47,63] over a period of 4-5 weeks after exposure to UV, there is increased melanization of the melanosomes and increased cell division of melanocytes which in turn leads to an increased number of melanosomes transferred to the keratinocytes.[5,6,47] Under chronic UV radiation, the melanocyte density can increase by 3- or 4-fold leading to a significant increase in melanin deposition in the epidermis and in darkening of the skin.[5,6,47]

UV can also stimulate the production of α-MSH and ACTH in the skin.[4-6,64] In response to UVB, keratinocytes and melanocytes are induced to increase expression of α-MSH and ACTH which stimulate production and activity of the MC1R on melanocytes leading to increased production of eumelanin, enhanced melanogenesis and darkening of the skin.[5,6,65] This response to UV is a protective one, because melanin functions to absorb UV which has harmful effects on skin and subcutaneous tissues.[1,5,6,47,48] Melanin in the skin can scavenge free radicals and minimize the toxic effects of reactive

oxygen species (ROS) generated by UV and help protect cellular DNA, proteins and lipids from damage; thus, deposition of melanin in the epidermis has a photoprotective effect against sunlight.[1,5,6,47,48]

Disorders in Skin Pigmentation

There are a number of important biological processes which are critical for normal pigmentation of the skin. Disturbances in any of these processes can result in pigmentary disorders which include both increased and decreased melanization of the skin. These disorders are ultimately due to changes in the number or activity of melanocytes present in the basal layer of the epidermis, to altered synthesis of proteins/enzymes needed in the formation or melanization of melanosomes in the melanocytes, or to changes in the transfer of melanosomes from the melanocytes to the keratinocytes. Just a few of the disorders in which these processes are defective and which result in either hypopigmentation or hyperpigmentation will be discussed.

Hypopigmentary Disorders

Vitiligo is one of the most common acquired hypopigmentary disorders.[28,66-72] It is characterized by loss of epidermal melanin in the skin which is due to selective destruction of melanocytes in the basal layer of the epidermis.[28,66-72] These areas are usually very well demarcated and depigmentation is complete. Loss or reduction in the number of melanocytes can result from defects in pigment cell differentiation, proliferation, migration and/or survival.[66-72] This disorder is almost always acquired.[68,71-75] The prevalence of vitiligo varies markedly; it has a prevalence of approximately 1-2% of the world population.[66,67,72] The pigmented skin of vitiligo patients shows micro-

depigmentation and vacuolization of melanocytes and/or keratinocytes.[75] Ultrastructural studies show the absence of melanocytes in the vitiliginous lesions.[71] The etiology and underlying pathogenesis of vitiligo remain poorly understood and its progression is unpredictable.[28,66,67,71,72]

Vitiligo is thought to be a multifactorial disorder involving the interplay of several factors. Several hypothesis have been proposed as to the underlying mechanisms involved.[66,68,69,71-75] Two of the main ones are the autoimmune response hypothesis and the autocytoxic/metabolic hypothesis.[66,68,71,73-75] The autoimmune hypothesis proposes that there is an autoimmune response against melanocytes leading to a loss of melanocytes.[66,68,69,71-73,76-78] Autoantibodies to proteins associated with melanocytes, such as tyrosinase and tyrosinase related proteins, may be present thus leading to loss of function of the major enzyme involved in melanin synthesis and to death of melanocytes.[70,79] A role for cellular immunity playing a role in vitiligo is further supported by evidence that specific cytotoxic T cells (CD8+ T cells) against tyrosinase and other melanocyte proteins are found in some vitiligo patients and are associated with the destruction of melanocytes.[71,72,76-79]

The autocytotoxic/metabolic hypothesis proposes that intrinsic metabolic defects in nonlesional melanocytes lead to intracellular oxidative stress which is an intracellular signal for melanocyte degeneration.[68,71,73] Oxidative stress could also be generated by various extrinsic triggers such an sunburn and exposure to toxic chemicals which lead to melanocyte death.[68,73] In support of this, individuals with vitiligo have been reported to have compromised antioxidant responses.[68,73] In addition, recent studies suggest that E-cadherin, which mediates the adhesion between melanocytes and keratinocytes,

is absent or deficient at melanocyte membranes and that this abnormality is associated with detachment of the melanocytes from the keratinocytes in the epidermis; this dissociation is accelerated after oxidative and mechanical stress.[74] This could thus be a factor in loss of melanocytes from the basal layers of the epidermis.[74] As a result, there are a number of factors that could be important in the pathogenesis of vitiligo and the loss of melanocytes in the epidermis. The various pathways proposed may not be mutually exclusive and may converge, ultimately leading to destruction or loss of melanocytes.

Albinism is a congenital disorder characterized by hypomelanosis in most normally pigmented tissues resulting from defects in melanin biosynthesis.[4,71,80,81] There are several clinical types of albinism. In the most common type, oculocutaneous albinism (OCA), there is reduced synthesis of melanin in melanocytes in the skin, hair and eye.[4,71,80,81] In OCA1, which is one of the most prevalent clinical phenotypes of OCA, deficiencies in melanogenesis are the result of loss of functional activity of tyrosinase due to mutations in the *TYR* gene which encodes tyrosinase.[71,80,81] A large number of different mutations have been reported in the *TYR* gene in this disorder.[71,80,81] Only Stage I and Stage II melanosomes are present; internal matrix formation in the melanosomes is normal, however, loss of tyrosinase activity leads to failure of synthesis of melanin (Figure 1).[81] In different clinical phenotypes of OCA, however, tyrosinase is present indicating that there is a different cause for lack of melanin synthesis.[81] Cutaneous lack of pigmentation in the skin in OCA is mainly due to lack of melanization of the melanosomes. This loss of melanin in Individuals with albinism can lead to massive solar damage and numerous ultraviolet light-related skin cancers.[71,80,81]

Piebaldism is a congenital autosomal dominant disorder, characterized by a white forelock and leukoderma on the frontal scalp, forehead, ventral trunk, and/or extremities.[80-82] The cutaneous lack of pigmentation in the ventral white patches and white forelock is due to a significant or complete loss of melanocytes in the epidermis and hair bulbs.[80-82] This is caused by improper migration of melanoblasts from the neuronal crest in the embryo and/or their failure to differentiate into melanocytes (Figure 1).[80-82] Loss of melanocyte formation in the skin is due to mutations in the c-*KIT* gene, which encodes a tyrosine kinase transmembrane receptor on the melanoblast surface.[81,83-86] These mutations decrease the ability of the c-KIT receptor to be activated by the c-KIT ligand (stem cell factor, steel factor or mast cell growth factor) expressed by epidermal keratinocytes and initiate a cascade of signaling events to upregulate melanoblast proliferation, migration and/or differentiation into melanocytes during embryogenesis.[80-82] In piebaldism, loss of melanocytes is caused by a loss-of-function in c-KIT receptors on melanoblasts which results in failure of the melanoblasts to migrate to the skin and differentiate into melanocytes leading to loss of melanization of the skin.

Hermansky-Pudlak Syndrome (HPS) is a rare autosomal genetically heterogeneous disorder in which individuals present with the clinical findings of OCA and are characterized by hypopigmentation of the skin, hair and eyes accompanied by additional nonpigmentary findings (e.g. bleeding diathesis, immune-deficiency, lung fibrosis).[1,71,81,87,88] This is a disorder of melanosome biogenesis which results from mutations in genes that encode proteins involved in membrane trafficking of melanosome related proteins to maturing melanosomes (Figure 1).[1,71,81,87,88] Electron

microscopy has shown that macromelanosomes as well as stage I to III melanosomes may be present, but not stage IV melanosomes.[71] These deficiencies in melanosome biogenesis contribute to the loss or reduction in pigmentation of the skin in individuals with HPS.[1,71]

Griscelli syndrome (GS) is a rare autosomal recessive disorder which is characterized by diffuse pigmentary dilution in the skin and silvery-gray hair, in addition to some nonpigmentary abnormalities (e.g., neurologic impairment, immune abnormalities, and a hemophagocytic syndrome).[2,71] This is due to melanosomes congregating in the center of the epidermal melanocytes as a result of failure of melanosomes to be transported within the melanocyte dendrites and transferred to the surrounding keratinocytes (Figure 1).[2,71]

There are three types of GS (GS1, GS2, GS3) each one having a defect in a different gene involved in melanosome transport in the melanocyte.[2,71.89-91] These genes code for one of three proteins: Rab27a, a GTPase on the melanosome surface; melanophilin, an adaptor protein which binds to Rab27a and in turn recruits a third protein, myosin-Va to the melanosome.[2,17,24-27,71] Myosin-Va then binds to actin in the tip of the melanocyte dendrite linking the actin network to the melanosome.[2,17,24-27,71] This linkage provides a dynamic interaction between the actin network and the melanosome and plays a key role in localization of melanosomes to the tip of the melanocyte dendrite and their transfer from the melanocyte to the keratinocyte (Figure 1).[2,17,24-27,71] In the absence of any one of these proteins, melanosome transfer to the keratinocytes does not take place. The cellular deficiencies present in GS thus show the

importance of transport of melanosomes within melanocyte dendrites for transfer of melanin to keratinocytes and for normal pigmentation of the skin.

Pityriasis alba is an acquired hypopigmentation disorder which is characterized by hypopigmented macules and patches, typically located on the face but which may appear on the shoulders and arms.[71,86,92] It is thought to result from edema between epidermal cells due to an inflammatory process occurring from low-grade eczematous dermatitis.[89] The edema between cells prevents melanosome transfer from melanocytes to the surrounding keratinocytes, resulting in patches of skin which show a striking reduction in pigmentation (Figure 1).[86] This demonstrates the importance of contact between the melanocytes and keratinocytes in order for melanosomes to be transferred to the keratinocytes and for melanization of the epidermis to occur. Separation of epidermal cells (keratinocytes) by edematous fluid, as can occur in pityriasis alba, interferes with this process and results in patches of hypopigmentation.

Hyperpigmentation Disorders

Skin hyperpigmentation may result from numerous causes. Perhaps, the most common and/or significant hyperpigmentation disorders occurring in the epidermis are post-inflammatory hyperpigmentation, melasma, and Addison disease. Dermal hyperpigmentation can also occur, particularly after inflammation, and will be briefly discussed. Dyspigmentations due to aberrant melanocytic proliferations are numerous and are beyond the scope of this paper.

Postinflammatory hyperpigmentation is an extremely common acquired disorder in which there is an excess of melanin pigment production following cutaneous

inflammation or injury.[93-95] It may occur due to hyperplasia of epidermal melanocytes, to hyperfunction of epidermal melanocytes, or to both together (Figure 1) .[93-95] This leads to increased melanogenesis and to increased melanin deposition in the epidermis (e.g., following atopic dermatitis or acne) or in the dermis (e.g., following lichen planus).[93-95] In the latter, melanin enters the dermis via a damaged basement membrane where it is phagocytosed by dermal macrophages, forming melanophages.[93,94] These macrophages may also migrate into the epidermis where they phagocytose melanosomes and then return to the dermis.[93,94] This increased melanin deposition leads to formation of hyperpigmented macules and patches in the skin.[93-95]

A number of factors produced during inflammation, such as prostaglandins, leukotrienes, cytokines and inflammatory mediators, may play a role in this response and the increased melanogenesis which occurs.[94-97] The hyperpigmentation which is produced secondary to inflammation may persist for weeks to months in the epidermis and the melanin within dermal melanophages may persist for longer periods of time, such as years.[93] Due to the Tyndale light scattering effect (also known as the Rayleigh phenomenon), such pigment located deeper in the dermis may also impart a blue coloration to the affected skin seen clinically.

Melasma is a common acquired disorder characterized by symmetrical patches of hyperpigmentation on the skin, most commonly occurring on the face.[6,94,98-100] Areas of hyperpigmentation are due to increased deposition of melanin in the epidermis and dermis, where it is found in dermal macrophages (Figure 11).[6,98-100] No increase in number of melanocytes is observed in these areas but epidermal melanocytes show greater activity with increased production of melanosomes, increased tyrosinase

activity, increased synthesis of melanin, especially eumelanin, in the melanosomes, and increased transfer of melanosomes to keratinocytes (Figure 1).[6,99] Genetic predisposition is considered as one of the main causes involved in the development of melasma along with UV irradiation and endocrine factors.[6,94,98-100] It is most prevalent in women.[6,98-100] During pregnancy, elevated levels of estrogen, progesterone and MSH have been associated with melasma.[6,94] A number of signaling pathways are thought to be involved in the upregulation of tyrosinase and MITF, resulting in the stimulation of melanogenesis and the development of melasma.[6,100] These pathways and the factors triggering them are still being investigated.

Addison disease is an endocrinopathy in which there is acquired hypermelanosis.[94,101,102] It results from damage to the adrenal glands resulting in a deficiency in glucocorticoids and mineralocorticoids.[95,101,102] In greater than 50% of the patients it is an autoimmune disorder in which autoantibodies to the adrenal cortex lead to its destruction.[95,101,102] Hyperpigmentation of the skin is the most striking cutaneous sign of the disorder.[95,101,102] The adrenal cortical insufficiency and the resulting decreased production of corticosteroids leads to a compensatory overproduction of adrenocorticotropic hormone (ACTH) by the pituitary.[95,101,102] ACTH has the same active heptapeptide core as MSH and as a result can bind to the melanocortin-1 receptor on melanocytes and stimulate melanogenesis leading to increased synthesis of tyrosinase and melanin and increased transfer of melanosomes to keratinocytes in the epidermis (Figure 1).[95,101,102,104] Hyperpigmentation occurs and is typically striking and accentuated in sun-exposed areas, flexural folds and skin creases, including the creases on the palms.[94,103,104]

Pigmentary incontinence is a term used to describe a condition in which melanin is deposited in the dermis.[98] This condition usually follows inflammation in which there is basal cell damage at the dermal-epidermal interface of the skin (e.g., lichen planus, Riehl's melanosis, lupus erythematosus, incontinentia pigmenti, and fixed drug eruption).[98] This may lead to free melanin pigment, no longer in melanocytes or in keratinocytes, becoming deposited into the dermis (Figure 12A). The deposited melanin is then phagocytosed by dermal macrophages, known then as "melanophages." These melanophages are usually found in the upper dermis but may descend to the level of the deep papillary vascular plexus where they may persist for months to years or even decades (Figure 12 B). A characteristic slate-gray pigmentation of the skin is observed.[98]

There are conflicting explanations for the mechanism of development of pigmentary incontinence, particularly as to the origin of the melanosomes observed within the macrophages and the process of transfer of melanosomes from the epidermis to the dermis. There are reports of melanosomes in melanocyte dendrites being taken up by macrophages in the dermis or of being discharged directly into the dermis.[98] Other studies have suggested that, in the inflammatory process, keratinocytes degenerate and are phagocytosed by macrophages, which have migrated into the epidermis from the dermis. The macrophages containing the phagocytosed dyskeratotic cells and their melanosomes then migrate back to the dermis.[98] Thus there are a number of very interesting possibilities as to how the melanin found in the dermis during inflammatory processes is actually deposited there.

Other causes of hyperpigmentation – There are numerous and reflect much excellent research but are beyond the scope of this paper. The reader is directed elsewhere.

Conclusions

Deposition of melanin in the epidermis and its processing within the keratinocytes involves a number of steps critical for pigmentation in the skin. Of particular importance is the migration of melanoblasts from the neural crest to the dermis during embryonic development, their subsequent development into melanocytes and migration of the melanocytes into the epidermis. Cellular processes within the melanocyte lead to unique sorting and trafficking pathways involving synthesis of tyrosinase and other melanosomal proteins, formation of melanosomes, and development of a fully melanized melanosome. Interaction of the melanosome with cytoskeletal structures in the melanocyte such as microtubules and actin filaments is critical for its movement to the tip of a melanocyte dendrite and its incorporation into phagocytic vesicles important in the donation of melanin to the surrounding keratinocytes.

The interaction of a melanocyte with its surrounding group of keratinocytes to form an epidermal melanin unit and the transfer of melanosomes from melanocytes in this unit to the keratinocytes is distinctive to the skin pigmentary system and essential for melanin pigmentation in the skin. Levels of melanization of the skin can be influenced by a number of factors: synthesis of melanin in melanosomes, melanosome size, transfer of melanosomes to the keratinocytes, and degree of degradation of melanosomes in the keratinocytes. These processes can be under genetic control, as is

seen in racial differences in skin coloration, or under hormonal control as observed by the effects of MSH or ACTH on melanocytes.

Environmental factors can also play an important role as seen after exposure of skin to UV irradiation. Defects in any one of the fundamental processes given above which are involved in melanin deposition and processing in the epidermis can lead to both skin hypopigmentary and hyperpigmentary disorders. Knowledge of these processes and further studies on their mechanistic role in the development of these disorders is critical for obtaining a better understanding of methodologies for their treatment.

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Figure Legends

Figure 1. Stages in melanocyte development, melanosome formation and melanization, and melanin transfer to keratinocytes. Melanoblasts develop in the neural crest and migrate to the dermis of the skin [1]. In the dermis, the melanoblasts differentiate into melanocytes [2]. These melanocytes then migrate to the epidermis [3] where they can undergo mitotic division [4]. Within the melanocyte, tyrosinase is synthesized by the rough endoplasmic reticulum and transported to the Golgi complex where it is modified [5]. Tyrosinase and tyrosinase-related proteins bud off as vesicles from the Golgi and then further develop into stage I melanosomes [6]. Melanization of the melanosomes takes place and stage II, III and IV melanosomes form [7]. The melanosomes are transported along microtubules via the motor protein kinesin to the tip of the melanocyte dendrite, where the melanosome, via Rab27a and melanophilin, interacts with myosin-Va, which associates with the peripherial actin network in the melanocyte dendrite. Transfer of melanosomes from the melanocyte to the surrounding keratinocytes takes place [8]. As the keratinocytes differentiate and move upwards in the epidermis to the stratum corneum, the melanosomes start to degrade [9]. In hypopigmentary disorders of the skin, there are deficiencies in different aspects of the processes described above: *Piebaldism* - improper migration of melanoblasts from the neural crest [1] and/or failure to differentiate into melanocytes [2]; *Albinism* – defects in

tyrosinase synthesis [5] and failure of melanization of melanosomes [7]; *Hermansky-Pudlak* syndrome – defects in melanosome biogenesis [6]; Griscelli syndrome – failure of transfer of melanosomes to keratinocytes [8]; *Pityriasis alba* – deficiency in transfer of melanosomes to keratinocytes [8]. In *vitiligo*, the areas of hypogmentation are due to destruction or loss of melanocytes (not shown). In hyperpigmentary disorders of the skin, there are also deficiencies in several aspects of these processes: *Postinflammatory hyperpigmentation of the skin* – hyperplasia of epidermal melanocytes [4], increased melanogenesis [7], and increased transfer of melanosomes to the keratinocytes [8]; *Melasma* – increased tyrosinase actitivy [5], increased formation of melanosomes [6], increased melanization of melanosomes [7], increased transfer of melanosomes [8]; *Addison disease* – increased tyrosinase synthesis [5], increased melanin synthesis [7], increased transfer of melanosomes [8]. In addition, several of the hyperpigmentary disorders can lead to melanin deposition in the dermis such as: postinflammatory hyperpigmentation, melasma, and pigmentary incontinence (not shown).

Figure 2. Basal epidermal cells and melanocytes in the skin stained with dihydroxyphenyalanine (DOPA). A. An epidermal sheet of skin from a guinea pig ear was obtained by separation of the epidermis from the dermis. After incubation with DOPA, tyrosinase in the basal melanocytes converts DOPA to black DOPA-melanin. **B**. A vertical cross section through the skin showing the melanocytes in the basal layer of the epidermis making contact with the surrounding keratinocytes.

Figure 3. Stages in the development of human melanosomes containing eumelanin. Stage I melanosomes are spherical vesicles with tyrosinase, tyrosinase-

related proteins and an amorphous matrix. Stage II melanosomes are elongate and develop proteinaceous fibrils. In stage III melanosomes, melanin is deposited on the fibrils. In stage IV of human melanosomes, melanin deposition is complete. (Courtesy of Dr. George Szabo)

Figure 4. Models for deposition of melanin in the epidermis. A. In the *cytophagocytosis or dendritic phagocytosis model*, the tip of the melanocyte dendrite is pinched off and taken up by cytophagocytosis by the keratinocyte. **B.** In the *membrane fussion or tunneling nanotube model*, the tip of the melanocyte membrane fuses with the keratinocyte membrane and a tunneling nanotube serves for transfer of melanosomes from the melanocyte to the keratinocyte. **C.** The *shedding-phagocytosis model* proposes that melanosomes are shed in vesicles from the melanocyte and taken up by phagocytosis into the keratinocytes. **D.** The *exocytosis/endocytosis model* proposes that individual melanosomes are exocytosed by the melanocyte dendrite and endocytosed by the keratinocytes. (From Wu, X, Hammer, JA. Melanosome Transfer: it is best to give and receive. Current Opin Cell Biol. 2014; 29:1-7. with permission. Copyright, Elsevier)

Figure 5. Association of a melanocyte with a keratinocyte. Electron micrograph of a section of a melanocyte and keratinocyte from guinea pig ear skin in co-culture. The tip of the melanocyte (M) dendrite is in contact with a keratinocyte (K). Microfilaments FM) are present beneath the keratinocyte cell membrane. (From Wikswo, MA, Szabo, G. Effects of cytochalasin B on mammalian melanocytes and keratinocytes, J. Invest. Dermatol. 1972; 50:163-169. with permission. Copyright, The Williams & Wilkins Co.)

Figure 6. Pinching off of the tip of a melanocyte dendrite in contact with a keratinocyte. Electron micrograph of a section of a melanocyte and keratinocyte in co-culture obtained from guinea pig ear skin in co-culture. The tip of the melanocyte dendrite is in contact with a keratinocyte and is being pinched off during phagocytosis by the keratinocyte.

Figure 7. Tip of a melanocyte dendrite phagocytosed by a keratinocyte. Electron micrograph of a cross-section of a keratinocyte in co-culture with melanocytes from guinea pig ear skin,. The keratinocyte is phagocytosing the tip of the melanocyte dendrite which contains numerous melanosomes.

Figure 8. Electron micrograph of a melanocyte dendrite. Section of a melanocyte dendrite in a co-culture of melanocytes with keratinocytes obtained from guinea pig ear skin. Actin microfilaments are present beneath the melanocyte membrane surface. Melansomes are aligned along a microtubule in the dendrite.

Figure 9. Electron micrograph of a kerationocyte. Section of a keratinocyte in co-culture with melanocytes from guinea pig ear skin. Actin microfilaments are present beneath the keratinocyte membrane surface. (From Wikswo, MA, Szabo, G. Effects of cytochalasin B on mammalian melanocytes and keratnocytes, J. Invest. Dermatol. 1972, 50:163-169. with permission. Copyright, The Williams & Wilkins Co.)

Figure 10. Fate of melanosomes transferred from melanocytes to keratinocytes in skin from diferent racial groups. Electron micrograph of sections of the epidermis from human skin from different racial groups showing the differences in distribution of melanosomes transferred from the melanocytes into keratinocytes. **A.** In Caucasian

skin, melanosomes are arranged in groups of two or more and are surround by a membrane; **B.** In Asian skin, melanosomes are arranged in groups in the keratinocytes. There are fewer melanosomes per group but they are larger in size than those in Caucasian skin; **C.** In African/American skin, the melanosomes in the keratinocytes are predominately single rather than in groups. They are larger than those found in Caucasian or Asian skin and contain more melanin. Variations in these patterns are also observed (From Szabo, G, Gerald, AB, Pathak, MA, and Fitzpatrick, TB. Racial differences in the fate of melanosomes in human epidermis. Nature.1969; 222: 1081-1082. with permission. Copyright, Nature Publishing Group.)

11. Photomicrograph of melasma. Increased pigmentation in the skin is primarily in the keratinocytes in the basal layer (unstained; their natural color is brown) due to increased activity of the melanocytes, increased production of melanin and increased transfer of melanin to the keratinocytes. There is also a lesser amount of melanin pigment that began in the epidermal basal layer but which is now located in the dermis (pigment incontinence) secondary to increased permeability of the dermal-epidermal interface due to inflammation (dermatitis) in the epidermis. (Hematoxylin and eosin, x380)

12. Photomicrograph of pigment incontinence. A. Pigment is observed in the dermis due to transfer of melanin (brown) from the epidermis into the papillary (superficial) dermis secondary to inflammation in the epidermis “dermatitis”. This inflammation makes the dermal-epidermal interface more permeable to melanin deposits, so that they end up in macrophages (melanophages) in the papillary dermis. (Melanin A, Mart 1, immunohistochemical stain, x 980). **B.** Progressive movement of

melanin (seen as brown granules) into the superficial dermis is observed on the right side of the figure. Movement into the central and deeper parts of the dermis is seen on the left and central parts of this figure where is is found in melanophages. Hematoxylin and eosin x560.

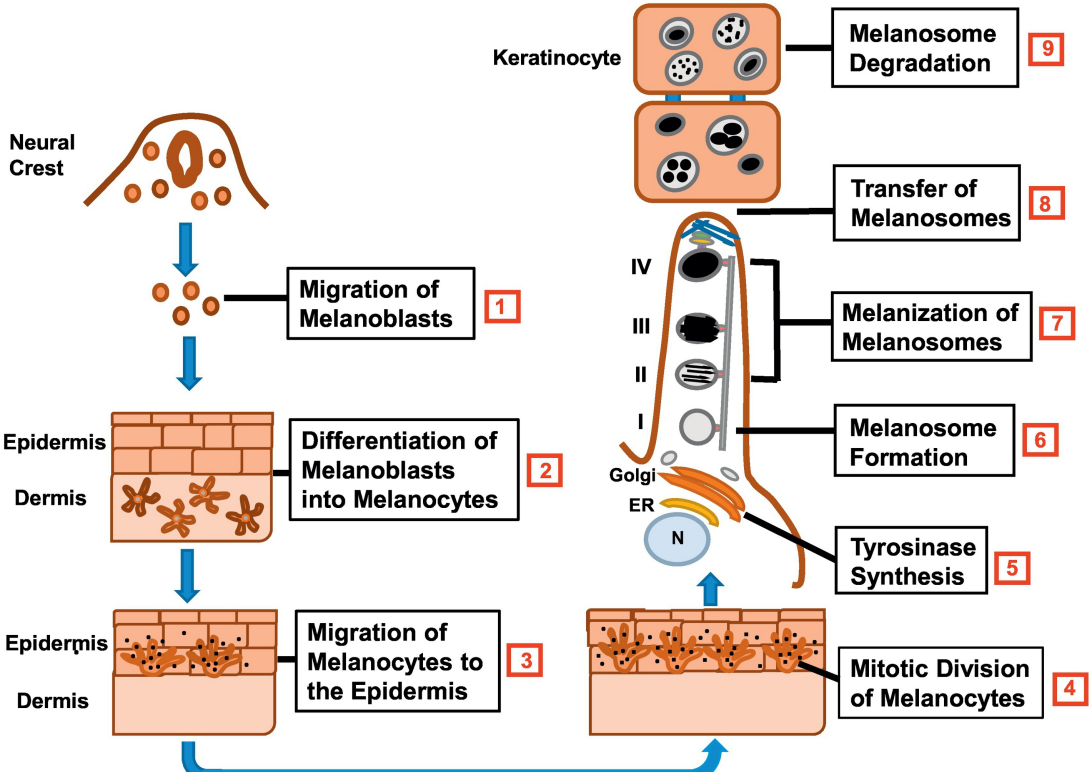

Neural Crest
Migration of Melanoblasts
1
Epidermis
Dermis
Differentiation of Melanoblasts into Melanocytes
2
Epidermis
Dermis
Migration of Melanocytes to the Epidermis
3
Mitotic Division of Melanocytes
4
Tyrosinase Synthesis
5
Golgi
ER
N
Melanosome Formation
6
IV
III
II
I
Melanization of Melanosomes
7
Transfer of Melanosomes
8
Keratinocyte
Melanosome Degradation
9

Figure 1

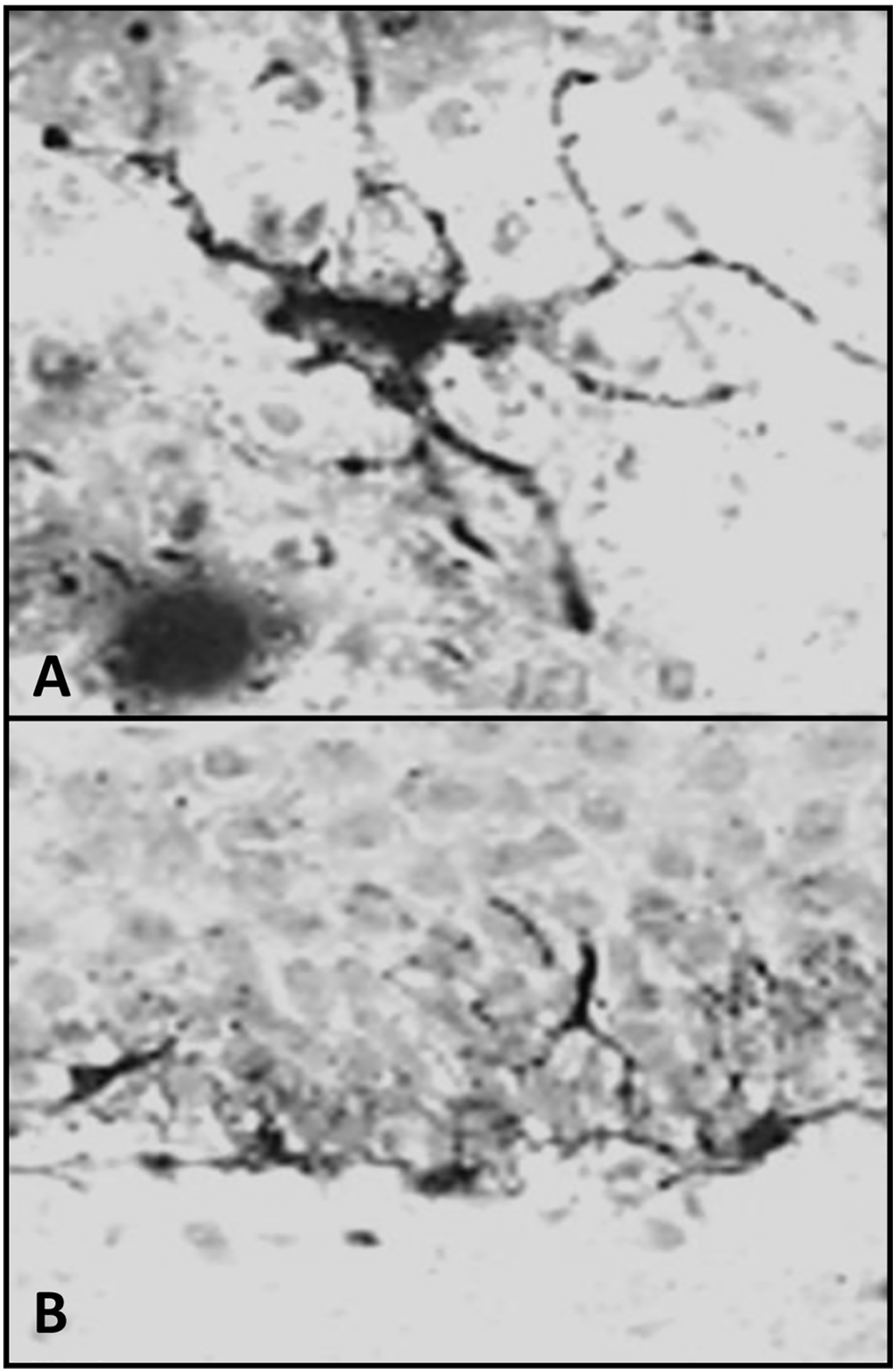


Figure 2

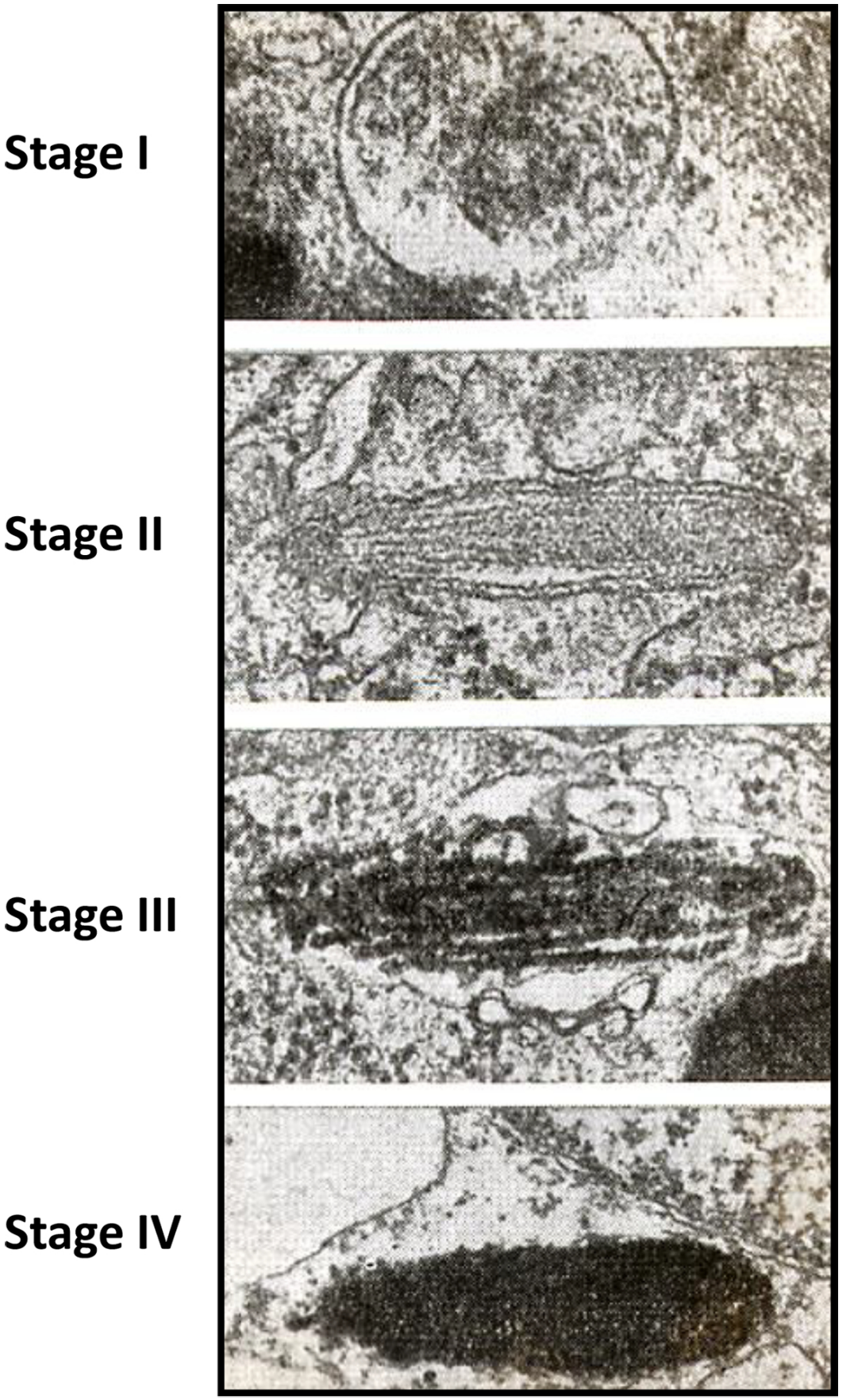


Figure 3

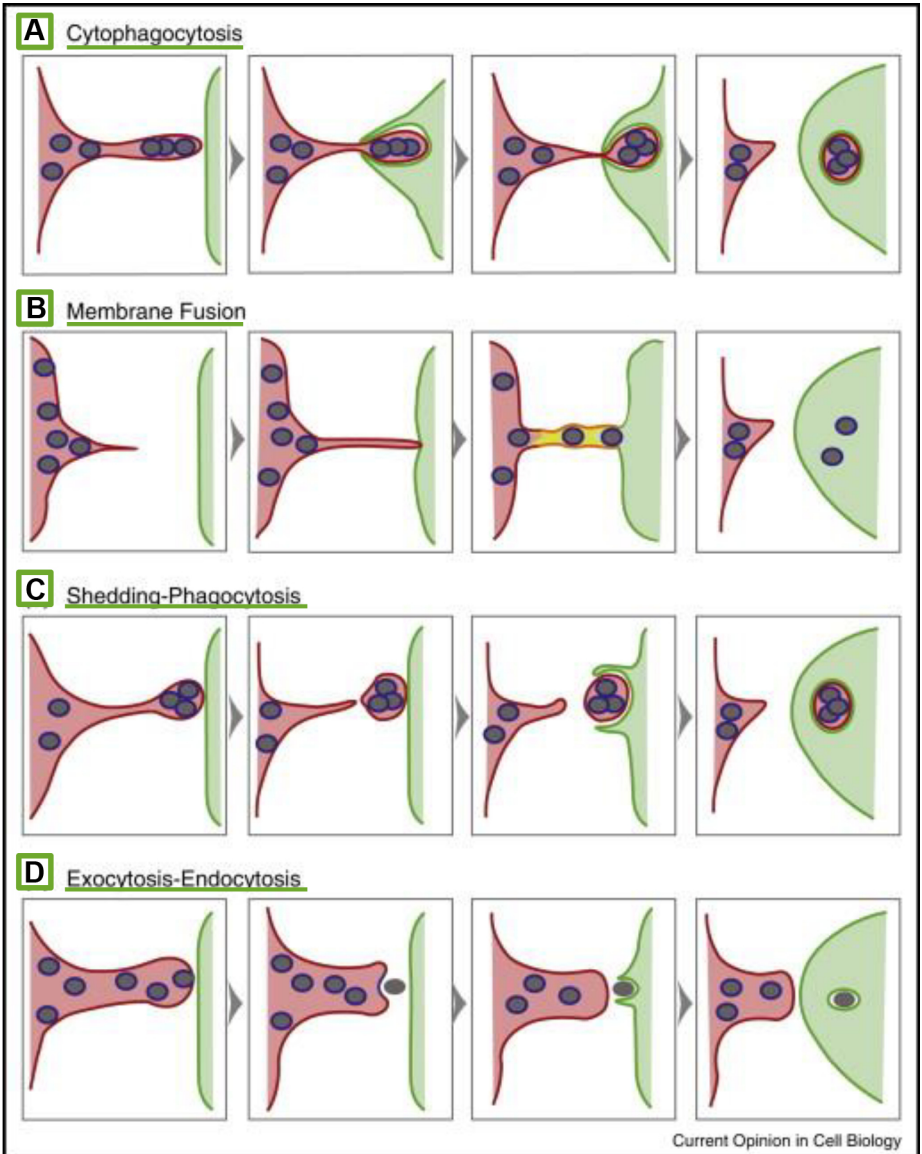

A
Cytophagocytosis
B
Membrane Fusion
C
Shedding-Phagocytosis
D
Exocytosis-Endocytosis
Current Opinion in Cell Biology

Figure 4

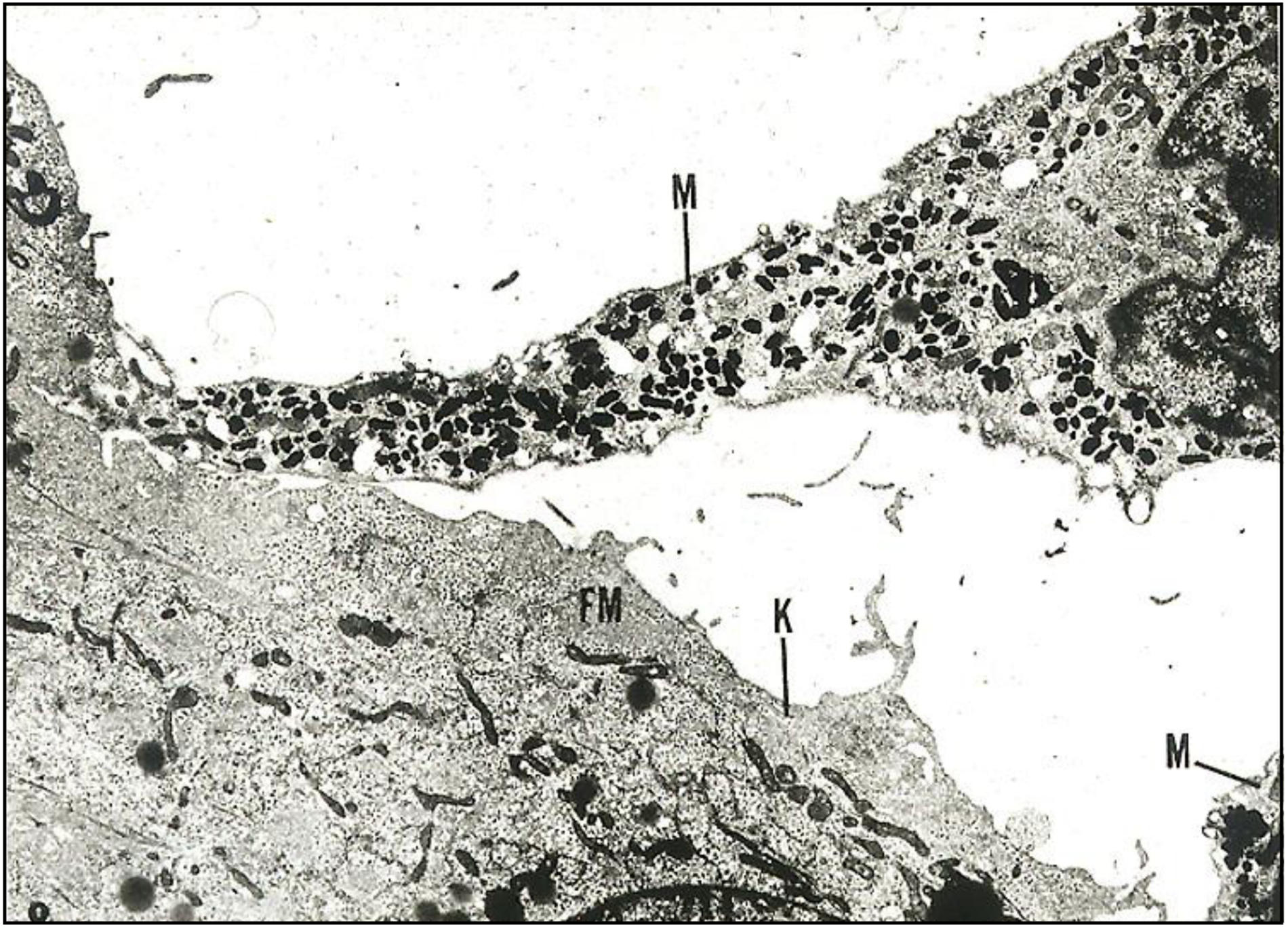


Figure 5

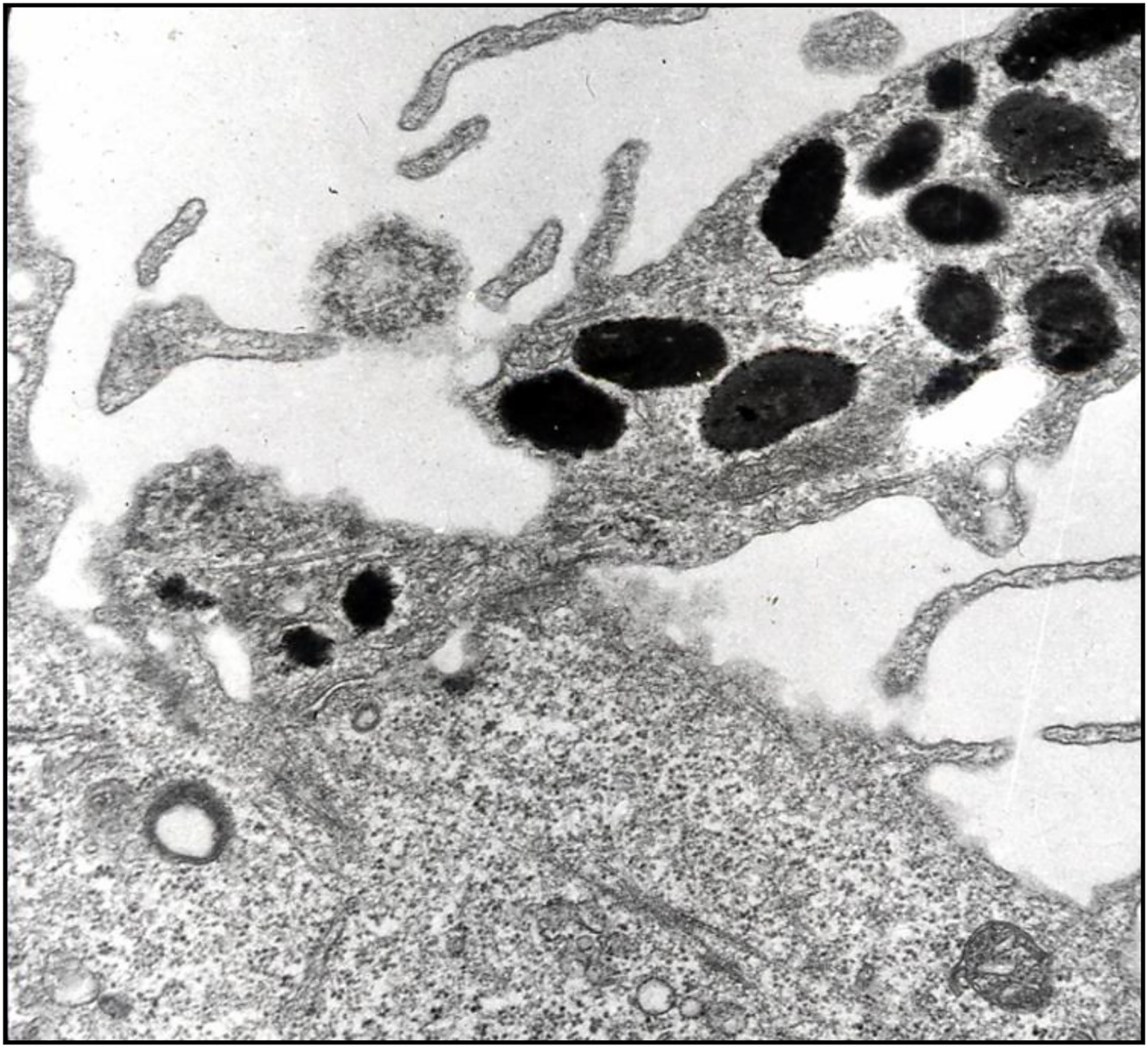
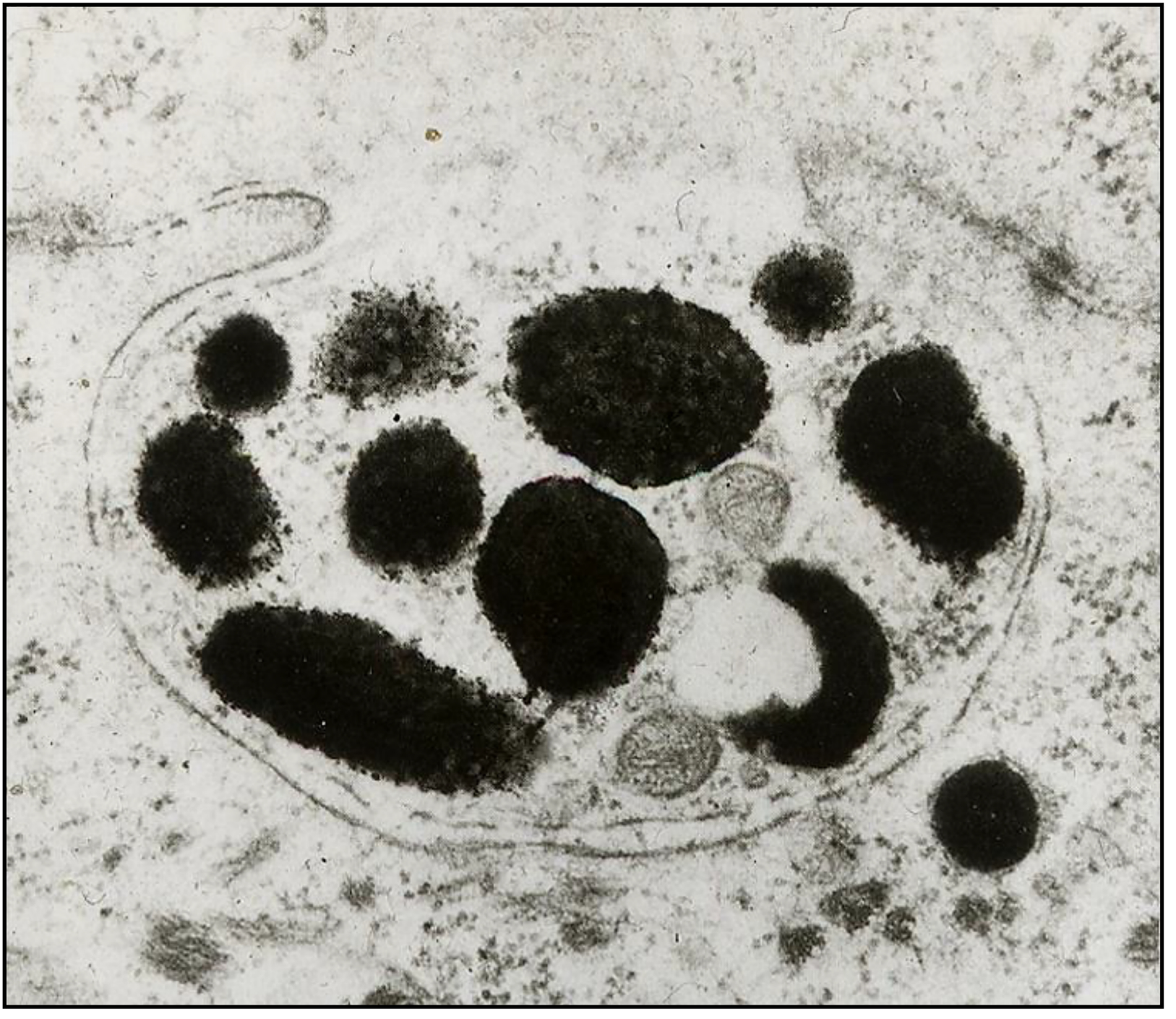


Figure 6



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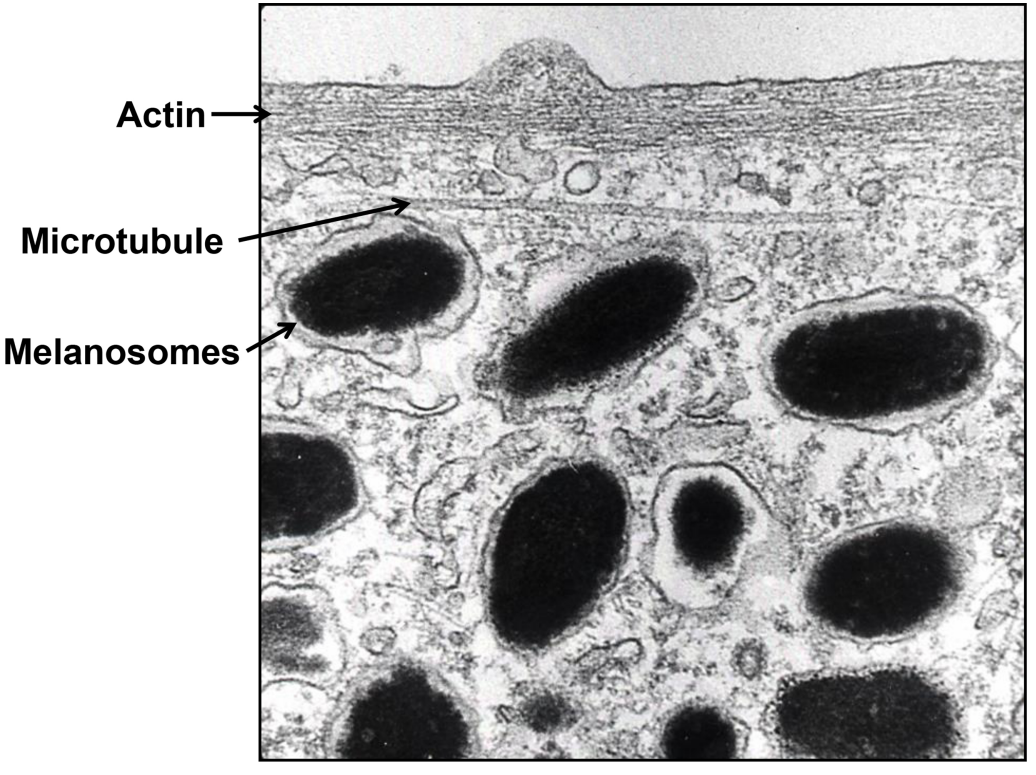

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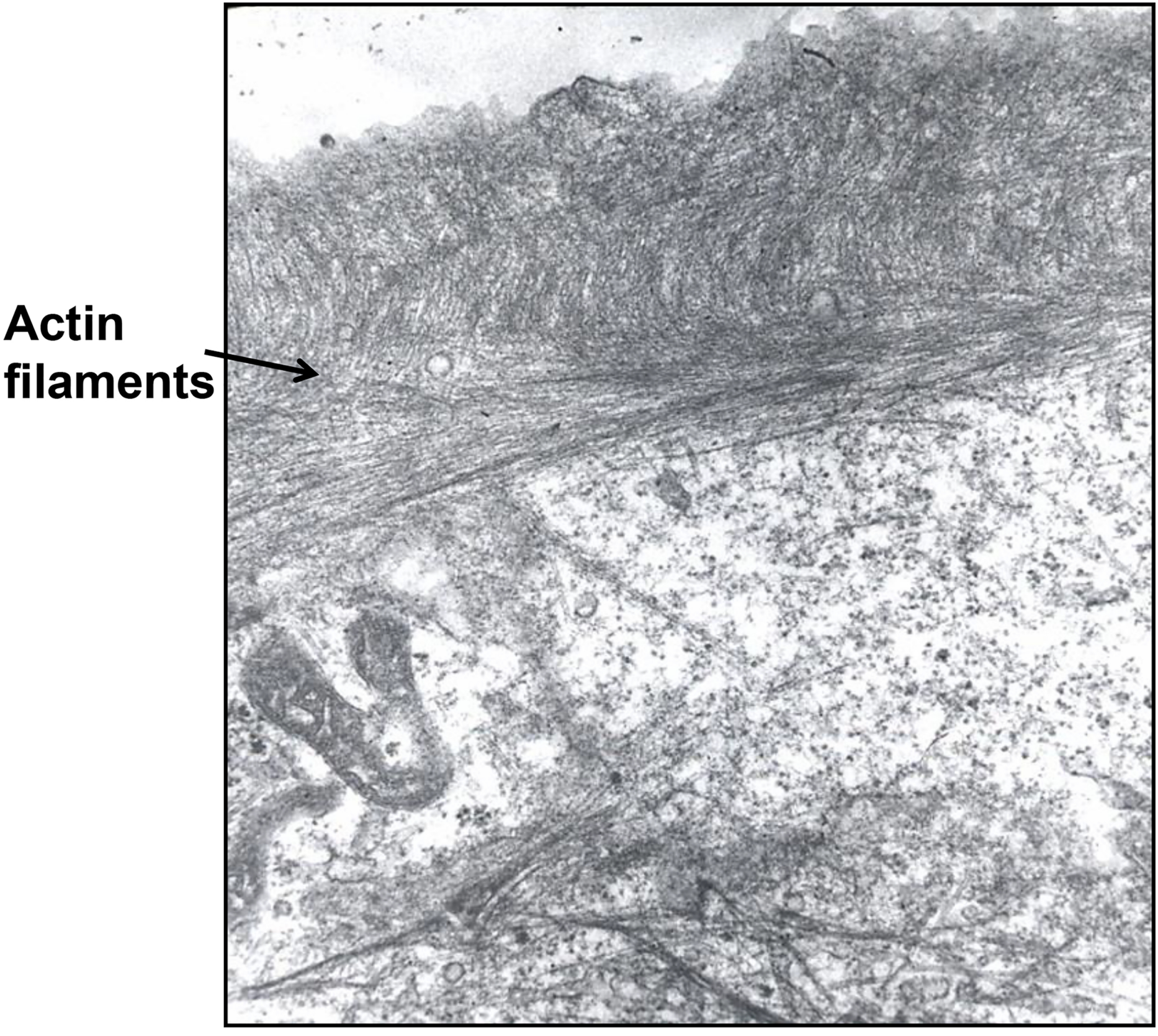

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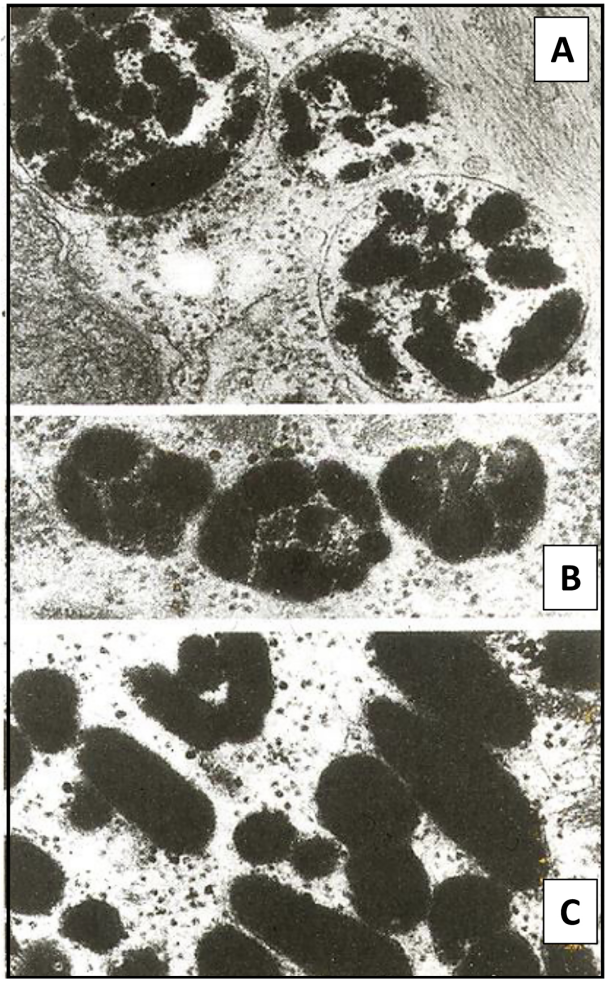


Figure 10

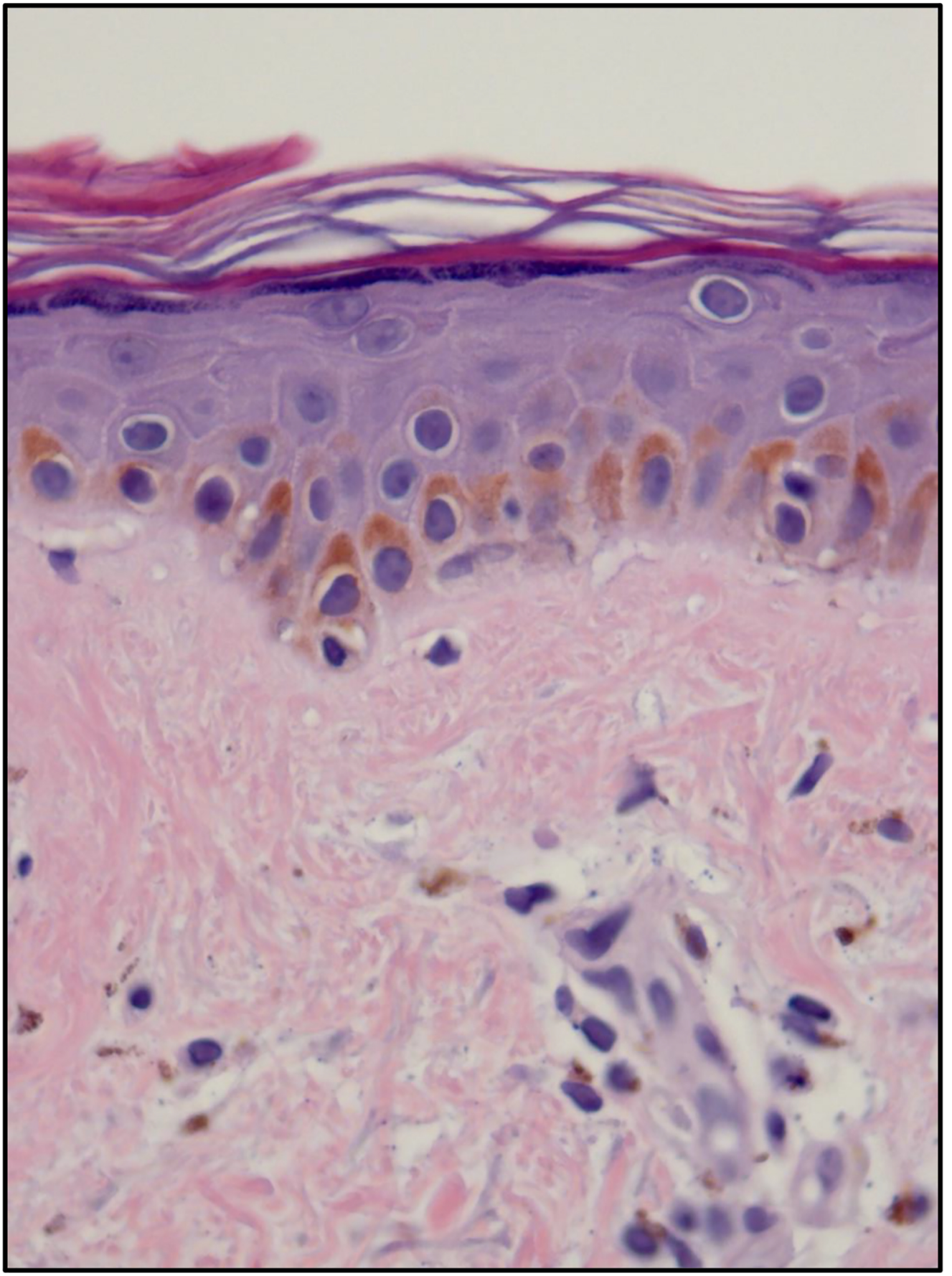
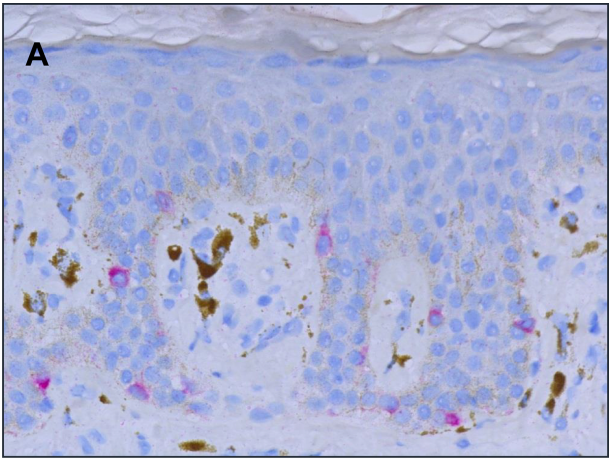


Figure 11



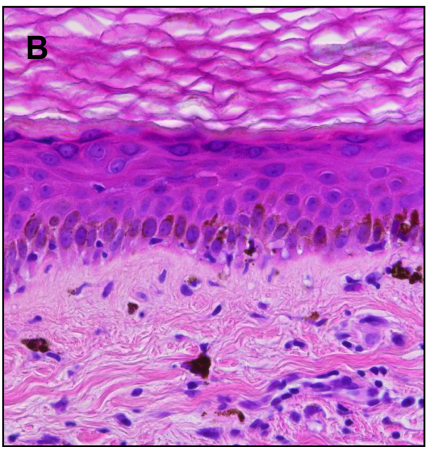


Figure 12