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MELANOMA STUDIES *

I. THE DOPA REACTION IN GENERAL PATHOLOGY

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Introduced in 1917 by the distinguished dermatologist of Zurich, Bruno Bloch, [1-4] and defended by him vigorously ever since, [5-15] the dopa reaction has been used chiefly by the dermatopathologist (Becker,[16, 17] Peck, [18-20] and Kissmeyer [21, 22]). It deserves to be more widely known and practiced both by the histologist and by the general pathologist. Following the simple method devised by Blackberg, which is described in detail in the second paper of this series, the dopa reaction ** can be carried out easily and accurately in any laboratory.

The dopa reaction is specific for two kinds of cells, for melanoblasts (a term which includes all melanin-producing cells as distinguished from mere phagocytes), and for myelogenous leucocytes (cells which have no known connection with melanin production). Both of these cells contain a ferment, an oxidase, which converts dopa to melanin. The newly formed melanin colors the cell black. This blackening of the reacting cell is the dopa reaction. The ferment of the melanoblast is dopa-oxidase — specific for dopa — it oxidizes nothing else. The ferment of the leucocyte is a polyphenol-oxidase, oxidizing many phenols to colored products.

Figure 1 shows the dopa reaction of a Hodgkin's lymph node. The black spots are the eosinophil and neutrophil leucocytes. Lymphocytes and collagen are colorless.

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** The word "dopa" is Bloch's abbreviation for 3, 4-dioxyphenylalanin.

Figure 2 shows the dopa reaction of normal Caucasian skin. In the colorless derma are a few black leucocytes. In most sections of normal skin there are a few scattered leucocytes; they have no significance. The chief point of interest is the blackening of certain cells in the basal layer of the epidermis and in the matrix of the hair — that is to say — the sites of melanin production. These are the melanoblasts revealed by the dopa reaction.

The melanoblasts of the epidermis assume many shapes — round, cuboidal, columnar and branched. Traditionally the branched cells of the epidermis are called dendritic. Often they contain melanin, often not. Their common property is the ability to oxidize dopa to melanin, blackening in dopa solutions. Bloch holds that in this dopa-oxidase which converts dopa to melanin he has discovered the agent which manufactures natural melanin in mammalian skin. The dopa reaction thus becomes an indicator of the presence of the natural oxidase. Leucocytes being excepted, it follows that every dopa-positive cell is an active melanoblast.

After many experiments on all sorts of tissue, normal and pathological, pigmented and non-pigmented, I accept Bloch's view as the most satisfactory working hypothesis yet offered to explain the production of melanin in human skin. The illustrations will show some of the evidence which led to this conclusion.

Our material consisted of freshly excised surgical specimens from many parts of the human body. Our first and fundamental conclusion was that, leucocytes being excepted, dopa-positive cells are found only in those tissues where melanin is being produced, or where it can be produced under appropriate stimulation. These tissues normally are the skin and the mucous membranes of ectodermal origin; pathologically they are pigmented moles and malignant melanomas.*

Melanin in Excess

Having agreed with Bloch that dopa-positive cells are found only in melanin-producing tissue, in our next finding we were obliged to

* The pigment of the eye constitutes a special field which has been little cultivated. Since the dopa reaction requires fresh tissue, or tissue that has been fixed in 5 per cent formalin for five hours at the longest; and since it is customary to fix specimens of human eye much longer than that, there are practical difficulties in attempting dopa reactions with this material. A thorough study of the dopa reaction of the embryonic eye was made by Miescher.[22]

agree with him again that wherever melanin is being produced in excess, there the dopa-positive cells are increased in number and often in complexity of dendrites also. Figure 3 is the dopa reaction of normal negro skin from the breast. The dopa-positive cells are more numerous than in the normal Caucasian skin of Figure 2. In this negro skin most of the dopa-positive cells are round and devoid of dendrites, negativing the common belief that a melanoblast must be dendritic. Figure 4 shows circumanal skin from another negro. The dopa-positive cells are numerous; many of them are dendritic. Figure 5 shows the increased number of dopa-positive cells in the epidermis of a dark brown patch of von Recklinghausen's disease in Caucasian skin. All of these melanoblasts are round, without dendrites. Figure 6 shows Caucasian skin that has been tanned by exposure to X-ray. Here melanin production is very active. The dopa-positive cells of the epidermis are numerous. The many leucocytes in the corium are an expression of the X-ray dermatitis.

Thus, it matters not whether the pigmentation be racial (negro), or pathologically congenital (von Recklinghausen) or acquired, or a reaction to radiation from without (sunlight, radium, thorium, X-ray), the increased production of melanin is attended by an increase in the dopa-positive cells.

Figure 7 shows the dopa reaction of a pigmented mole, illustrating Bloch's conception of the distinction to be made between active and latent melanoblasts. This distinction will be discussed under the caption Latent Melanoblasts. In the upper zone of this mole there is abundant melanin; here the nevus cells are strongly dopa-positive. Passing downward there is less and less melanin and correspondingly the dopa reaction grows fainter and soon disappears. Three-fourths of the cells in this nevus are producing no visible melanin; these cells are dopa-negative. The limitation of melanin production to the upper layers of a pigmented mole is common. It is seen again in Figure 8. In both of these moles the melanin-producing cells are round and devoid of dendrites.

Dendritic Cells

The common belief that melanoblasts must be dendritic is an error carried over from the frog histology which has long dominated human melanogenesis. It is true that active melanoblasts are often dendritic, but they are not necessarily so. In the pigmented skins

and in the moles illustrated, and in malignant melanomas, the active melanoblasts may be of any shape — round, cuboidal, stellate or dendritic. The shape of the cell has absolutely nothing to do with its melanin-producing power or with its malignancy. Figure 9 shows the dopa reaction of another pigmented mole removed from the arm of a negress. This unusual mole consists exclusively of dendritic cells, all of them dopa-positive. The clinical history of such moles differs in no way from that of moles composed of round or other non-dendritic cells.

Melanin Absent

Having become convinced that an increase in the number of dopa-positive cells goes hand in hand with increased activity of pigment formation, we studied the opposite condition — decrease and absence of melanin. Figure 10 shows the dopa reaction from the edge of a patch of vitiligo of negro skin. On the left is seen normal pigmented negro epidermis with its dopa-positive cells. Then comes a zone of bizarre dendritic cells, as is usual at the margin of pigmentation or depigmentation. On the right the colorless skin begins. Here the dopa-positive cells have disappeared, together with the melanin. This disappearance of the dopa-positive cells in vitiligo was described first by Bloch,[24] who reported a similar disappearance of dopa-positive cells from the hair matrix of graying hair.[25]

Figure 11 shows the dopa reaction from the edge of a black spot on the white ear of a guinea-pig. Melanin and dopa-positive cells are abundant in the black spot, totally absent from the white skin. Bloch [7] and his school have shown repeatedly that there are no dopa-positive cells in albino skin and that no amount of radiation will elicit either melanin or dopa-positive cells in such skin, although in non-albino skin it is a simple matter to produce both of them by radiation (Lutz [26]). Thus, whether the absence of melanin be congenital (albino) or acquired (vitiligo), where there is no melanin there are no dopa-positive cells; or, to put it the other way, where there are no dopa-positive cells no melanin is made.

Melanin Reappears

The final proof that dopa-positive cells are concerned in melanin production is that they are the invariable precursors of melanin's appearance or reappearance. Among dermatologists it is well known

that some patches of vitiligo can be stimulated temporarily to repigmentation by exposure to radiation (Buschke,[27] Buschke and Mulzer,[28] With [29]). In such repigmentation of vitiligo, both Bloch and Kissmeyer [30] have reported that the dopa-positive cells reappear first; melanin follows. In my own observations of the repigmentation of scars, this sequence is invariable; it is especially striking in negro skin. Bloch [7, 25] has described the same sequence in the human embryo, Miescher [23] in the eye of the embryo chick, rabbit and guinea-pig, Peck [19] in tanning of human skin by thorium radiation. The dopa-positive cells appear first, melanin next, never in the reverse order. The inference is that the dopa-positive cells are essential to the production of melanin.

The Acanthoses

In his early experiments Bloch soon found dopa-positive dendritic cells to be abundant among the epithelium of the acanthoses. The word is Unna's; it applies to the thickening of the prickle-cell layer of the epidermis seen in psoriasis, papilloma, condyloma and similar lesions. Usually there is no excessive pigmentation, often no melanin whatever. This observation has been confirmed by all dopa workers. I have seen it repeatedly. In fact, the massing of dopa-positive dendritic cells a little distance back from the margin of a granulating wound might be ascribed to the acanthosis always present in this zone quite as justly as to their being forerunners of pigmentation.

According to Kyrle,[31] that rare spirit too early lost to dermatopathology, the cells of the epidermal basal layer have two functions, melanin formation and proliferation. It seems that stimulation of either function increases the number and complexity of the dopa-positive cells. I have been unable to correlate this acanthotic increase of these cells with their pigment function. This phase of their activity awaits adequate explanation.

Mucous Membranes

Among mucous membranes, production of melanin is confined to those of ectodermal origin. Adachi [32] found melanin in the epithelium of the mucous membrane of the cheek, lower lip, prepuce and vagina. He was limited to hand-cut, unstained sections. Using both the silver and the dopa reaction, Redslob [33] found melanin and dopa-positive cells in normal human conjunctiva, Ramel [34] in normal hu-

man "buccal mucosa" (site not stated). Becker [16] found both melanin and dopa-positive cells in Adachi's locations and added the normal mucous membrane from the middle of the cheek and from the pharynx at the level of the hyoid bone. Laidlaw and Cahn [25] found dopa-positive cells and melanin in normal human gum (Fig. 12); Laidlaw (unpublished) in normal anal canal. The presence of melanoblasts explains the occurrence of primary melanoma in these mucous membranes and also the absence of primary melanoma in non-ectodermal mucous membranes which normally harbor no melanoblasts and make no melanin.

In blonds, melanin and dopa-positive cells of the mucous membranes are scanty, as they are in blond skin; in the mucous membranes of negroes and brunets, they are abundant, an observation already made by Adachi in regard to melanin. To the naked eye, such a mucous membrane may look pink and without a trace of pigmentation; nevertheless, on microscopic examination melanin will be found in abundance. Ramel noted racial pigmentation of the mouth in Tziganes; Cahn observed similar racial pigmentation of the mouth in negroes and in a negroid type of Bavarian Jew.

Chromatophores of the Derma

In the upper layers of the derma of every normally pigmented skin there are seen cells of various shapes containing melanin. These cells never give the dopa reaction. Consequently according to the dopa hypothesis they contain no oxidase and they cannot have produced the melanin which they contain. Often these cells are obviously phagocytic; they seize and retain any pigment that happens to enter the skin, such as tattoo pigment, blood pigment and gunpowder. Miescher [36] proved their phagocytic power for melanin and their long retention of it by injecting melanin into living human skin and excising bits of skin at various intervals afterward. To distinguish them from melanoblasts, these cells are called pigment carriers, chromatophores.

Within certain limitations, to be mentioned presently, the dopa reaction is the one reliable method of distinguishing the melanoblast from the chromatophore. This distinction may be seen in any negro skin or pigmented Caucasian skin. Miescher [37] and von Albertini and Walthard [38] have used the dopa reaction to identify melanoblasts in the metastases of malignant melanoma.

Latent (Dopa-negative) Melanoblasts

The use of the dopa reaction as a specific stain for melanoblasts stumbles over the difficulty that the cell capable of producing melanin is not always and everywhere dopa-positive; it does not always contain the oxidase. This is seen readily in pigmented moles and in melanomas where broad areas of the tumor are free from visible melanin and from dopa-positive cells. Here a negative reaction means nothing; only the positive cells count. (The situation is different with the chromatophores of ordinary pigmented skin. Thousands of microscopic sections examined by competent dopa workers have never once revealed a positive dopa reaction in the chromatophores of the upper derma. We may accept them as permanently lacking the melanin-producing oxidase).

In his first publication, Bloch [1] wrote that in malignant melanoma the cells around the growing margin of the tumor were most apt to be dopa-positive, while many of the cells in the center of the tumor were negative — an observation confirmed by Miescher [39] and by von Albertini and Walthard.[38] Both Bloch and Miescher point out that the dopa-positive melanoblast, whether dendritic or non-dendritic, does not always contain melanin, neither is the melanin-containing cell always dopa-positive. These discrepancies are explained best by Bloch's ferment hypothesis, according to which a distinction must be made between the oxidase (which carries out the dopa reaction), and the finished melanin (which does not carry out the reaction). The dopa-positive factor — the oxidase — appears in the cell some time before the resulting melanin is visible (see caption Melanin Reappears); the melanin itself may remain in the cell long after the oxidase has disappeared (Miescher's experimental injection of melanin into human skin).

My own experience is in strict harmony with the ferment hypothesis; but, whatever the explanation, there remains the fact that the dopa reaction must be used with this precaution, that not all cells capable of producing melanin are at all times dopa-positive. In her studies of pigmentation of the skin of the embryo and newly born gray mouse, Steiner-Wourlisch [40] found that even in this normal, progressive pigmentation the production of melanin is not continuous, and the dopa reaction is not continuously present; there are intervals of rest.

Mongol Cells

These ribbon-like cells buried deep in the corium over the sacrum and along the backs of infants of all races are true melanoblasts, the only melanoblasts of mesodermal origin in normal human skin. Bloch [7] and Bahrawy [41] found them to be dopa-positive. As an anomaly I saw them in the neurofibromatous skin of von Recklinghausen's disease from over the sacrum of a Caucasian girl 18 years of age. As shown in Figure 13, they were dopa-positive. They blackened with silver also, proving their scanty pigment to be melanin. Melanomas arising from these cells and from their analogues, the cells of blue nevi, are the only melanotic tumors of human skin to which the name melanosarcoma can justly be applied, a view first formulated by Darier,[42] prompted by a suggestion from Bloch.

Not every nevus that looks bluish black conforms histologically to the Tièche-Jadassohn blue nevus.[43] Even epidermal melanin in the tips of long rete pegs will look bluish and not brown if there is little or no melanin in the surface epithelium (Fig. 12). Sato [44] has made a similar observation of ordinary nevus nests that were deeply placed in the corium. The diagnosis of blue nevus, and the sequent melanosarcoma, should rest on microscopic examination.

Sarcoma and Carcinoma

The dopa reaction has no relation to malignancy, as such. In repeated tests of various forms of non-melanotic sarcoma and carcinoma, I have found the tumor cells to be consistently dopa-negative.

Epithelioma

Recalling Kyrle's dictum that the function of the epidermal basal cells is twofold, proliferation and melanin production, it might be expected that the progeny of these cells in forming an epithelioma would show some melanoblastic characteristics, especially since acanthosis or thickening of the prickle-cell layer is attended by an increase in the dopa-positive cells. This expectation is fulfilled. As in all tumors, function is performed imperfectly and irregularly. In some epitheliomas I find no dopa-positive cells; the dopa-positive cells of the overlying skin stop some distance back from the edge of the ulcer as they do in non-malignant granulating wounds. In other

epitheliomas the dopa-positive cells continue in the surface epithelium over the tumor, or a few scattered dopa-positive cells are seen among the tumor epithelia, chiefly in the basal layer. As in the acanthoses, these cells are mostly dendritic. Their presence in an epithelioma seems to have no significance.

Melanosis Coli

In melanosis coli the tunica propria of the mucosa contains many large, round, stellate and spindle cells loaded with yellow-brown granules. In the specimens which I have examined the pigment reacts like melanin in that the fine granules blacken quickly in silver, while the larger granules require a long time, perhaps several days. Current opinion is divided as to the nature of the pigment, but is agreed that it has been absorbed from the intestinal contents and phagocytized by these cells. The view that the cells are phagocytes is corroborated by Walthard's[45] finding them dopa-negative at autopsy.

Negative dopa reactions of autopsy material are open to suspicion, owing to the length of time that necessarily elapses between death and the immersion of the tissue in dopa. By the kindness of Doctor Janssen, I was able to immerse a specimen of diffuse melanosis of the upper part of the rectum in dopa within two hours of its excision from the living body. Microscopically the mucosa presented the typical appearance of melanosis coli. The pigment-bearing cells were dopa-negative. There were no dopa-positive cells, except polynuclear leucocytes which abound in this mucosa. I have treated many fresh surgical specimens of colon and rectum with dopa and also tested them for melanin with silver with consistent negative results. The conclusion is that there are no melanoblasts and no melanin in the colon or in the rectum above the mucocutaneous junction. It follows that the occurrence of primary melanoma above this line is highly improbable. If it occurs, it must spring from misplaced islands of ectoderm.

The Silver Controversy

Soon after Bloch's first publications Heudorfer[46] declared that there is nothing specific in the dopa reaction and that it merely duplicates pigment staining with silver, an assertion endorsed by Lem-

mel [47] and Meirowsky.[48] My own experience, covering hundreds of sections of skin stained with various silver techniques, and additional hundreds of dopa sections compared with silver staining of the same skin, agrees absolutely with Bloch's statement that the dopa reaction and the silver reaction are totally different things. To an experienced dopa worker it is obvious that Bloch's critics have been misled by overstained dopa sections. The correct reactions could never be mistaken for one another. Silver blackens melanin whereever found, in the derma as in the epidermis, in melanoblasts, phagocytes, and free in the lymph spaces indifferently. If the dopa-positive cells happen to contain melanin they will stain with silver; otherwise not. Dopa, on the other hand, singles out the active melanoblasts, leaving the melanin in the resting cells unstained. The only melanin that blackens with dopa is the melanin inside of a dopa-positive cell, on which the dopa-melanin is adsorbed or deposited.

The Dimethylparaphenylendiamin Controversy

On the publication of the dopa reaction Kreibich [49] declared that he had long secured similar effects from dimethylparaphenylendiamin (one of the components of the Schultze-Winkler formula), a position in which he is supported by Meirowsky,[48] who goes even further and finds the dopa reaction duplicated by a whole series of easily oxidizable phenols. After many trials with this most highly praised phenol of this group, I agree with Bloch [12] and with Walthard [45] that this phenol, like silver, stains only the melanin and not the protoplasm of the cell. Even when the reaction succeeds, the demonstration of the dendritic cells is far inferior to that of the dopa reaction, as Meirowsky himself admits.

The Mast Cell Controversy

Of the many controversies prompted by the dopa reaction, the oddest and the least necessary would seem to be the difference of opinion of equally well qualified observers over the reaction of mast cells. That master of dermatopathology, P. G. Unna, wrote long ago that mast cells abound in the snout of the white rat and in human neurofibroma. It is a simple matter to immerse fresh frozen sections of these tissues in dopa solution and counterstain them with cresyl

violet. In such sections it is seen beyond any possible doubt that Bloch scores once again. The mast cell, both in the white rat and in human neurofibroma, is dopa-negative. The reader may see this for himself in Figures 5 and 13, from the same von Recklinghausen neurofibroma. These sections contain myriads of mast cells, not one of which has become visible in the dopa solution.

SUMMARY

1. Bloch's dopa doctrine is endorsed as the best working hypothesis of melanin production in human skin.

2. The dopa reaction is indispensable in the study of pigmented moles, melanoma and the movements of melanin.

3. In the identification of melanoblasts with the dopa reaction, only the positive cells are significant.

4. The appearance of dopa-positive dendritic cells in non-pigmented acanthoses remains unexplained.

5. In the controversies which have arisen over the dopa reaction, Bloch's histological findings are corroborated.

In conclusion I must thank Professor Bloch for his kindness in sending me a portion of his dwindling store of dopa in the beginning of these studies two years ago; Dr. S. N. Blackberg of the Department of Pharmacology, who showed me how simple a matter the dopa reaction could be made to be; Dr. Jerome Webster for many fresh specimens from his plastic surgery clinic; and Professor Purdy Stout who generously places his choicest material at my disposal.

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DESCRIPTION OF PLATES

Plate 83

All figures are untouched photomicrographs of dopa reactions at pH 7.4 and at 37°C for from 3 to 4 hours.

Fig. 1. Hodgkin's lymph node. Myelogenous leucocytes black; lymphocytes and collagen colorless.

Fig. 2. Normal Caucasian skin from breast. In the basal layer of the epidermis there are blackened melanoblasts of various shapes, both round and dendritic. They are few in number and spaced far apart. The rest of the epithelium and the collagen are colorless.

Fig. 3. Normal negro skin from breast. In the epidermis the dopa-positive cells (melanoblasts) are more numerous than in Fig. 2. Almost all of them are round, not dendritic. The chromatophores of the corium are visible as pale gray spindle cells. They contain no ferment and do not blacken in dopa.

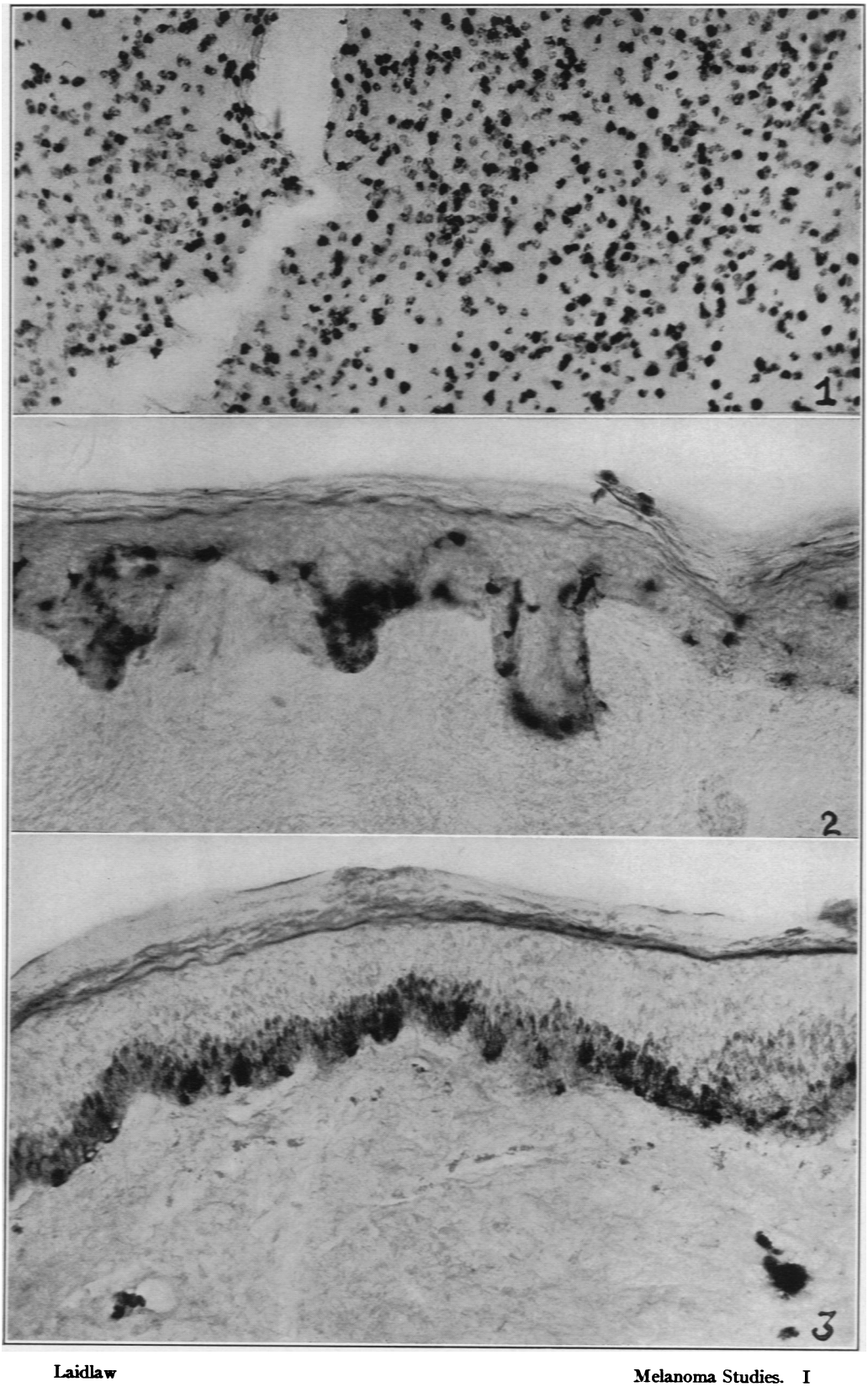
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PLATE 84

FIG. 4. Normal circumanal skin from another negro. The dopa-positive cells of the epidermis are very black, very numerous and most of them are dendritic. In this corium there are many chromatophores loaded with melanin; none of them blacken in dopa.

FIG. 5. Dark brown patch of von Recklinghausen pigmentation of Caucasian skin. Melanoblasts of the epidermis very numerous. Almost all of them are round, a few are dendritic.

FIG. 6. Caucasian skin tanned by X-ray. Dopa-positive cells of the epidermis much more numerous than in the normal epidermis of Fig. 2. In the papillary layer of the corium there are many black leucocytes, an expression of the X-ray dermatitis.

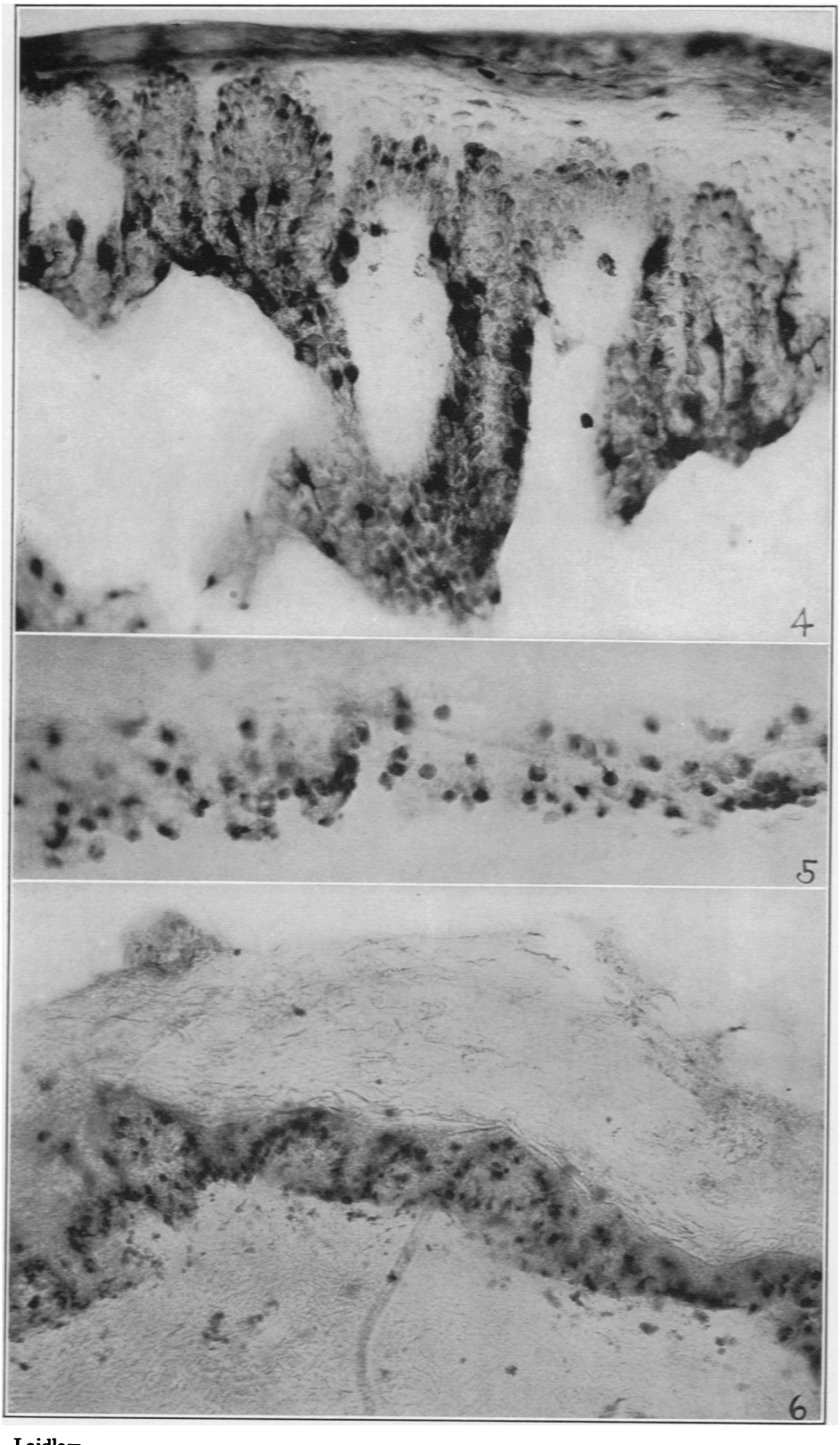
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PLATE 85

FIG. 7. Pigmented mole. In the center is a hair in its follicle. There is much melanin in the upper layers of the mole and here the nevus cells are strongly dopa-positive, very black. Passing downward the melanin decreases and correspondingly the dopa reaction becomes fainter. In the lower part of the mole, where no melanin is being produced, the nevus cells are dopa-negative.

FIG. 8. Another pigmented mole with the same features as Fig. 7. In both of these moles the active melanoblasts, the nevus cells, are round and free from dendrites.

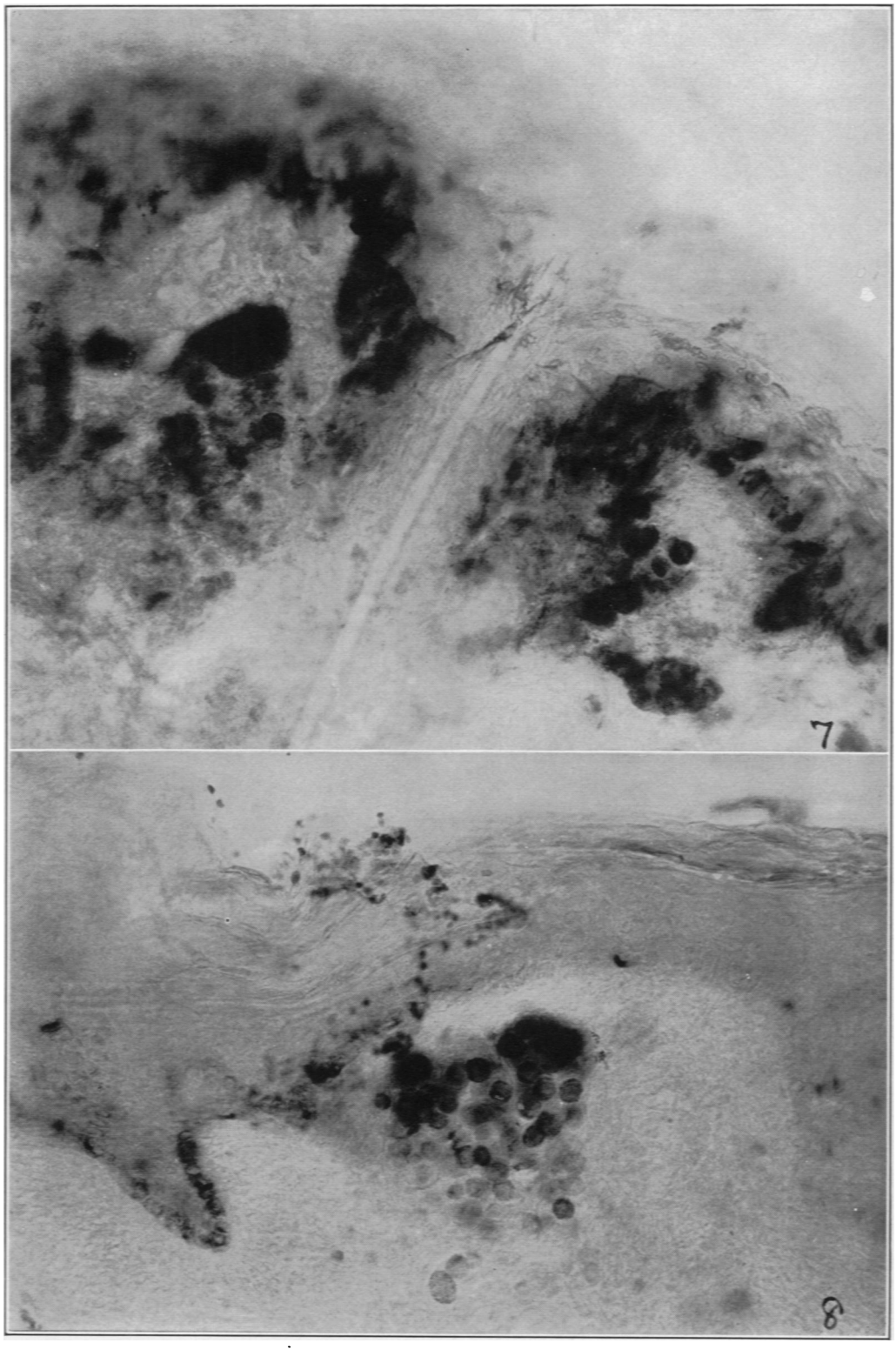
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PLATE 86

FIG. 9. Unusual pigmented mole consisting entirely of dendritic cells, all of them dopa-positive. From the arm of a negress.
(FIGS. 5, 7, 8 and 9 from patients of Dr. Webster.)

FIG. 10. Margin of patch of vitiligo of negro skin. On the left, normal negro epidermis with abundant melanin and dopa-positive melanoblasts. In the middle, the zone of bizarre dendritic cells usually seen at the margin of active pigmentation or depigmentation of the epidermis. On the right, the colorless epidermis; both melanin and dopa-positive cells have disappeared. (Patient of Dr. Marie Karelitz.)

FIG. 11. Edge of black spot on white ear of guinea-pig. In the epidermis of the black spot, melanin and dopa-positive melanoblasts are abundant; they are totally absent from the epidermis of the white skin. At the margin of the black spot, where melanin becomes scanty, the outline of the dendritic melanoblasts is seen more clearly.

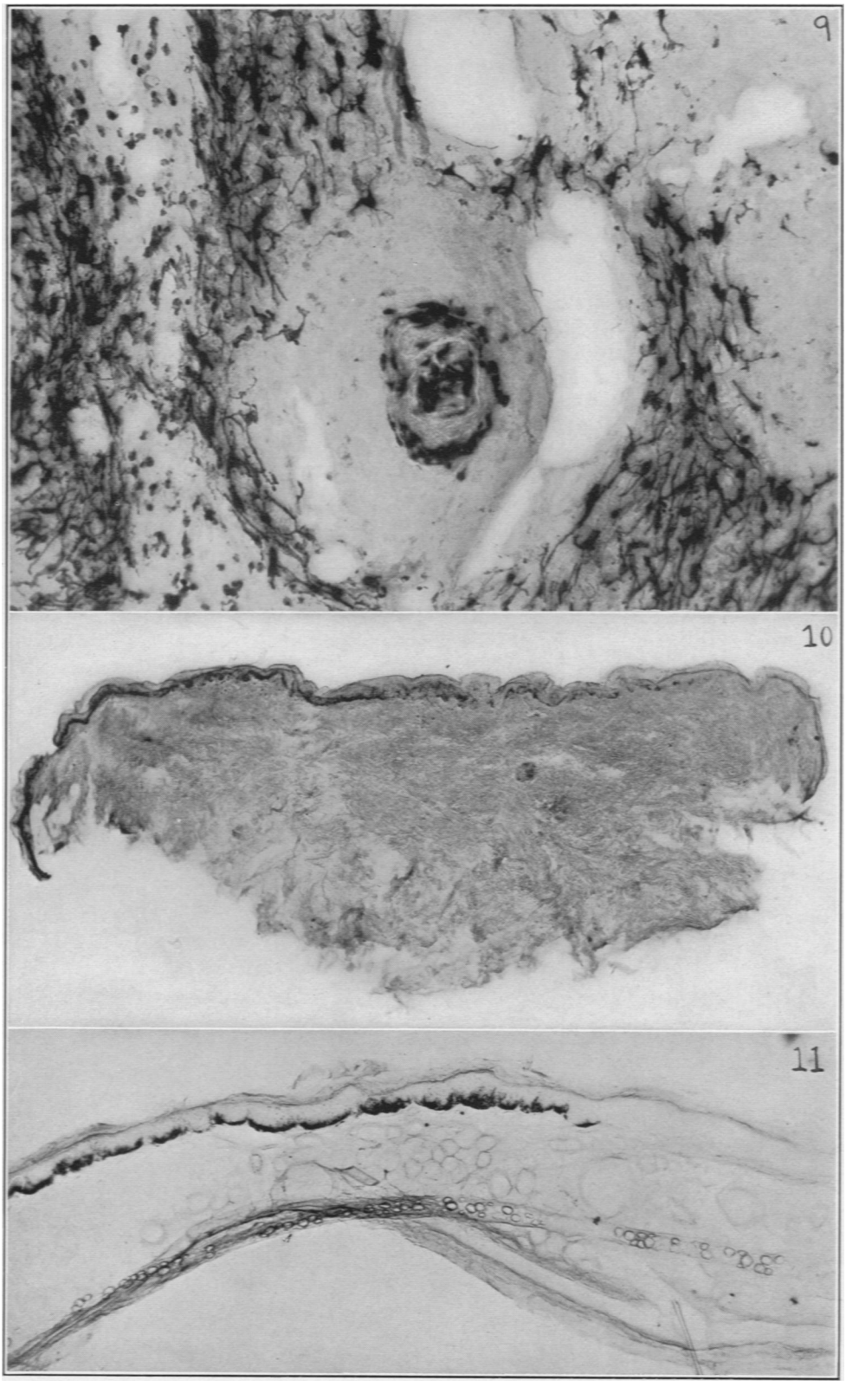
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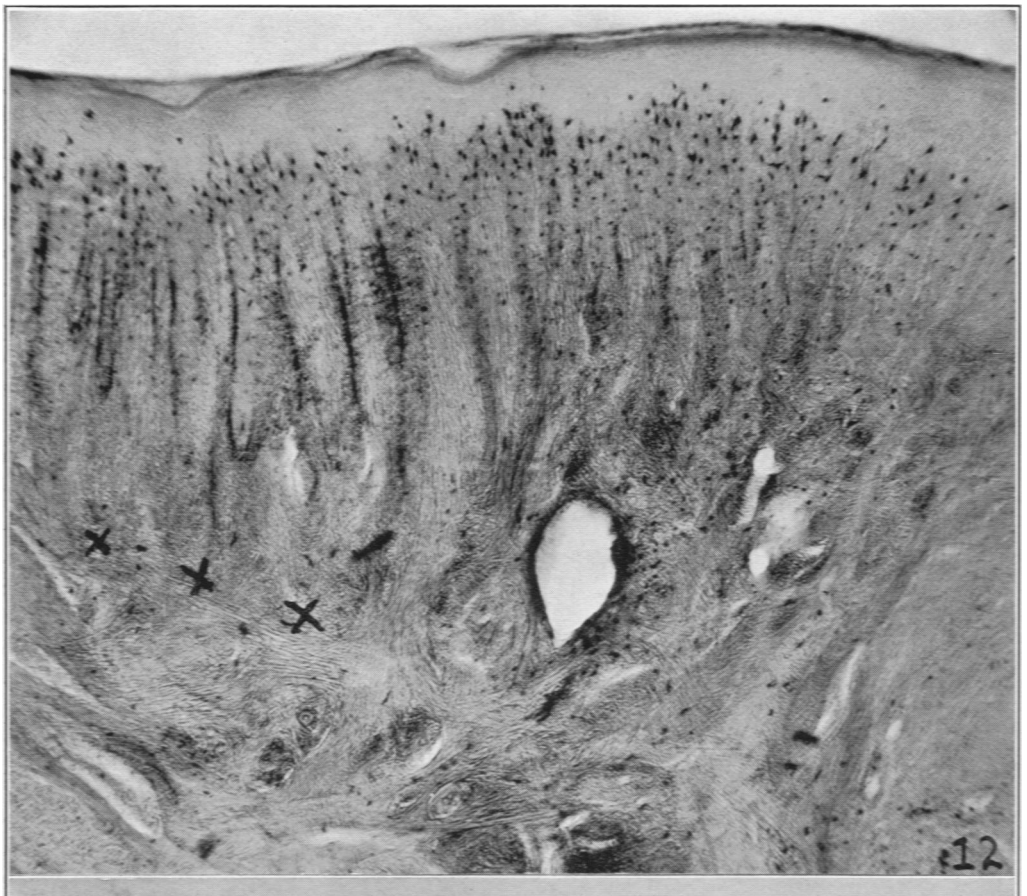
PLATE 87

FIG. 12. Slate-colored pigmentation of gum of Latin-American; black hair, dark eyes. In the middle third of the very thick stratified epithelium there are many dopa-positive dendritic cells. The melanin itself is situated chiefly in the lower end of the very long, slender rete pegs. The deep situation of the melanin and the absence of superficial pigment accounts for the blue color of the gum, although the melanin is exclusively in the epithelium and not in the corium, as in the Tièche-Jadassohn blue nevus. An X mark has been placed just below the tips of the longer rete pegs. (Patient of Dr. Lester Cahn.)

FIG. 13. Dopa-positive Mongol cells. Neurofibromatous skin in von Recklinghausen's disease from over the sacrum of Caucasian girl (Polish Jew) 18 years of age. The corium is very thick, averaging 1.5 cm. The groups of ribbon-like cells are situated deep in the corium, in its middle or lower third. They are filled with fine granules of melanin. (Patient of Dr. Webster.)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