

Human Melanocytic Naevi

By

PETER J. LEA¹ and ANDREW PAWLOWSKI²

¹Department of Anatomy and ²Department of Medicine, Faculty of Medicine,
University of Toronto, Ontario, Canada

Graphic design Jerk-Olof Werkmäster

Printed in Sweden by Almqvist & Wiksell, Uppsala 1986

The publication of this supplement was sponsored, in part, by:

Hitachi Scientific Instruments

Nissei Sangyo Canada Inc

89 Galaxy Blvd

Rexdale, Ontario

Canada

Contents

HUMAN MELANOCYTIC NAEVI. *By P. J. Lea and A. Pawlowski*

I. Electron Microscopy and 3-Dimensional Computer Reconstruction of Naevi and Basement Membrane Zone from Ultrathin Serial Sections	5
II. Depth of Dermal Protrusion; Comparative Measurements of the Basement Membrane Zone and Diameters of Intracellular Microfibrils using a Microcomputer Analysis System	17
III. Immunofluorescence and Immunoelectron Microscopy of the Basement Membrane Zone	23

Human Melanocytic Naevi

I. Electron Microscopy and 3-Dimensional Computer Reconstruction of Naevi and Basement Membrane Zone from Ultrathin Serial Sections

PETER J. LEA¹ and ANDREW PAWLOWSKI²

¹Department of Anatomy and ²Department of Medicine, Faculty of Medicine, University of Toronto, Ontario, Canada

Lea PJ, Pawlowski A. Human melanocytic naevi. I. Electron microscopy and 3-dimensional computer reconstruction of naevi and basement membrane zone from ultrathin serial sections. *Acta Derm Venereol (Stockh)* 1986; Suppl. 127: 5-15.

Ultrastructural studies were carried out to investigate epidermal and dermal components of human naevi. Serial sections from forty-three acquired human melanocytic naevi were examined in the electron microscope. Control naevi were obtained from guinea pigs, and melanoma from human subjects and guinea pigs. It was found that the epidermal melanocytes which form naevus nests, do not "drop down" and penetrate into the dermis, but remain in continuity with the epidermis. The naevus "nests" actually remain as part of the epidermis and are contained by the basement membrane derived from the epidermal-dermal junction. The three-dimensional aspect of naevus protrusion into the dermis was confirmed by computer reconstruction throughout the ribbons of serial sections for all forty-three biopsies examined. The results indicate that the present differentiation of melanocytic naevi into junctional, compound, dermal and blue naevus is not adequate.

P. J. Lea, Department of Anatomy, Faculty of Medicine, University of Toronto, Ontario, Canada M5S 1A8.

The "Abtropfung" theory (1), which led to the classification of melanocytic naevi into junctional, compound or dermal types, is still generally accepted (2) although the mechanism of "dropping off" is not understood.

The origin of naevus cells, which has been the subject of speculation for many years, has been narrowed down to melanocytes as the source (3). The connection to skin neural elements (4, 5, 6), especially in blue naevus (7), remains a controversial matter.

Melanocytes of the naevi have been found in close association with a basement membrane (7, 8). In "junctional" naevi, the epidermal-dermal junction serves as the basement membrane. In "intradermal" naevi, basement membrane has been reported as not always present (9), found as fragments (10), or as a conspicuous basement membrane (11).

The origin of this basement membrane and the significance of its 3-dimensional continuity in melanocytic naevi (12, 13) is reported in the appended ultrastructural observations.

The following terminology has been used throughout the papers comprising this series on melanocytic naevi. Melanocytes observed in nests, containing two or more cells, and or surrounded by a complete or partial basement membrane, were called naevus cells. Melanocytes and naevus cells were considered as melanin producing cells.

MATERIAL AND METHODS

Forty-three pigmented naevi were obtained from patients of twelve to fifty-three years of age. The naevi were removed from six months to approximately twenty years after having developed. Naevi were located on the scalp (2), face (23), neck and chest (11), arms and forearms (7). At the time of biopsy/removal, the naevi showed no inflammatory characteristics. The following additional specimens were obtained; 1 case of lentigo maligna, 1 primary human skin melanoma, 1 metastatic skin melanoma, 5 naevi and 5 skin melanomas from albino guinea pigs.

Naevi and melanomas in guinea pigs were induced with 9,10-dimethyl-1,2 benzantracene (14, 15). In each case, part of the material was processed for light microscopy and stained with haematoxylin and eosin.

For examination in the electron microscope, the material was immediately fixed in universal fixative (16), and processed for electron microscopy. Ultrathin serial sections were cut and mounted on single slot, formvar carbon coated grids. The sections were examined with a Philips EM300 electron microscope.

Three-dimensional computer reconstruction

In order to fully interpret the successive ribbons of sections in the serial section series, 3-dimensional reconstruction and or perspective computer plots were carried out (17).

The individual slotted, formvar carbon coated grids were carefully placed into special holders designed to fit the top loading specimen stage of a JEOL 100CX transmission electron microscope. Approximately the identical areas of specimen were photographed from sequential serial sections on Kodak 4489 electron microscope sheet film. Some emphasis was placed on trying to ensure that the tissue orientation was more or less consistent throughout the photographed series. The micrographs were taken at a magnification of 400 diameters. Any farther rotational alignment for the computer reconstruction was corrected by using the computer system. The negatives were subsequently measured, corrected for uniform density and re-photographed onto 35 mm roll film, in effect creating a movie. This roll of film was developed and mounted in a precision film transport mechanism. The individual frames were then digitized using a high resolution television camera, and stored in a frame grabber digital storage device for further image processing. The digitized frames were displayed sequentially and the relevant areas of interest delineated in the image. These outlined areas were then assembled on the display system as a 3-dimensional graphic. The graphic reconstruction was oriented as needed.

RESULTS

Conventional light microscopy resulted in three human naevi being classified as "junctional", nineteen as "compound", eighteen as "dermal" and three as blue naevi. It was difficult to classify these naevi into specific categories, as most of them demonstrated both epidermal and dermal components.

In all human and guinea pig naevi serial sections examined in the electron microscope, the epidermal-dermal junction was well preserved. Both the lamina lucida and the lamina densa were easily traced through the series of serial sections. No cell, or any part of a cell was observed attempting to cross the basement membrane. The interface between the basal keratinocytes and/or melanocytes and the connective tissue was convoluted by numerous ridges projecting from the epidermal cells (Fig. 1). Very often, keratinocytes were completely surrounded by dermal collagen. Following and tracing these keratinocytes through the serial sections, it was possible to find a direct joining of these cells to the epidermis. In the observed naevi, the melanocytes were actively prevented from making contact with the actual basement membrane, by keratinocytes. Areas in which the cell membrane of melanocytes contacted basement membrane were rarely observed. Invariably, cellular processes from keratinocytes were found between the melanin producing cell and the basement membrane (Fig. 2).

However, the depth to which keratinocytes protruded in the direction of the dermis was limited.

Hemidesmosomes and anchoring fibrils were absent in the regions where melanocytes were in contact with the basement membrane. All the hemidesmosomes observed, were found on the processes extending from neighbouring keratinocytes.

Individual naevus cells were also found in the "dermis". These cells were completely surrounded by basement membrane consisting of both lamina lucida and lamina densa (Figs. 2, 3). Single naevus cells could be traced to another naevus cell or naevus cell nest, with subsequent confirmation of their direct, physical connection to the epidermis. Fig. 4

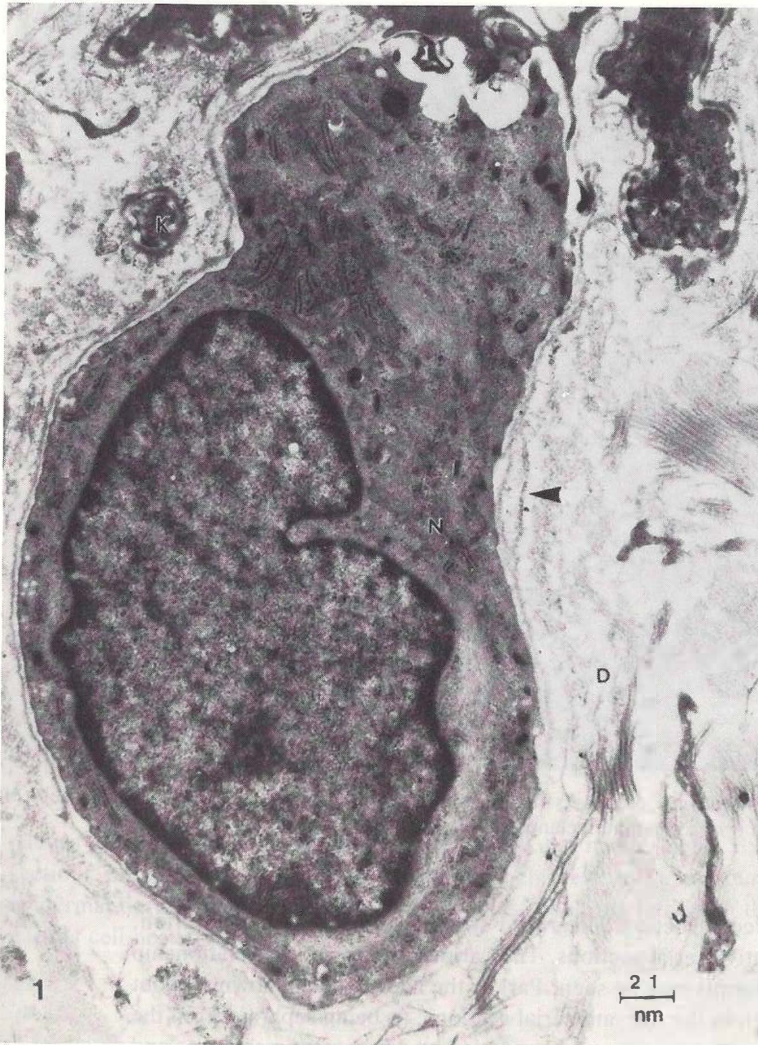


Fig. 1. Naevus cell (N), surrounded by partially doubled (arrow), basement membrane. Undulating epidermal-dermal junction gives the false impression that the keratinocyte (K), is entirely located in the dermis (D). $\times 54\,700$.

A, B, C shows the interrelationship of naevus cells and nests in two neighbouring hair follicles, H1 and H2. These illustrations were selected from a ribbon of eighty-five serial sections, as being the three micrographs to show the continuity of the epidermis as illustrated through the seventeen serial grids examined in the electron microscope. Naevus cells and small nests were incorporated into the structure of follicle H2, whereas a group consisting of a few naevus cells, was completely "separated" from the hair follicle H1 by collagen (Fig. 4 A). Fig. 4 B shows a medial thinning of follicle H2. The naevus cell nest next to follicle H1 now has more cells in the plane of section than in Fig. 4 A. Fig. 4 C shows a nest which has become "detached" from follicle H2, and a nest which has "joined" onto follicle H1.

Three-dimensional computer reconstruction

Following the assessment of ribbons of serial sections to show the continuity of "dermal" naevus nests with the epidermis, the same series of serial sections were processed for 3-

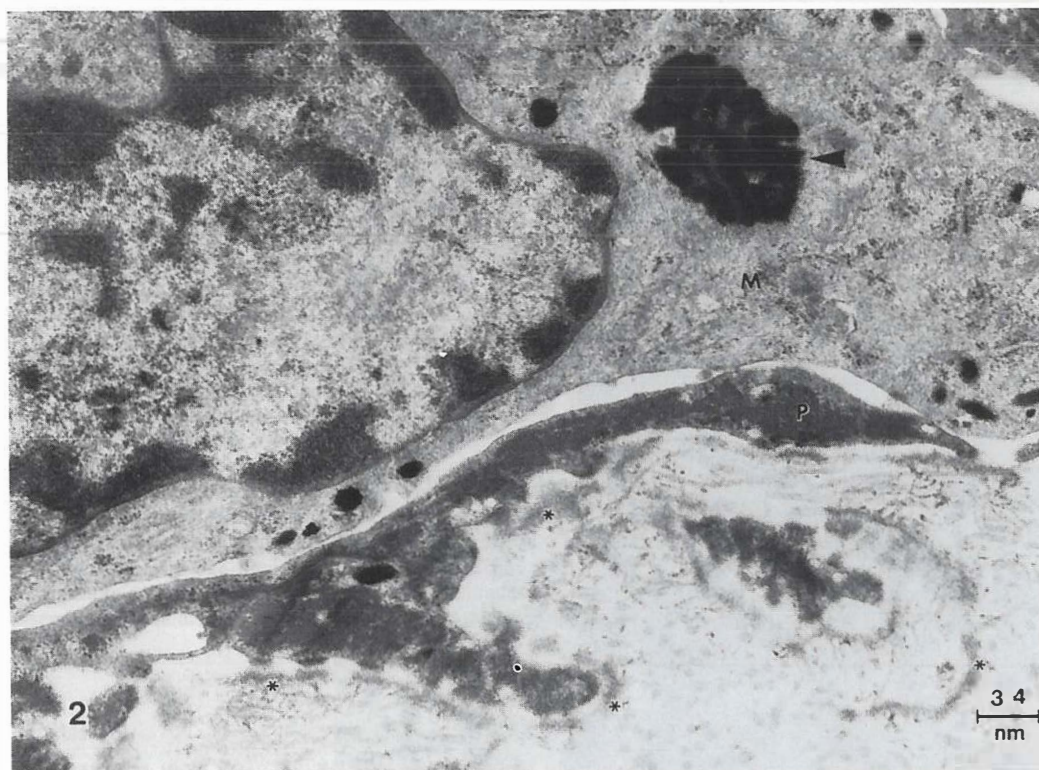


Fig. 2. Keratinocytes have long cytoplasmic processes (*P*), preventing contact of the melanin producing cell (*M*) with the epidermal-dermal junction (*). Compound melanosome is indicated with arrows. $\times 33\,000$.

dimensional computer aided reconstruction. Diagram 1 shows a complete reconstruction, consisting of fifty-four consecutive serial sections. The various aspects of the relationship of naevus cell nests to the epidermis may be seen. Part of the naevus nest, shown without shading, near the centre, is seen in the relevant serial sections, as being separate from the epidermis, i.e. located entirely in the dermis and surrounded by a basement membrane and connective tissue.

As the serial section series progresses, the "island" or "nest" of naevus cells in the dermis, confirmed at high magnification as being surrounded by a complete basement membrane, approaches more and more closely to the epidermal-dermal junction, until the basement membrane around the dermal "nest" and the basement membrane separating the epidermis and dermis, join. In the next section, the epidermis is again seen to be continuous. The light shading clearly shows at which level the epidermal-dermal junction is continuous with the basement membrane surrounding the protrusion. In order to help understand the complex spatial relationship of the protruding naevus cells to the epidermis, Diagram 2 represents an artistic interpretation of the computer reconstruction shown in Diagram 1. The continuity of protrusions of naevus cells with the epidermis as they protrude into the dermis are clearly indicated. The dotted lines show the level at which the naevus cells, if sectioned at that level, would be seen as lying in the dermis, surrounded by connective tissue.

Naevus nests were covered by the basement membrane only at their outer border, i.e. between naevus nests and dermis (Fig. 5). No basement membrane was found between

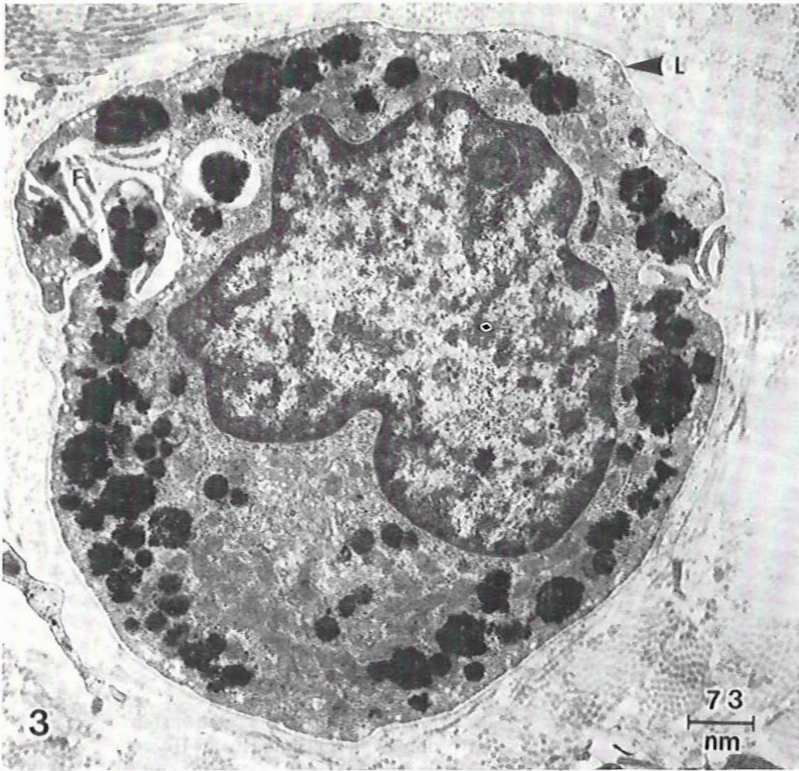
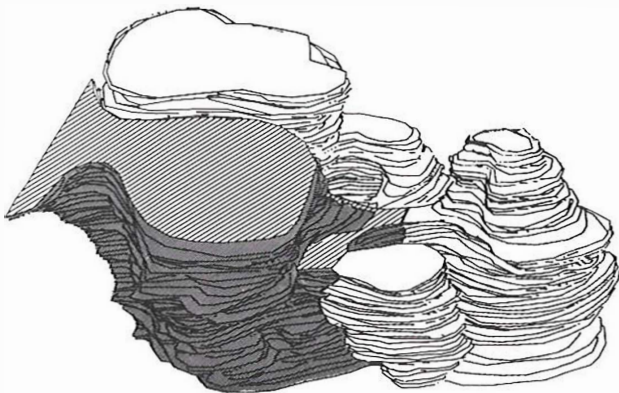


Fig. 3. "Intradermal" naevus cell with filiform extensions (F), circumscribed by lamina lucida and lamina densa of basement membrane (L). Some degradation of melanosomes seems to be taking place. $\times 16\,000$.

naevus cells or between epidermal keratinocytes and naevus cells. Examination of serial sections permitted the tracing of basement membrane as it contoured down from the epidermal-dermal junction and/or keratinocytes, adhering tightly to the periphery of the naevus cell nests (Fig. 5).



- ☐ Naevus cells not connected to epidermis in section plane, surrounded by basement membrane
- ☐ Naevus cells connected to epidermis in section plane
- ☒ Epidermal peg — normal epidermis
- ☒ Epidermal peg — section plane

Diagram 1. Human naevus (54 serial sections).



Fig. 4 A, B, C. Interrelationship of naevus cells in nests (*) to normal epidermal cells. H1 and H2 are hair follicles. The micrographs are representative of a series to show the "intradermal" naevus nest (A), the nest becoming larger (B) and the nest joining the epidermis (C). In each case, the nest is circumscribed by the basement membrane, i.e. the nests are in fact intrafollicular. $\times 44\,500$.

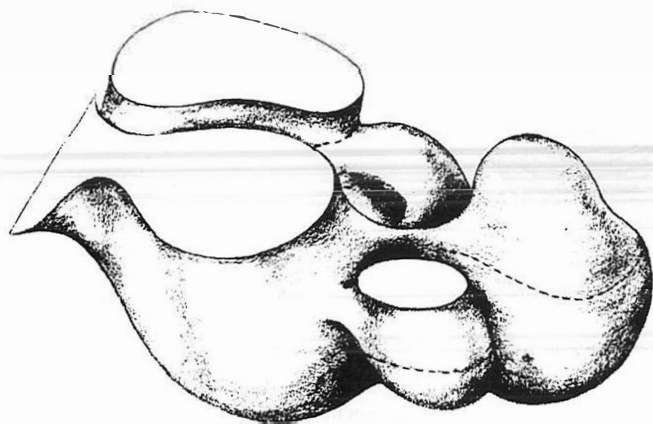


Diagram 2. Human naevus (3-dimensional perspective).

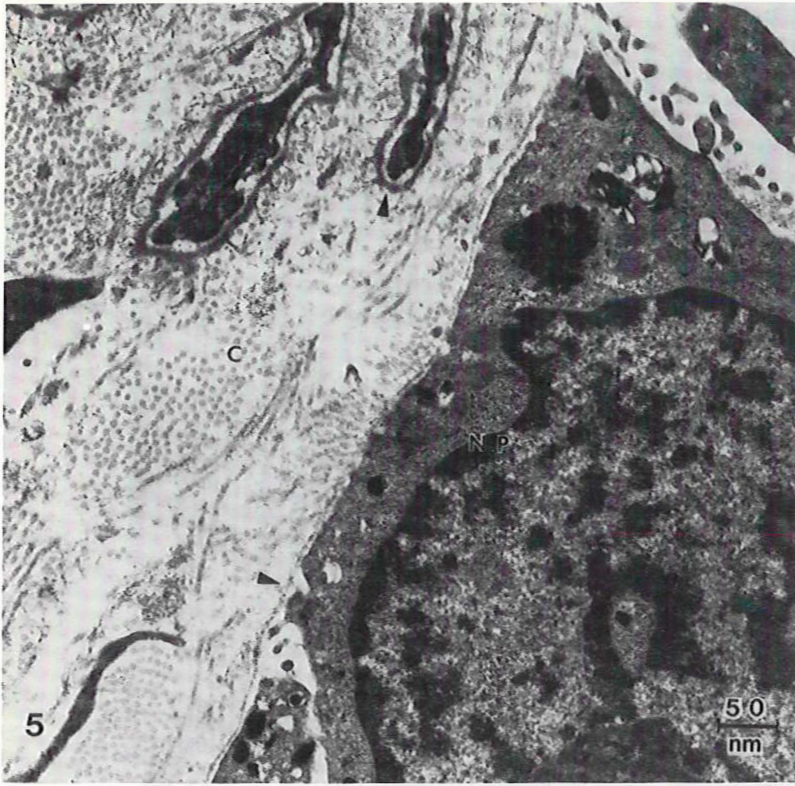


Fig. 5. Basement membrane (arrows) is seen to continue from the keratinocyte at the epidermal-dermal junction, to embrace a naevus nest. The basement membrane contours along the outer periphery of the nest only, e.g. along the naevus cell (NP), thus separating the nest from the dermal collagen. There is no basement membrane in between the naevus cells in the nest, or where the nest is continuous with the epidermis. $\times 23\,400$.

Only melanocytes were found in the naevus cell nests. Fibroblasts, macrophages and collagen when present, were always found to be on the dermal side of the basement membrane, i.e. not included in the epidermal nest (Fig. 6). Melanocytes in the more superficial regions of naevi contained melanosomes. Premelanosomes and "melanosomal complexes" were also observed (Figs. 2, 3, 5). "Nests" and cells which protruded deeply into the dermis show a gradual decrease in melanosome number, leading to their final disappearance.

In blue naevi, melanocytes were not grouped into large nests. However, many individual naevus cells and small nests were partly or completely contained by a basement membrane. The bulk of the blue naevus appeared to be formed by melanin containing macrophages. These macrophages were located outside the basement membrane.

The sections were carefully examined for Schwann cells and nerve fibres. Neural elements were scant in the vicinity of the epidermal-dermal junction in the human naevi. In the naevi deeper in the dermis, neural elements were ensheathed by their own basement membrane, which could not be found connecting to the basement membrane of the naevus cells or nests (Fig. 7). Schwann cells showed characteristic profiles of axons wrapped in membranes, interspersed by cytoplasm. Substructures resembling organized melanin were observed in Schwann cell cytoplasm.

In the control tissues, taken from both human and guinea pig melanoma, no basement membrane was present in the regions of tumour penetration or around single or grouped malignant cells in the dermis. In lentigo maligna, abnormal melanocytes had not penetrated into the dermis. A complete basement membrane separated malignant cells from the dermis.

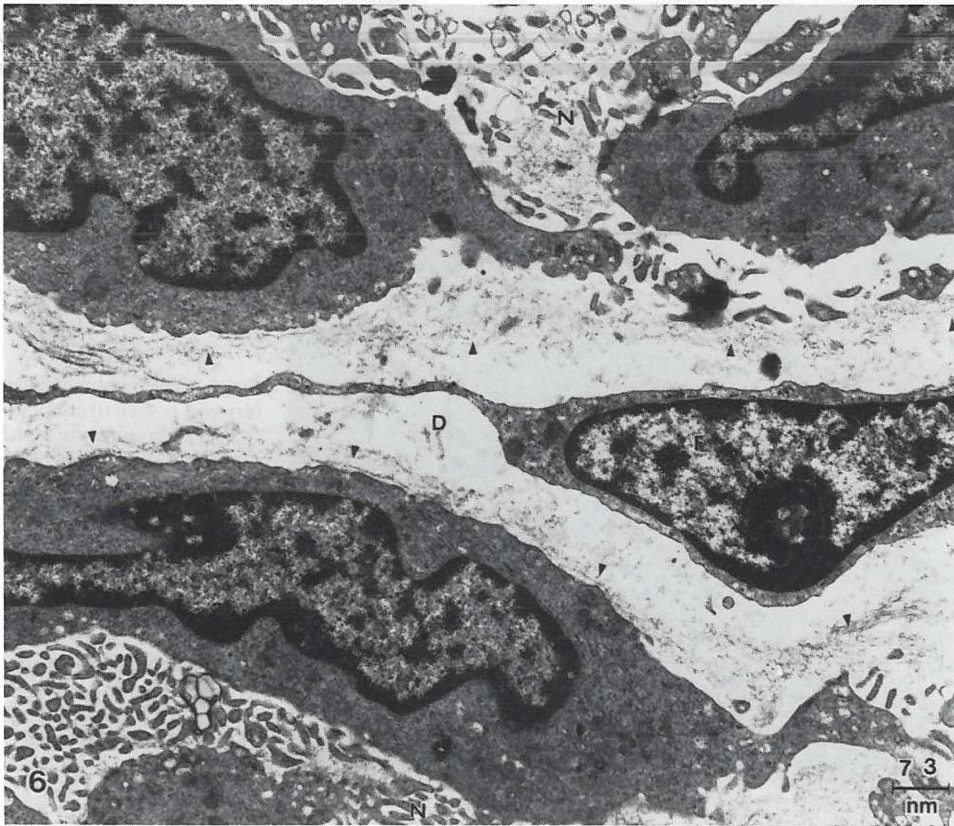


Fig. 6. Two naevus nests (N), separated from each other and the dermis (D) by basement membranes (arrows). In between them is a fibroblast (F) in the dermis. $\times 16\,500$.

Fully melanized melanosomes were not found in the melanoma cells. Premelanosomes showed considerable variation and irregularity in shape and internal structure. Large, dense masses of melanin were located in autophagosomes. Myelin figures were common.

Nervous tissue elements were not found in the areas infiltrated by melanoma cells.

DISCUSSION

Basement membrane can be synthesized by many cells, including endothelial, smooth muscle, Schwann and epithelial cells. The synthesis of basement membrane components by malignant tumours derived from these cell types has been investigated (18). Neither *in vivo*, during wound healing (19, 20), nor *in vitro* (21, 22, 23), was it possible to show the formation of basement membrane by melanocytes. Formation of basement membrane was also not found in tissue culture of human naevus cells (21, 24, 25). In contrast, during wound healing and in tissue culture, basal cells of the epidermis produce a basement membrane (25, 27). Keratinocytes appear to be the source of this newly formed membrane.

Furthermore, it was shown that dermal viability was not essential for the synthesis of basement membrane (28, 29).

The organizing sites for the deposition of basal lamina are the hemidesmosomes (28),

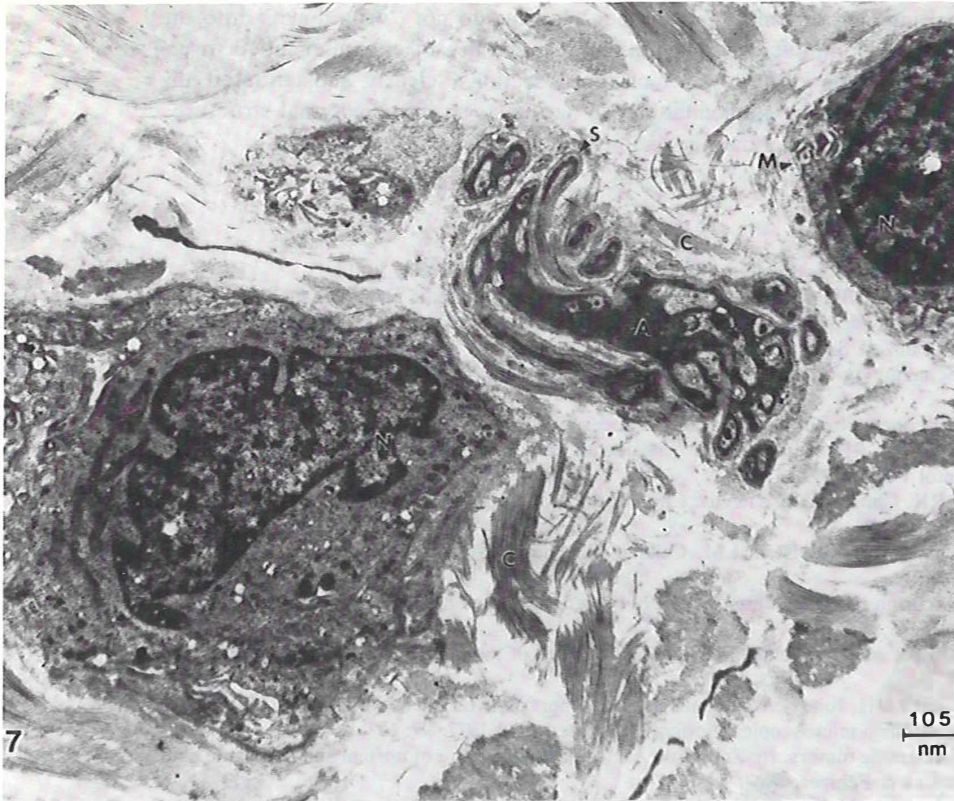


Fig. 7. Small axons (A) in the dermis, completely separated by a Schwann cell basement membrane (S), and collagen (C) from the membrane (M) of the naevus cell (N). $\times 10900$.

which are characteristically found in association with keratinocytes, but not with melanocytes (30). The presence of hemidesmosomes at the epidermal-dermal junction, and their absence where the naevus cells have protruded deeply into the dermis, provides further proof that keratinocytes are the source of basement membrane.

Duplication or multilayering of basement membrane as observed frequently in naevi, could be the result of an attempt by the epidermal-dermal junction to either accommodate more cells, or help to contain the epidermal cells within a weakened basement membrane. A change in the normal contour of the epidermal-dermal zone by a cluster of naevus cells results in the protrusion of the naevus into the dermis. Keratinocytes were found along the basement membrane of this protrusion into the dermis for only a certain distance, after which the naevus cells were accompanied only by basement membrane. The three dimensional architecture of these protrusions, surrounded by the basement membrane of the epidermal-dermal junction and filled with naevus cells, permits the consideration of all acquired melanocytic naevi as comparable to epidermal appendages.

Naevi, which on light microscopic grounds would be classified as "dermal", exhibited, in all the naevi examined, the same epidermal continuity. The serial section series showed some "dermal" nests becoming smaller and smaller and finally disappearing from the next consecutive section. These sections were considered as having been cut in a direction away from the epidermal connection.

Ultrastructural studies of serial sections showed that melanocytic naevi do not cross the

epidermal-dermal junction basement membrane, and thus do not "drop down" into the dermis, but remain in continuity with the epidermis. The use of computer aided three-dimensional reconstruction of the series of serial sections, clearly substantiated the interpretation of the pigmented congenital human naevus as an intraepidermal structure.

There is no involvement of dermal nerves in the morphology of the naevi.

REFERENCES

1. Unna PG. Naevi and naevocarcinoma. *Berliner Klin Wochenschr* 1893; 30: 14.
2. Elder DE, Green MH, Bondi EE, Clark WH Jr. Acquired melanocytic naevi and melanoma. The dysplastic naevus syndrome. In: Ackerman AB, ed. *Pathology of malignant melanoma*. New York: Masson Publ., USA, 1981: 129.
3. Lever WF, Schaumburg-Lever G. *Histopathology of the skin*. 6th ed. Philadelphia: Lippincott, 1983: 681.
4. Masson P. My conception of cellular nevi. *Cancer* 1951; 4: 9.
5. Shelley WB, Arthur RP. Nerve fibres, a neglected component of intradermal cellular nevi. *J Invest Dermatol* 1960; 34: 59.
6. Winkelmann RK. Cholinesterase nevus: Cholinesterases in pigmented tumours of the skin. *Arch Dermatol* 1960; 82: 17.
7. Bhawan J, Chang WH, Edelstein LM. Cellular blue nevus. An ultrastructural study. *J Cutan Pathol* 1980; 7: 109.
8. Curran RC, McCann BG. The ultrastructure of benign pigmented naevi and melanocarcinomas in man. *J Pathol* 1976; 119: 135.
9. Gottlieb B, Brown AL, Winkelmann RK. Fine structure of the nevus cell. *Arch Dermatol* 1965; 92: 81.
10. Eady RAJ, Gilkes JJH, Jones EW. Eruptive naevi: report on two cases with enzyme, histochemical, light and electron microscopical findings. *Br J Dermatol* 1977; 97: 267.
11. Mishima Y. Melanotic tumors. In: Zelickson AZ, ed. *Ultrastructure of normal and abnormal skin*. Philadelphia: Lea & Febiger, 1967: 388.
12. Lea PJ, Pawlowski A. Melanocytic nevi in humans. A new histogenic concept based on transmission electron microscopy. In: *Biological, molecular and clinical aspects of pigmentation*. XII Int Pigm Cell Conf. University of Tokyo Press, 1985: 641.
13. Lea PJ. Application of ultrastructural analysis to the study of human melanocytic naevi. Doctoral dissertation, California Coast University, 1985.
14. Pawlowski A, Haberman HF, Menon IA. Junctional and compound pigmented nevi induced by 9,10-Dimethyl-1,2-benzanthracene in skin of albino guinea pigs. *Cancer Res* 1976; 36: 2813.
15. Pawlowski A, Lea PJ. Nevi and melanoma induced by chemical carcinogens in laboratory animals: similarities and differences with human lesions. *J Cutan Pathol* 1983; 10: 81.
16. McDowell EM, Trump BF. Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 1976; 100: 405.
17. Stevens JK, Trogadis J. Computer reconstruction in neuronal studies. *Adv Cell Neurobiol* 1984; 5: 341.
18. Barsky SH, Siegal GP, Janotta F, Liotta LA. Loss of basement membrane components by invasive tumors but not by their benign counterparts. *Lab Invest* 1983; 49: 140.
19. Giacometti L, Montagna W, Bell M, Hu F. Epidermal melanocyte proliferation in skin wound healing and after ultraviolet irradiation in Rhesus monkeys. In: *Pigmentation: Its genesis and biological control*. Appleton-Century-Crofts, 1972: 451.
20. Bell E, Sher S, Hull B, Merrill C, Rosen S, Chamson A, Asselineau D, Dubertret L, Coulomb B, Lapiere C, Wusgens B, Neveaux Y. The reconstruction of living skin. *J Invest Dermatol* 1983; 815: 11.
21. Hu F, Staricco RJ, Pinkus H, Fosnaugh RP. Human melanocytes in tissue culture. *J Invest Dermatol* 1957; 28: 15.
22. Glimcher ME, Garcia RI, Szabo G. Organ culture of mammalian skin and the effects of ultraviolet lights and testosterone on melanocyte morphology and function. *J Exptl Zoo* 1978; 204: 229.
23. Gilchrist BA, Vrabel MA, Flynn E, Szabo G. Selective cultivation of human melanocytes from newborn and adult epidermis. *J Invest Dermatol* 1984; 83: 370.
24. Kraemer M, Yuspa S, Clark W, Townsend K, Green M, Neises G, Hearing Y. Reduced DNA repair in cultured melanocytes and naevus cells from a patient with xeroderma pigmentosum. *J Invest Dermatol* 1985; 84: 294.

25. Halaban R, Ghosh S, Duray PH, Lerner AB. Growth responses of normal and malignant human melanocytes. Abstract. 1st International Conference on Skin Melanoma, Venice, 1985: 219.
26. Prunieras M, Regnier M, Fougere S, Woodley D. Keratinocytes synthesize basal-lamina proteins in culture. *J Invest Dermatol* 1983; 74: 815.
27. Hirone T, Taniguchi S. In vitro synthesis of basal lamina by epidermal cells. *J Invest Dermatol* 1983; 80: 341.
28. Briggaman RA. Basement membrane formation and origin with special reference to skin. In: Prunieras M, ed. *Epidermal keratinocyte differentiation and fibrillogenesis. Frontiers of matrix biology*. Vol. 9. Basel, Munich: S. Karger, 1981: 142.
29. Briggaman RA. Epidermal-dermal interactions in adult skin. *J Invest Dermatol* 1982; 79: 21 s.
30. Daroczy J, Feldman J. Microfilaments of the human epidermal-dermal junction. In: Prunieras M, ed. *Frontiers of matrix biology-epidermal keratinocyte differentiation and fibrillogenesis*. Vol. 9. Basel, Munich: S. Karger, 1981: 155.