

## AN ELECTRON MICROSCOPICAL STUDY OF BLOCH-SULZBERGER SYNDROME (INCONTINENTIA PIGMENTI)

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**Abstract.** A study of a case of Bloch-Sulzberger syndrome at the pigmented papule stage was made with optical and electron microscopes. Many atypical melanocytes were found in the upper epidermis and it is likely that they, rather than dyskeratotic cells, are the vacuolated cells seen in optical microscopical studies. The melanocytes contained two abnormal structures: giant vacuoles and myelin figures. Many gaps were found in the basement membrane and offered a possible explanation for pigmentary incontinence. In addition the dendritic process of a melanocyte was found in the upper dermis suggesting that melanocytes may be discharging melanin directly into the dermis.

The Bloch-Sulzberger syndrome is a disorder of skin and often other organs, which is now generally accepted as being a developmental anomaly of both ectodermal and mesodermal tissues (3, 6, 7, 9, 11, 12, 15). The most striking clinical finding in this syndrome is the appearance of bizarre skin pigmentation and it is this that attracted the attention of dermatologists. It has been postulated that the pigmentation is a post-inflammatory reaction (8, 14) and is the result of an excess of melanin in both the basal layer of the epidermis and the upper dermis. Certain authors (9, 13, 14) have remarked on the presence of abnormal "vacuolated" cells in the upper epidermis. The present study describes the first ultrastructural investigation of the skin lesion at the pigmented papule stage of this syndrome.

### CASE HISTORY

A female child born at full term at the Maternity Unit of the United Oxford Hospitals on 30 Dec. 1969. First baby. Birth weight 6 lb. 15 oz. The day after delivery, her mother noticed that the skin of the hands and feet was very dry and there were two small vesico-pustular lesions on the right forearm. The baby was transferred

to the Infectious Disease Unit with a putative diagnosis of Pemphigus Neonatorum. She was treated with chlorhexidine gluconate baths and parenteral Ampicillin. The baby was afebrile and thrived normally. She was feeding well and gaining weight. On 9 Jan. 1970, she was seen by the Dermatology Department who made a diagnosis of Bloch-Sulzberger syndrome and stopped antibiotics. By this time the baby had numerous groups of papules, situated symmetrically on both legs and arms, scalp and lower trunk, and linear pigmented streaks were beginning to appear in these areas. There was no evidence of any other abnormalities. Routine examination showed a normal haemoglobin, sedimentation rate, and platelet count. The white cell count was also normal with no increase in eosinophils. There was no bacterial growth on culture of the material from the pustules. Chromosome studies of this case were normal at the age of 2 months. There was no family history of skin or neurological diseases and there is no consanguinity between the parents.

### MATERIALS AND METHODS

A biopsy was taken from the pigmented papules on the flexor aspect of the right leg (Fig. 1) on the 12th day of life. The specimen was divided into three parts, one each for routine histopathology, dopa reaction and ultrastructural study.

#### *Optical microscopy*

After fixation in 10% formalin and routine processing the sections were stained with haematoxylin and eosin, PAS, trichrome and Masson silver impregnation for melanin (20). Dopa reaction was carried out on the fresh specimen by the method of Laidlaw & Blackberg (17).

#### *Electron microscopy*

After slicing, the specimen was fixed in 1% osmium tetroxide buffered to pH 7.3 in *s*-collidine at 4°C for 2 hours. Tissue slices were dehydrated in ethanol and embedded in epoxy resin according to the method of Luft (18). Thin sections were cut with glass knives and a Du-Pont diamond knife on a Huxley ultramicrotome and mounted on uncoated copper grids. These were stained with saturated uranyl acetate for 10 min then stained

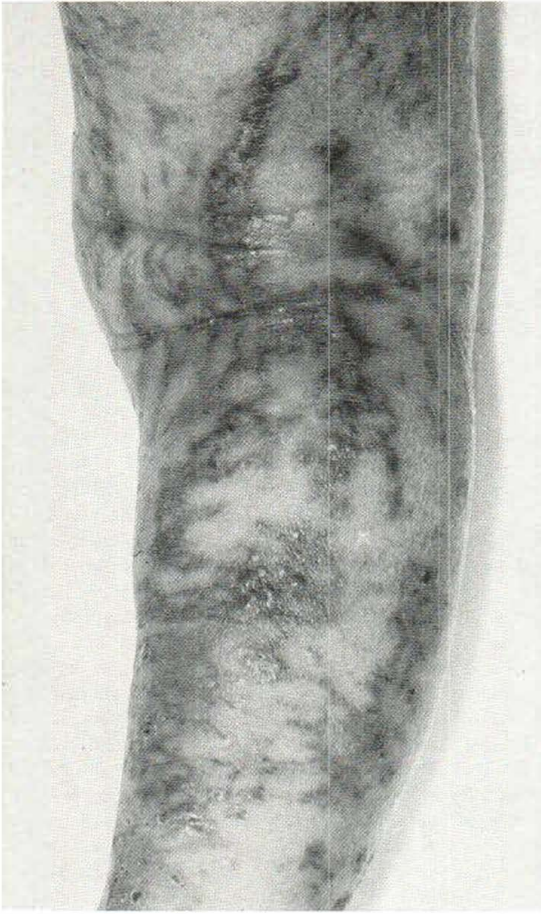


Fig. 1. Clinical picture showing pigmented streaks and papules on the flexor aspect of the right leg.

with Reynolds lead citrate (23) for 15 min. Micrographs were taken with an EM-6B electron microscope.

### RESULTS

The H.E. section (Fig. 2) showed slight hyperkeratosis and mild acanthosis. In the upper epidermis there were numerous vacuolated cells with eccentric basophilic nuclei. The basal cells showed wider spaces in certain areas. A moderate number of small round cells were seen in the upper dermis.

Masson silver impregnation stain (Fig. 3) confirmed excess melanin in the basal layer and upper dermis found by other workers. There were some melanophages in the upper dermis. There was also an excess of melanin in the upper epidermis and the stratum corneum. The distribu-

tion of dopa-positive melanocytes in the basal layer was normal and no dopa-positive cells could be detected in the upper epidermis.

At electron microscopical level, the intercellular spaces between keratinocytes in the epidermis were widened but their desmosomes were both intact and structurally normal. Some of these keratinocytes contained phagocytised melanosomes. An unexpected finding in the upper epidermis was the presence of many melanocytes (Fig. 4). In addition to the normal premelanosomes and melanosomes these cells contained giant vacuoles up to  $2.1 \mu$  across and myelin figures (Fig. 5). No acantholytic or dyskeratotic cells were detected. The basal layer of the epidermis showed moderate intercellular oedema, but the half-desmosomes of the basal cells were preserved. The melanocytes in the basal layer were structurally normal. There were several gaps in the basement membrane and the dendritic process of a melanocyte, containing premelanosomes and melanosomes, was present

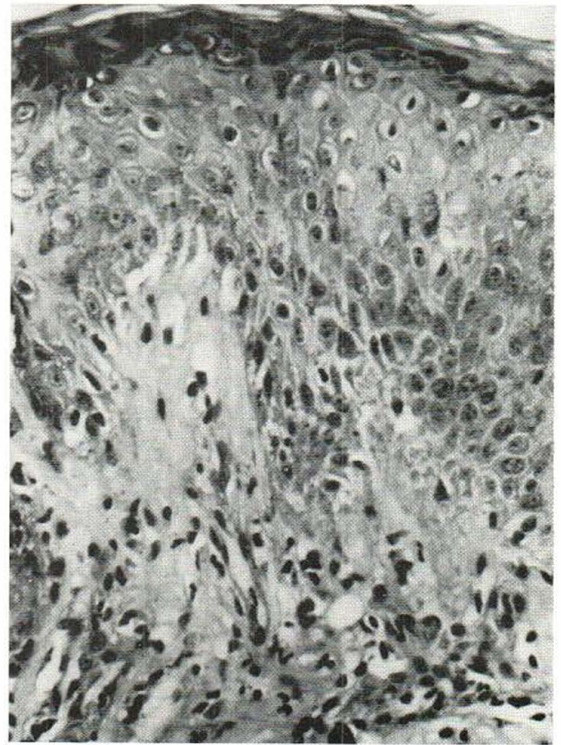


Fig. 2. H.E. stain showing numerous vacuolated cells in the upper part of the epidermis, wider spaces of the basal layer and inflammatory cell infiltration of the upper dermis.  $\times 300$ .

below the basement membrane in the upper dermis (Fig. 6). The upper dermis (Fig. 7) contained a considerable number of melanosomes many of which were inside phagocytes while others, lying in the intercellular spaces, were surrounded by lysosomes.

### DISCUSSION

At optical microscopical level the nature of the vacuolated cells in the upper epidermis in the Bloch-Sulzberger syndrome is uncertain and some workers (9, 13, 14) have labelled them "Dyskeratotic Cells". In H.E.-stained sections of normal skin melanocytes in the basal layer frequently appear vacuolated, the so-called "clear cell", it is therefore reasonable to assume that they should show similar vacuolation if they are present in the upper epidermis in this condition. The positive dopa reaction occurs exclusively in normal active melanocytes and is therefore not seen in these atypical cells. At electron microscopical level dyskeratotic cells have a characteristic structure (19, 29), and in the present investigation no such cells were seen, but numerous atypical melanocytes were found in the position in the upper epidermis in which these "Dyskeratotic Cells" have been described as occurring. It is probable therefore that the vacuolated cells in the upper epidermis of the Bloch-Sulzberger syndrome are atypical melanocytes and not dyskeratotic cells.

These atypical melanocytes showed two structural abnormalities, giant vacuoles and myelin figures. Neither has been previously reported to occur in melanocytes but one of the authors has detected myelin figures in human malignant melanoma cells (31). Myelin figures are known to occur in areas where phospholipid metabolism is taking place (24) and frequently seen for example in xanthoma cells (21). The size of vesicles is about 0.5  $\mu$  in the normal melanocyte and since phospholipids are present in considerable quantity in early normal melanogenesis (10), the giant vacuole and myelin figures in these melanocytes may be due to abnormal metabolism of phospholipids during melanogenesis. Whether this abnormal structure appears after the melanocyte has lost its connexion with the basal layer or is in anyway responsible for its being shed could not be deduced from the present study.

Bloch (1), Sulzberger (27) and Doorninck (8)

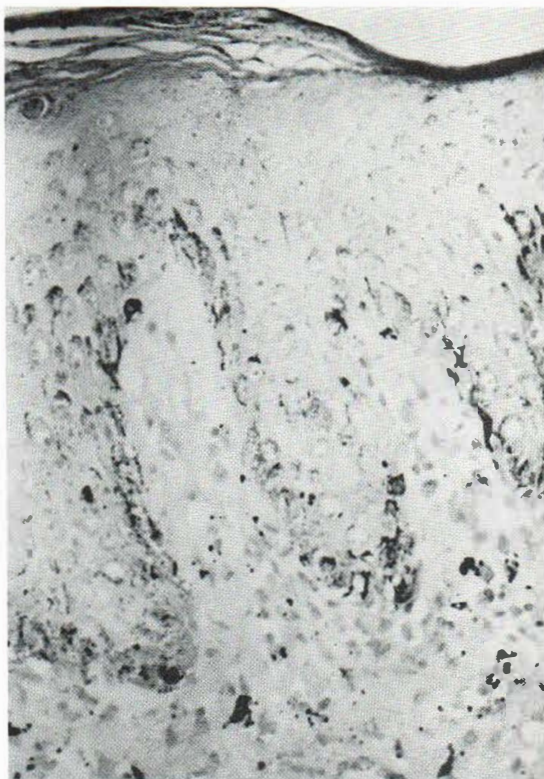


Fig. 3. Masson's silver impregnation for melanin showing melanin granules in the stratum corneum, a considerable number of melanin granules in the epidermis, and melanin granules and melanophages in the upper dermis.  $\times 300$ .

pointed out the excessive melanin in the dermis and basal layer of the epidermis and suggested that the bizarre clinical appearance of pigmentation in this disorder might be due to the leakage of melanin into the upper dermis instead of being carried upwards into the epidermis. In our Masson's silver impregnation sections a definite excess of melanin was present in the upper epidermis and the stratum corneum, and so another cause of the bizarre pigmentation pattern could be that pigment occurs in excess throughout the whole epidermis as well in the dermis.

The mechanism of melanin transfer into the dermis has not been elucidated in detail but the optical microscopical findings of basal layer necrosis with excess melanin in the upper dermis gave rise to the concept of pigmentary incontinence. Our finding of gaps in the basement membrane shows that the barrier is broken. Similar gaps have been found in ultrastructural studies of sub-

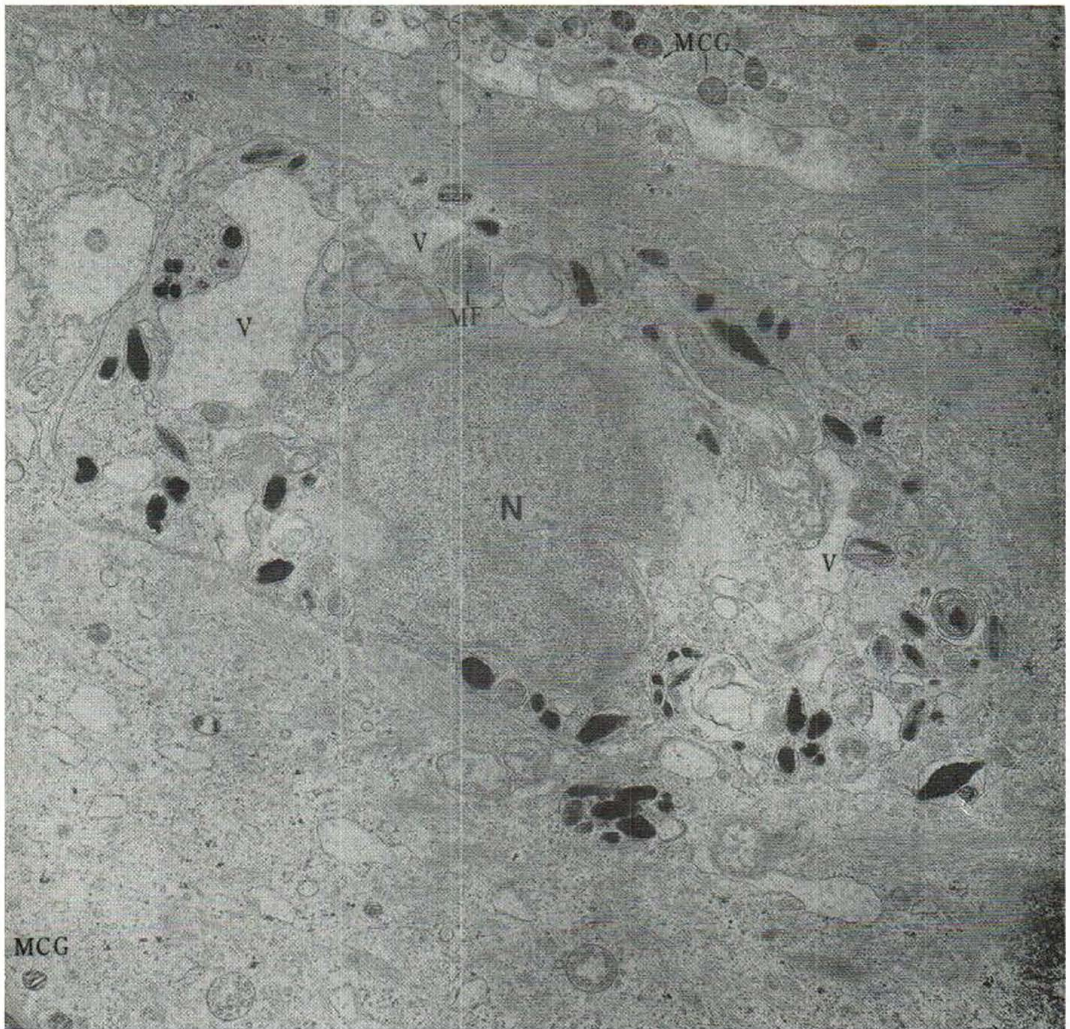


Fig. 4. A melanocyte containing giant vacuoles (V) and a myelin figure (MF) in the upper part of the epidermis.

Note numerous membrane-coating granules (MCG) surrounding the melanocyte. N, nucleus.  $\times 17\,500$ .

epidermal bullae (4, 22, 28) psoriasis (2, 5) and squamous cell carcinoma (16, 26) without marked pigmentary incontinence. Although the precise mechanism of focal discontinuity in the basement membrane is not clear, it has been suggested that physical force resulting from the increased pressure of intercellular fluid produced by liquefaction of the basal cells and rapid turn-over of the epidermal cells may destroy the basement membrane and form gaps. It is known that the normal epidermal basement membrane contains neutral polysaccharide (25) and alkaline phosphatase (30). These may be subject to attack by lytic enzymes

released by damaged epidermal cells and thus the normal biochemical composition of the basement membrane is altered and dissolution occurs.

The finding of a dendritic process of a melanocyte in the upper dermis suggests an additional mechanism by which melanin passes through gaps in the basement membrane. Melanocytes may be discharging melanin directly into the dermis through processes which have passed through breaches in the basement membrane and are lying in the upper dermis.

Our findings show a greater degree of dysfunction of melanocytes than has previously been

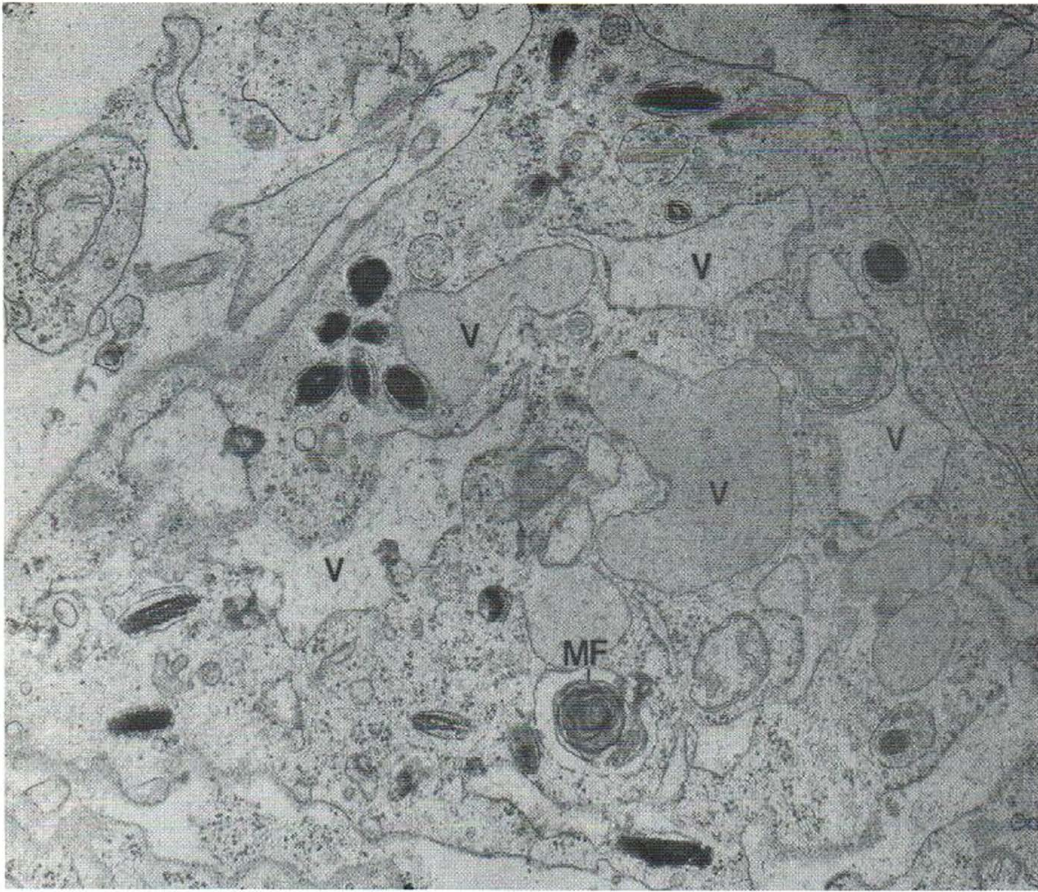


Fig. 5. A high magnification of a part of a melanocyte containing numerous giant vacuoles (V) and a myelin figure (MF).  $\times 35\ 000$ .

suspected. However, only after ultrastructural studies have been undertaken in the other diseases in which pigmentary incontinence occurs, will it be possible to say whether these changes are specific to the Bloch-Sulzberger syndrome or are part of any disease showing the "pigmentary incontinence" phenomenon.

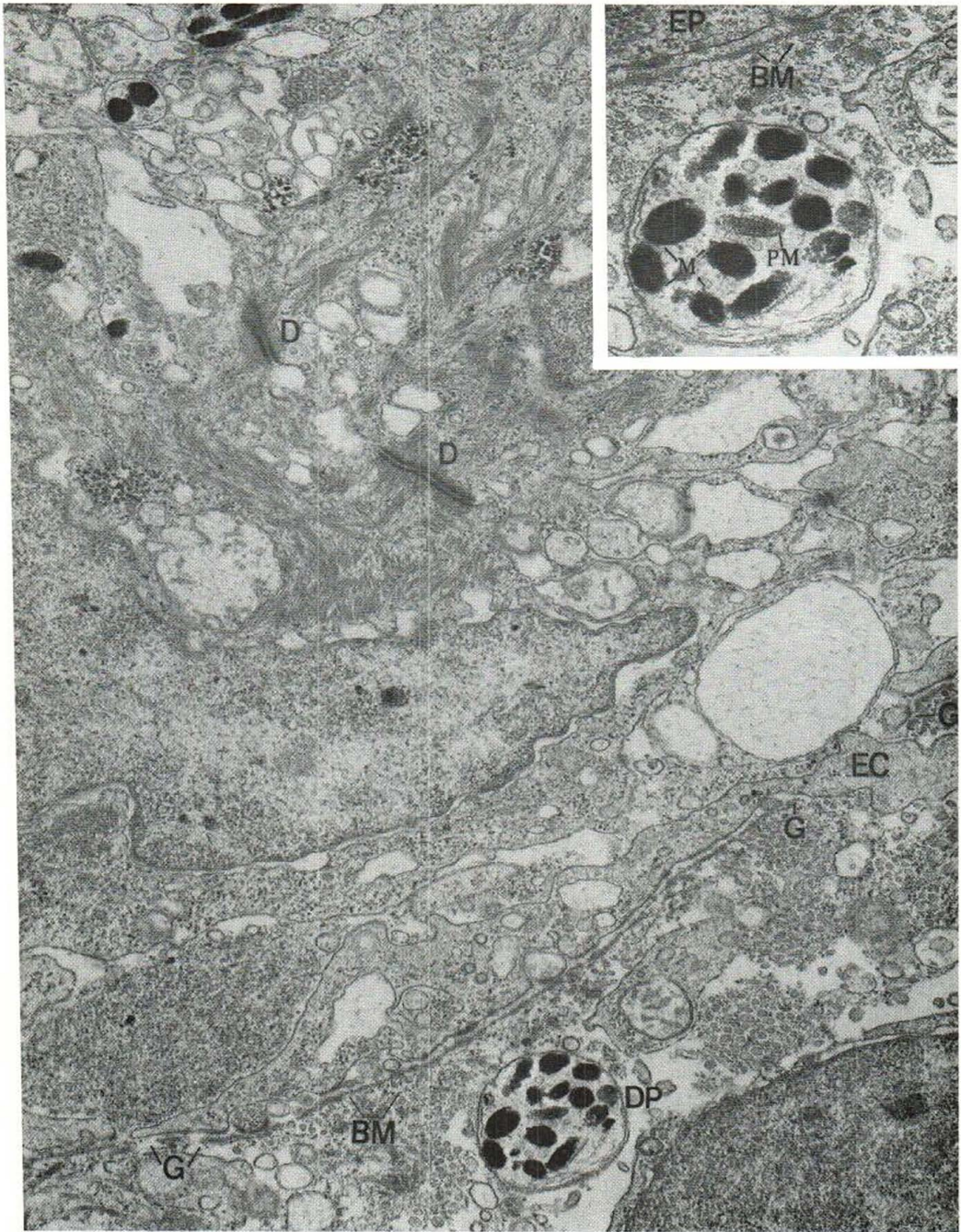
(This case was presented to the Royal Society of Medicine, London on 19 March, 1970.)

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*Fig. 6.* Micrograph of the basal layer of the epidermis showing normal desmosomes (*D*) but increased intercellular spaces between the basal cells. The basement membrane (*BM*) is broken, several gaps (*G*) being demonstrated. A part of an epidermal cell (*EC*) is intruding into

the upper dermis and a dendritic process (*DP*) of a melanocyte is also present in the upper dermis.  $\times 25\ 000$ . *Inset:* A high magnification of the dendritic process showing premelanosomes (*PM*) and melanosomes (*M*). *EP*, epidermis.  $\times 40\ 000$ .



Fig. 7. Basement membrane (BM) showing discontinuity. Several melanosomes (M) surrounded by lysosomes are present in the upper dermis. EP, epidermis.  $\times 22\ 500$ .

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