

## TWO SIBLINGS WITH EPIDERMODYPLASIA VERRUCIFORMIS WITH LARGE CLEAR CELLS IN THE EPIDERMIS: ELECTRON MICROSCOPE AND IMMUNOLOGICAL FINDINGS

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**Abstract.** In two Arab brothers presenting the characteristic clinical picture of epidermodysplasia verruciformis (EV), histological examination revealed large clear cells in the granular layer and uppermost part of the prickle cell layer of the epidermis. The report of this histological picture in two cases by other authors in the past had aroused considerable discussion as to the true diagnosis. The finding of large clear cells in our two confirmed cases of EV supports the opinion that this does not necessarily contradict the diagnosis of EV. Electron microscope investigation revealed groups of particles in the nuclei of a few keratinocytes in the granular layer which were compatible with papova virus particles. The changes seen in the clear cells support the theory of the viral etiology of EV. The immunological studies showed intact humoral immunity but impaired cellular immunity.

**Key words:** Epidermodysplasia verruciformis; Epidermodysplasia verruciformis—viral etiology; Epidermodysplasia verruciformis—immunological investigations; Epidermodysplasia verruciformis—electron microscope findings

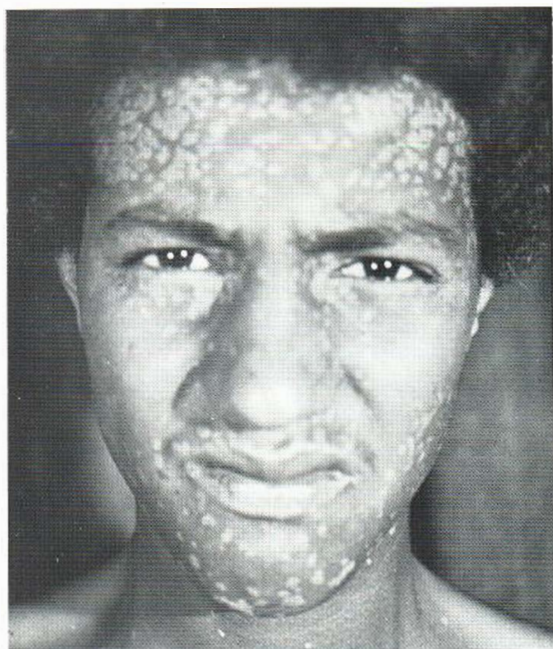
Epidermodysplasia Verruciformis (EV) is a rare disease, first described by Lewandowsky & Lutz in 1922 (1). The lesions may be more or less generalized, although in some cases they may be confined to the dorsal aspects of the hands and feet. The clinical and histopathological picture is identical with that of verrucae planae (especially when the lesions are present on the face and hands) but the papules which form the rash may vary considerably in form, size and even color, sometimes (particularly when the lesions appear on the trunk and extremities) being similar to verrucae vulgares. This multiformity of the clinical picture is striking, especially when compared with the usual uniformity of the histopathological findings.

We have had the opportunity of observing two brothers in whom a biopsy taken from a typical lesion revealed a histological picture so unusual that

the histopathologist was unwilling to accept the clinical diagnosis, although unable to offer any other. The clinical picture and anamnesis were so typical of EV, however, as to exclude any other diagnosis.

### CASE REPORT

An Arab boy aged 15½ years was seen in our out-patient clinic because of an asymptomatic eruption on the face, neck, chest and all four extremities. It had shown no change in form or distribution since its first appearance in infancy. The boy was hospitalized for investigation.



*Fig. 1. Well-defined flat, elliptic or polygonal papules greyish-white in colour covering the major part of the face of the patient first referred.*



Fig. 2. Close-up of the papules.

Physical examination showed a symmetric papular eruption which was particularly dense in the facial region, including the peripheral area of the scalp, as well as on the dorsal aspects of the hands, on the neck, upper chest and abdomen. Similar papules were scattered on the skin of the forearms, knees and legs. The well-defined papules were flat, greyish-white in color, 5–10 mm in size, elliptical or polygonal in shape and without scales (Figs. 1, 2).

Routine laboratory tests, including erythrocyte sedimentation rate, total blood count, liver and renal func-

tion tests and urinalysis were all within normal ranges. *Entamoeba histolytica* and *ascaris* were found in the stools. Serologic tests for syphilis were negative. X-rays of the chest and lungs were without pathological findings. Investigation of the serum proteins, including immunoelectrophoresis, showed normal values for both proteins and immunoglobulins. The level of T lymphocytes in the peripheral blood (E rosette forming cells) was below normal (45%) and that of B lymphocytes (EAC rosette forming cells) was within the normal range. Intradermal skin tests with common antigens, including trichophytin (1:50), candidin (1:50), tuberculin (1:2000) and streptodornase-streptokinase, showed delayed hypersensitivity only with candidin. Chromosomal analysis was normal.

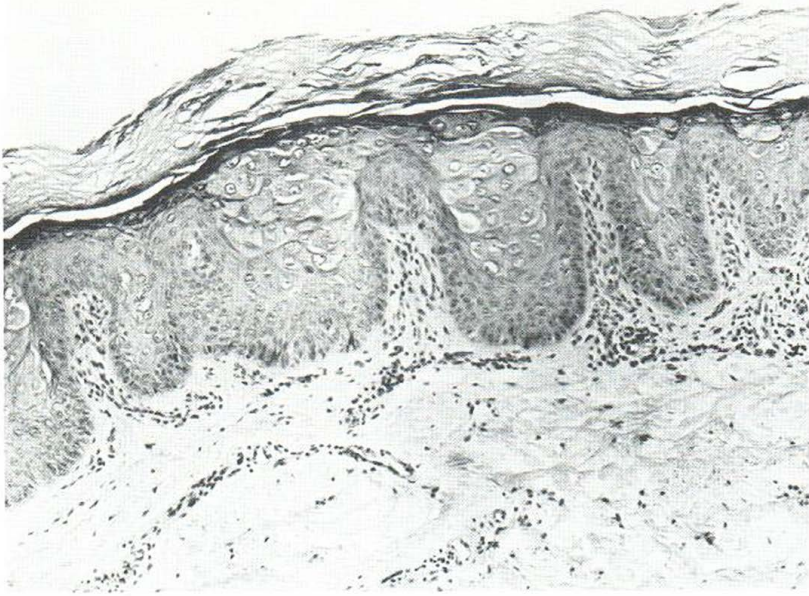
The familial anamnesis revealed that whereas the patient's parents and 7 siblings were all in apparent good health, one brother had suffered from a similar skin condition from infancy. This brother was therefore also subjected to investigation.

Physical examination of the brother revealed the presence of a scanty, symmetrical papular eruption of the face, neck, forearms and hands (Fig. 3). The characteristics of the papules were the same as those seen in his brother. Routine laboratory tests, as above, as well as the stool examination were without pathological findings. Serologic tests for syphilis were negative. X-ray of the chest was without pathological finding. Investigation of serum proteins, including immunoelectrophoresis, showed normal values for both proteins and immunoglobulins. The percentage of T lymphocytes in the peripheral blood was low (40%) and that of B lymphocytes normal. Intradermal skin tests as above, were all negative.

In both brothers, biopsy of typical lesions yielded identical histological and electron microscope findings. The specimens were divided into two parts. One was fixed in 4% formalin and embedded in paraffin and sections were stained with hematoxylin and eosin. The second part was fixed in 3% phosphate-buffered glutaraldehyde, post-fixed in 2% OsO<sub>4</sub> and embedded in Epon. Ultrathin



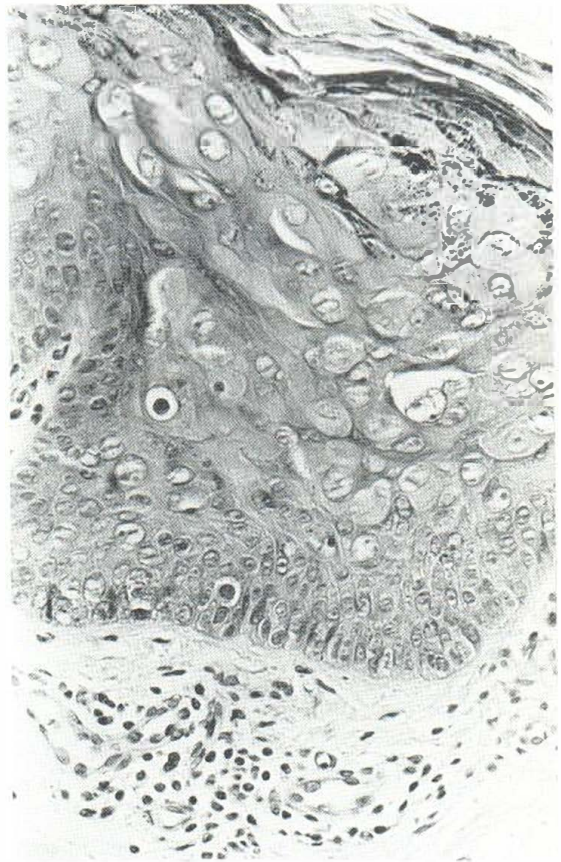
Fig. 3. Similar papules on the forehead of the patient's brother.



*Fig. 4.* Basket-weave hyperkeratosis and acanthosis as well as large groups of clear cells in the upper squamous and granular layers. Hematoxylin-eosin,  $\times 100$ .



*Fig. 5.* Large clear cells in the upper and mid-epidermis, with large keratohyalin granules in the granular layer. H & E,  $\times 200$ .



*Fig. 6.* Large clear cells in the upper epidermis and a few dyskeratotic cells in the lower part of the epidermis. Hematoxylin-eosin,  $\times 200$ .

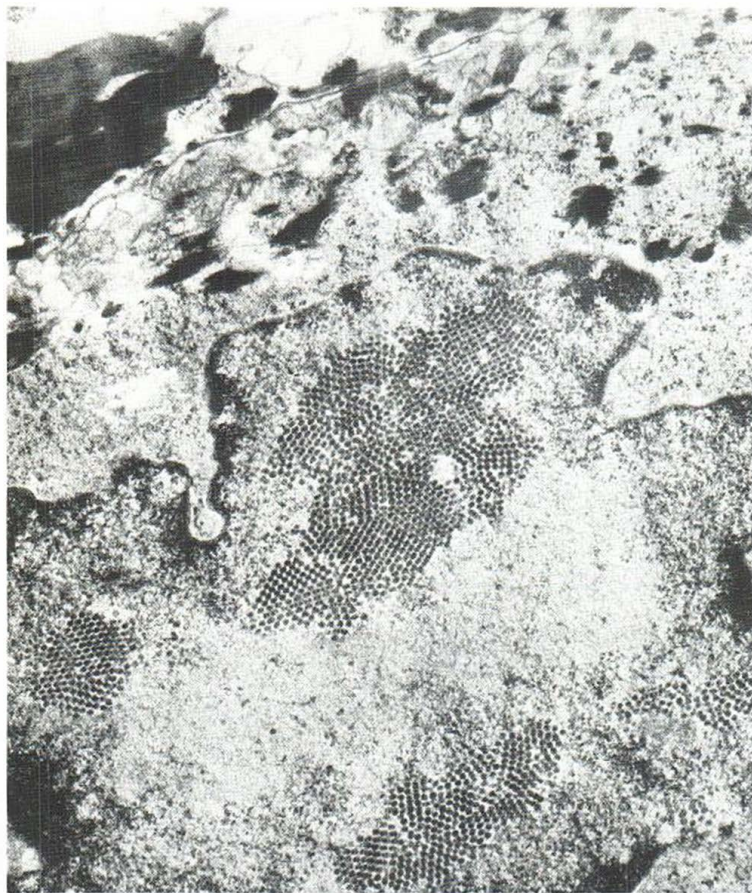


Fig. 7. Nucleus with groups of virus particles and devastation area at the center.  $\times 18\,000$ .

sections were double stained with uranyl acetate and lead citrate.

#### A. Histology

The epidermis showed a basket-weave horny layer, hypergranulosis and acanthosis. The rete ridges were thickened. The basal and lower squamous layers appeared normal. The main lesions were located in the upper squamous layer and the granular layer. Here there were groups of large, round or elliptic clear cells having a large round nucleus, a prominent nucleolus and abundant, slightly basophilic cytoplasm (Figs. 4, 5). These large cells contained an abundance of round basophilic keratohyalin granules. No intranuclear or intracytoplasmic inclusion bodies were found. A few dyskeratotic cells were seen among the keratinocytes in the lower part of the epidermis (Fig. 6). In the dermis there was a slight perivascular lymphomonocytic infiltration.

#### B. Electron microscope findings

A few of the keratinocytes in the granular layer showed an irregular nucleus with a clear space ("devastation area") lacking chromatin in its center. Observed along the margins of these devastated areas were groups of virus particles arranged in semi-crystalline patterns (Fig. 7), identi-

cal with the papova virus particles described by Jablonska et al. (2), Cornelius et al. (3) and Yabe et al. (4). These viruses, however, were not seen in the large clear cells present in the squamous and granular layers. The large clear cells were characterized by abundant cytoplasm containing a fine granular material (Fig. 8). Only a few short, irregular bundles of tonofilaments were observed (Fig. 9), in sharp contrast to neighbouring normal keratinocytes which contained numerous large groups of tonofilaments. A few large keratohyalin granules were seen. The nuclei of the clear cells were large, with one or two nucleoli and no heterochromatin. Melanosomes were only rarely found in the clear cells. Desmosomes were seen between the large clear cells and normal neighbouring cells but the tonofilament-desmosome complex was absent in the clear cells (Fig. 9). A few Langerhans' cells were seen between clear cells and occasionally there were a few dyskeratotic cells with abundant, thick interweaving bundles of tonofilaments (Fig. 10).

## DISCUSSION

In 1962 Johnson et al. (5) described two patients who were apparently suffering from epidermody

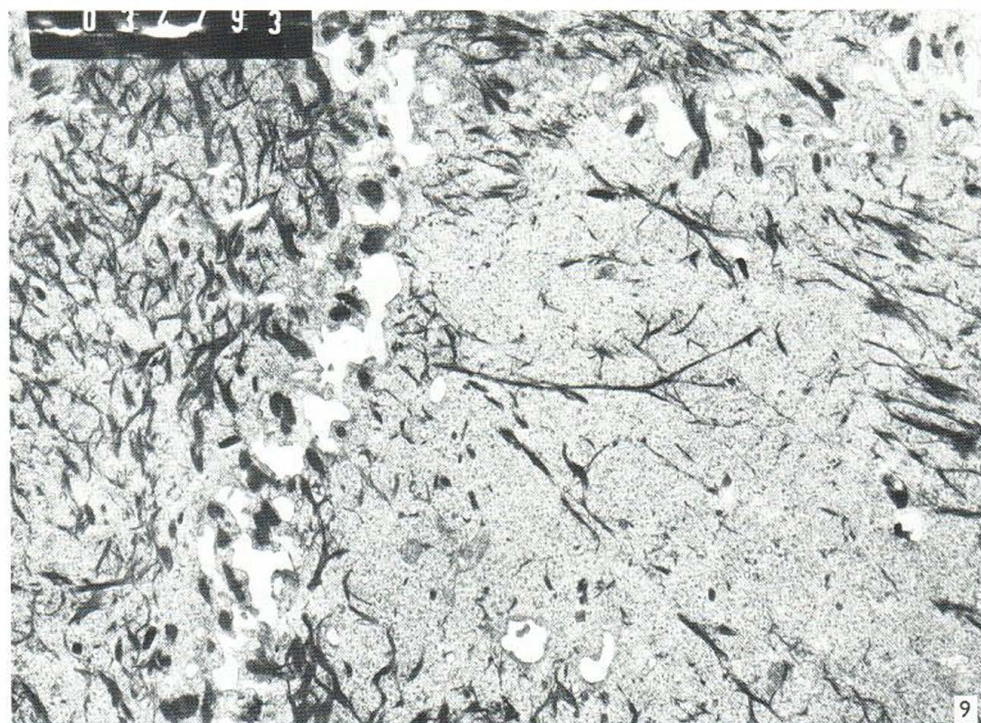
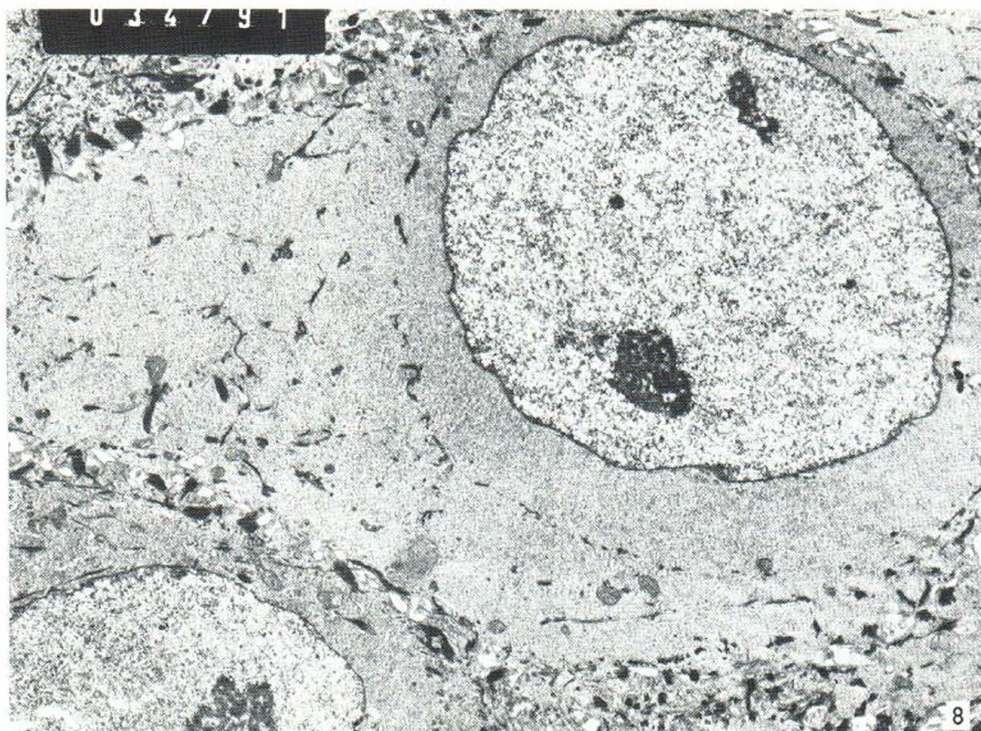


Fig. 8. Clear cell with large nucleus. The cytoplasm contains fine granular material with only a few small bundles of tonofilaments.  $\times 3\,300$ .

Fig. 9. Clear cell with a few thin straight bundles of tonofilaments and desmosomes with neighbouring keratinocyte. Note absence of desmosome-tonofilament complex.  $\times 5\,600$ .

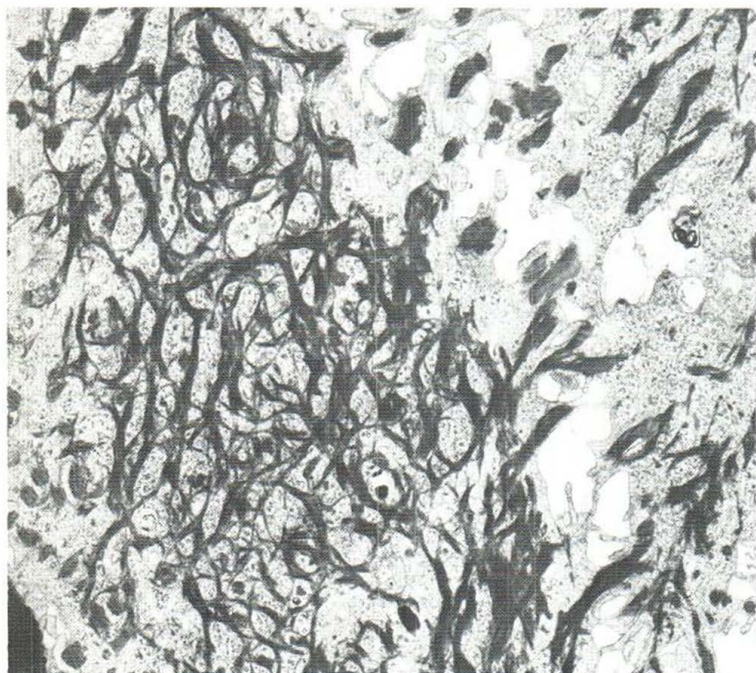


Fig. 10. Dyskeratotic cell with abundant thick tonofilament bundles.  $\times 8200$ .

splasia verruciformis but in whom the histological picture was so strange that it was considered incompatible with this disease. The perplexing nature of these findings is reflected in the title of their paper: "Two Cases of Unusual Vacuolar Degeneration of the Epidermis", with an interrogative subtitle "Epidermodysplasia verruciformis?". In the discussion devoted to this paper in the same issue, Montgomery (6) maintained his belief that these two cases were a variant of EV. Ellis (7) raised the possibility that the two cases might conceivably constitute a separate nosological entity, though he concluded that they did not, thus supporting Montgomery. Cawley (8) also stressed the "resemblance to epidermodysplasia verruciformis". The obvious obstacle in reaching a consensus for this diagnosis in these two cases was the unusual histopathological picture, hitherto undescribed in this disease.

Unlike these two cases of Johnson et al. (5), in which other diagnoses such as Darier's disease had also been considered, the two cases seen by us were from the beginning considered without doubt to be epidermodysplasia verruciformis, on the basis of the clinical picture. Striking in the histological picture was the finding of round or elliptic clear giant cells with a large nucleus containing 1–2 prominent nucleoli and basophilic cytoplasm in the

granular layer and among the underlying prickle cells of the epidermis. The presence of this finding, which is identical with that described in the cases of Johnson et al. (5), in our indubitable cases of EV would appear to confirm that their two cases were also actually EV.

There has been prolonged controversy as to the etiology of EV. Some authors feel that the evidence indicates its being an epithelial nevus (1, 9–11), a viewpoint supported by the frequent history of parental consanguinity and appearance of the lesion in other members of the family as well as its usual development shortly after birth. On the other hand, the resemblance of the lesions to verrucae suggests a viral etiology. The latter possibility is strongly supported by positive auto- and hetero-inoculation trials (12, 13) and has come to be generally accepted, leading to the proposal of a new term, "verrucosis generalisata" (14–16), which is compatible with a viral etiology, to replace the term "epidermodysplasia", which suggests the nevoid character of the disorder. An attempt to reconcile the two views has been made by Ruiters and Van Mullem (17) who believe that EV should be regarded as a genodermatosis in which the skin anomaly predisposes to infection with the virus.

The finding of the large clear cells in the

epidermis in both our cases and those of Johnson et al. (5) may constitute a clue to the solution of this problem. In the discussion on the cases of Johnson et al., Pinkus (18) suggested that "inasmuch as only individual cells were involved, while many others in between remained normal, . . . this might not be a nevus disturbance of the epidermis but rather a virus disease". Pinkus also conjectured as to "whether the large intranuclear bodies that looked like nucleoli might represent inclusion bodies". Montgomery (19), however, stated that "the vacuolar changes in the two cases reported by Johnson et al. are unique in my experience and are consistent with the broad group of epithelial nevi". Thus, even in regard to the giant clear cells, opinions are divided and no uniform interpretation has been achieved.

In this respect, the electron microscope studies carried out in our two cases may make a more definite contribution towards the solution of this dilemma. These revealed groups of particles in the nuclei of a few keratinocytes in the granular layer of the epidermis which comply with the criteria for papova virus particles (2-4). The fact that these particles were found in the upper granular layer and not in the clear cells may indicate the coexistence of two separate lesions—clear cells and virus-containing cells. The clear cells, however, show the typical cytopathic changes known to be induced by virus, i.e. a massive enlargement of the cell and the disappearance of organelles and of tonofilaments, even in the desmosomal complex (20). These findings taken together may be considered to support the theory of a viral etiology for EV.

The immunological studies in our two patients showed intact humoral immunity but impaired cellular immunity. The percentage of T cells in the peripheral blood was below normal and there was cutaneous anergy to the common antigens tested. These results are compatible with the report of Prawer et al. (21) of immunodeficiency in two siblings with EV. These still remains the question as to whether these findings reflect an inherited abnormality in immunoreactivity or whether the impaired immunocompetence is the result of viral infection. Considering the otherwise good health and well-being of these two brothers, it would appear that the immune damage is secondary.

In view of all the above, it is considered that the results of both the electron microscope and immunological investigations may be regarded as

supporting the theory of a viral etiology for epidermodysplasia verruciformis.

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