

## FURTHER HISTOLOGICAL INVESTIGATIONS ON MALIGNANT DEGENERATION OF CUTANEOUS LESIONS IN EPIDERMODYSPLASIA VERRUCIFORMIS

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**Abstract.** The histopathological changes in a skin lesion occurring in a patient with epidermodysplasia verruciformis (e.v.), in which malignancy was suspected, have been described in a preceding article. The lesion was found on the forehead of a 23-year-old patient and the histology showed the picture of an invasive bowenoid squamous cell carcinoma. A prominent feature of the histological picture of this lesion was the occurrence of broad bands of swollen "clear" cells identical in every respect with those considered characteristic for e.v. It was assumed that this invasive carcinoma had developed from an original e.v. lesion and represented a form known in the literature as epithelioma epidermodysplasique. The histopathological investigations of three additional biopsies of skin lesions, suspected of malignancy, which were also found on the forehead are described in the present article. Intraepidermal carcinomas were found. As the origin of these carcinomas in situ constituted a major point of interest a more detailed histological investigation of an original e.v. lesion was made. Here, attention was drawn to the unusual behaviour of epithelial cells in the lower layers of the epidermis. Two of the three additional biopsies showed pathological changes known to occur in senile keratosis and solar keratosis. Because of their poor quality no complete examination was made. In the remaining biopsy the picture of an incipient intra-epidermal carcinoma of the Bowen type was found. Here the histopathological changes showed several similarities with original e.v. lesions. A number of large clear cells was found immediately beneath the stratum granulosum. The micrographs suggested that they were pushed up by the atypically proliferating epidermis. In addition virus particles could be demonstrated in the sections with the electron microscope in relatively normal cells in the superficial layers of an area showing carcinomatous changes. The authors conclude, that both the invasive bowenoid carcinoma and the incipient intra-epidermal bowenoid carcinoma had developed from original e.v. lesions. The origin of the two other lesions showing changes known to occur in senile and solar keratoses remain in question. In all biopsies of the carcinomatous lesions (forehead) distinct actinic elastosis was observed. This finding is in accordance with the observation that malignant degeneration in e.v. predominantly occurs in

areas of the skin exposed to light. For this reason the occurrence of autochthonous solar carcinomatous changes beside malignantly degenerated e.v. lesions could not be excluded.

Ruiter & van Mullem (4) first demonstrated by electromicroscopy an intranuclear virus in the skin lesions of epidermodysplasia verruciformis (e.v.) in a 23-year-old man, institutionalized elsewhere because of imbecility. These findings were subsequently confirmed by, among others, Jablonska et al. (3) and Baker (2). Ruiter & van Mullem regarded the cutaneous condition in question as a genodermatosis in which the skin anomaly, among other expressions, reveals a striking decrease in resistance to a virus which is presumably identical with or related to verrucae vulgares (4). In a former publication in this journal, one of us described the histological changes of a cutaneous lesion in epidermodysplasia verruciformis (e.v.), in which malignant degeneration had occurred (5). A bowenoid squamous cell carcinoma with invasive growth was found, which still bore a histological resemblance to an original e.v. lesion. Areas which showed a striking resemblance to Bowen's disease were found in the strongly proliferated and acanthotic epidermis. They were separated by wide bands of swollen "clear" cells reaching from the stratum corneum to the corium. In the present paper this form of carcinoma will be discussed once more.

Besides malignant changes of the type reported above, successive biopsies revealed that other forms of malignant degeneration had also occurred. In the following these other forms will be described, while at the same time the question will

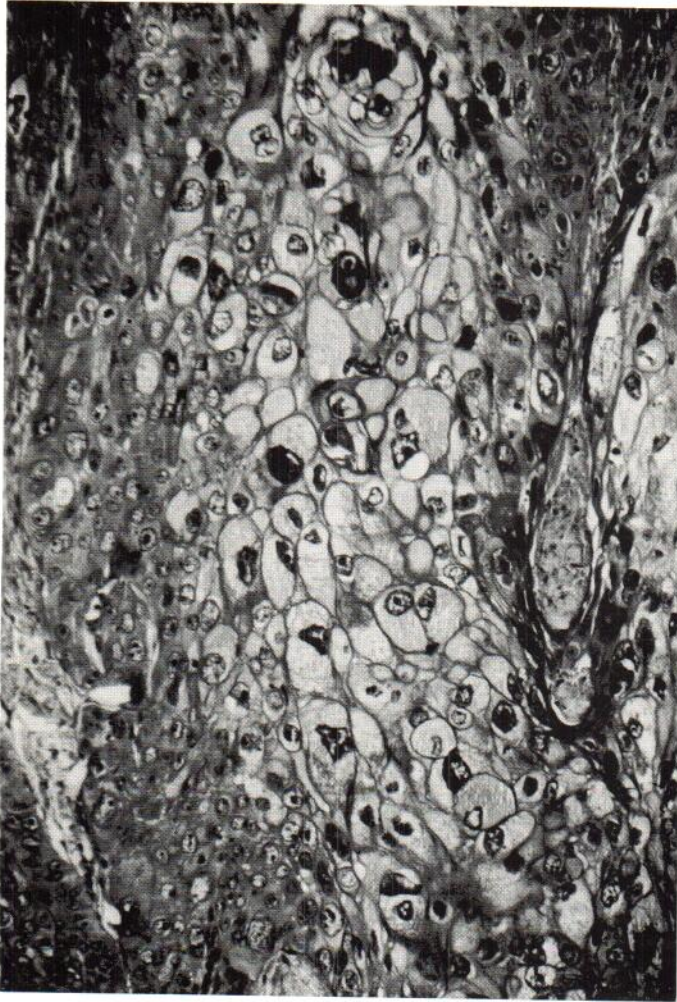


Fig. 1. Biopsy *a*: (Detail from invasive Bowenoid squamous cell carcinoma): Band of clear cells; the individual cells are demarcated from one another by membrane-like structures. Masson stain,  $\times 180$ .

be investigated as to what extent these changes may also be assumed to have developed from original e.v. lesions. All malignant changes found were confined to the skin of the forehead, viz. on areas exposed to sunlight.

#### MATERIALS AND METHODS

Punch biopsies were made of cutaneous changes occurring on the forehead, which were suspected of being malignant. One of these biopsies (*d*) was taken from a squamocrustaceous lesion. Two further biopsies (*b* and *c*) were taken from an eroded area the size of a 2-shilling piece, which became apparent after the thick, dry crusts had been removed. One-half of each biopsy was used for light microscopy, the other half for electron microscopical examination. The paraffin sections were stained with haematoxylin-eosin, with the Verhoeff stain and by the

PAS technique. Examinations were also carried out in polarized light. The material intended for electron microscopical investigation was prepared in the usual way. This examination was used principally for the detection of virus-containing cells.

#### RESULTS

Biopsy (*a*): The histopathology of the invasive (Bowenoid) carcinoma has already been described elsewhere (5). In the present investigations attention was given more particularly to the bands of large clear cells (Fig. 1), extending from the horny layer to the underlying corium and embedded in the broadened downward proliferating carcinomatous epidermis. It appeared that no intercellular bridges could be demonstrated be-

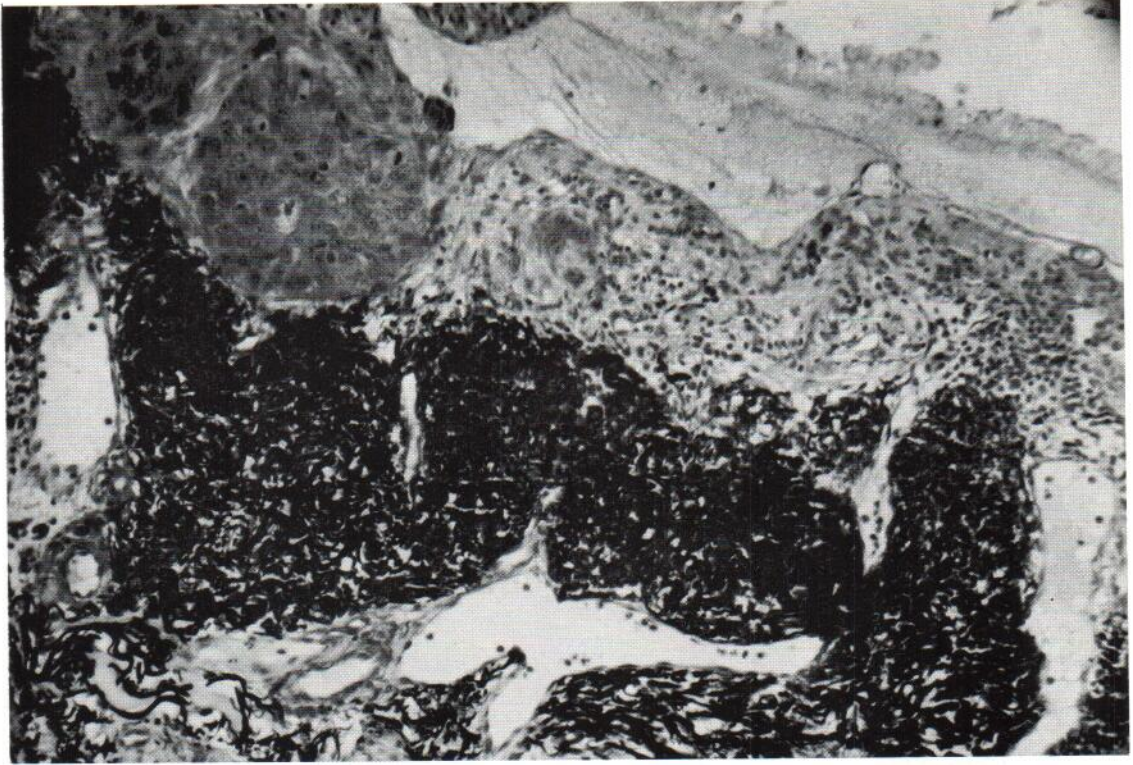


Fig. 2. Biopsy *b*: Pronounced (solar) degeneration of the collagen of the upper corium. Top left: remains of epidermis showing atypicality. Verhoeff stain,  $\times 210$ .

tween these clear cells and that they were separated from each other by a network of membrane-like structures, which did not show double refraction in the polarization microscope. The PAS reaction of the clear cells turned out to be negative. They were indetical in every respect with those found in the original lesions. In the upper parts of the dermis extensive degenerative changes of the connective tissue (solar elastosis) were found, which became apparent particularly with Verhoeff's stain.

Biopsies (*b*) and (*c*) showed intra-epidermal changes known to occur in senile and solar keratoses. Neither of these biopsies is included in this investigation because of their poor quality (being taken from an eroded lesion, large parts of the surface epidermis were missing). Here also extensive solar elastosis was observed (Fig. 2).

In biopsy (*d*) the histological picture of an incipient intra-epidermal carcinoma of the Bowen

type was found (Fig. 3). The histological changes were as follows: the epidermis was complete; it was irregularly thickened, forming epithelial buds extending into the corium; the basal layer was intact. The cells of the Malpighian layer were disorderly and atypical, with irregularly formed nuclei especially in the widened parts of the epidermis. Among them, large hyperchromatic specimens occurred. Occasionally, pathological epithelial cells in a whorled arrangement were seen. There were several mitoses. At the same time individual keratinization of epithelial cells was observed. The epidermis showed parakeratosis in some places, hyperkeratosis in others. Infiltrates of mononuclear cells could be seen in the dermis, just below the epidermis. Here too there were extensive degenerative changes of the upper layers of the collagen (solar elastosis). Some sections showed additional changes which will be more extensively discussed later.

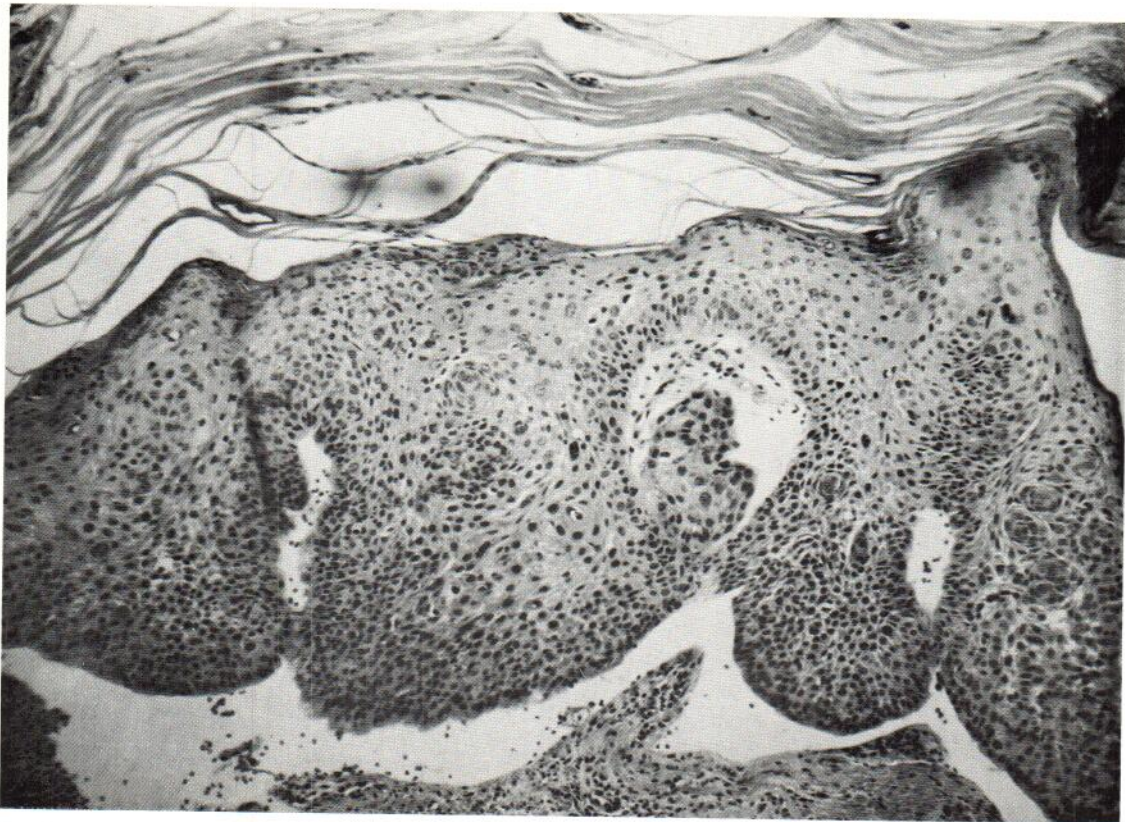


Fig. 3. Biopsy *d*: Intra-epidermal malignant changes of a bowenoid character. The epithelial cells show a disorderly arrangement and cell atypicity with irregularly formed

nuclei. Several hyperchromatic specimens are seen. Locally there is a whorled arrangement of pathological cells. The basal layer is intact. H and E stain,  $\times 60$ .

### DISCUSSION

Before discussing the epithelial changes, a few words should be said as to the extensive collagen changes found in the biopsies of the malignant changes observed. The extensive degeneration of collagen observed in the upper layers of the dermis must be considered to be so-called solar elastosis. This corresponds with the observation that the malignant lesions in e.v. occur almost exclusively on areas of the skin exposed to sunlight. Original e.v. lesions not exposed to sunlight (on breast) did not show these collagen changes. It is remarkable that this effect of light on the skin—occurring as a rule in older people—was observed here in a relatively young (23-year-old) patient. One might ask oneself whether the collagen changes in question are connected with a special (congenital) condition of the skin in e.v., which also finds its expression in a distinct decrease in resistance to the

virus of verrucae vulgares. At the same time it should be kept in mind that ultra violet light may induce cutaneous carcinomatous changes.

As to the epithelial changes, a prominent feature observed in the invasive Bowenoid carcinoma (biopsy *a*) consisted of the occurrence of bands of clear cells. As with the clear cells found in original e.v. lesions, they were separated from one another by membrane-like structures. Probably it is this form of squamous cell carcinoma in e.v. that is at times referred to as epithelioma epidermodysplasique (1).

Of the biopsies showing intra-epidermal malignant changes, biopsy (*d*), showing an incipient intra-epidermal carcinoma of the Bowen type, was studied in greater detail. For the proper evaluation of the histological changes found in this biopsy a more detailed examination of the morphological substrate of original skin lesions in e.v. appeared to be desirable.

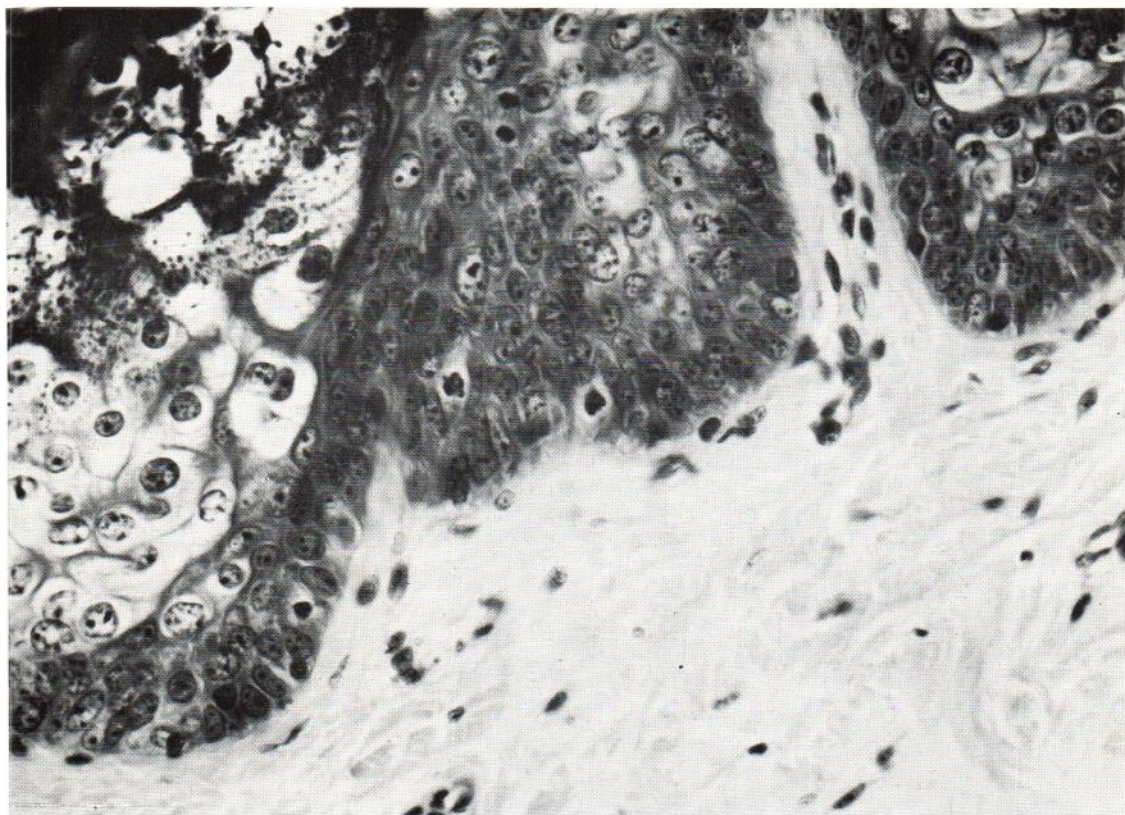


Fig. 4. Lower layers of an original e.v. lesion. There is a tendency to disorganization of the epithelial cells with a certain polymorphy from the side of the nuclei, which show a swollen appearance. Note various mitoses. On the

left, a number of "clear" cells. In the centre, the transitional zone between clear cells and underlying epithelial layers is just visible.

In original lesions not exposed to sunlight (from the breast of the patient) the epithelial layers situated below the nests of clear cells, the basal layer included, showed a tendency to local disorganization associated with a certain polymorphy on the part of the nuclei, which had a swollen appearance and as a rule displayed one or two large nucleoli. In addition there was increased mitotic activity (Fig. 4). As the lower layers of the epidermis are probably infected first, these changes may be the result of the viral infection. Further histological examination revealed between the lower layers of the epidermis and the nests of "clear" cells a transitional zone. In this zone the epithelial cells showed partial clarification of the cytoplasm, while intercellular bridges could still be demonstrated. In clear cells situated higher up, which were in the majority, intercellular bridges could no longer be demonstrated and the cells were separated from one another by membrane-like structures. In the uppermost of these clear cells numerous keratin granules occurred.

With regard to the incipient intra-epidermal bowenoid carcinoma one of the more remarkable

findings observed in investigating serial sections of this biopsy (*d*) was the occurrence in some sections of a number of large clear cells situated in the upper parts of the rete immediately beneath the stratum granulosum; these swollen "clear" cells could be easily distinguished from the underlying atypically proliferating epithelium (Fig. 5).

In such sections the lower layers of the clear cells still showed intercellular bridges while the outer layers they could no longer be demonstrated. Upper clear cells in original lesions show large numbers of keratin granules. In the clear cells occurring in the intra-epidermal carcinoma the amount of keratin appeared to be distinctly diminished but specimens near the stratum granulosum still showed a sprinkling of small keratin granules. The micrographs suggested that the clear cells were being pushed out by the underlying atypically proliferating epidermis. In Fig. 5 a large cell can



Fig. 5. Detail of a section from biopsy *d* (see also Fig. 3). At the top, a number of large "swollen" cells (arrows) resembling the "clear" cells of the original e.v. lesions. Underneath, the epidermis shows loss of normal architecture together with cell atypicality; the nuclei are of varying size with some large hyperchromatic specimens. H and E stain,  $\times 470$ .

actually be seen between the stratum granulosum and the stratum corneum. In the major part of the clear cells the cytoplasm of adjacent cells tended to fuse. Only a single specimen showed any indication of an enveloping membrane. Apparently the development of the "clear" cells had been partly disturbed by the abnormal behaviour of the underlying proliferating epidermis.

All in all sufficient evidence could be supplied indicating that the (incipient) intra-epidermal carcinoma examined had developed from an original e.v. lesion. Demonstration of virus in this incipient intraepidermal carcinoma also revealed a further argument in favour of this conception. Virus-containing cells (Fig. 6—electron microscope) could be located with the light microscope by

means of a  $0.5 \mu$  thick plastic section stained with acid fuchsin-crystal violet of Epon-embedded tissue, the remainder of which was subsequently used for electron microscopical examination. Virus could be detected in this way in cells of relatively normal appearance situated in the superficial layers of an area exhibiting intra-epidermal carcinomatous changes. These cells probably belonged to the original e.v. lesion, in which malignancy had developed. (In the frankly carcinomatous invasive lesion, in conformity with a publication by Jablonska et al., virus could no longer be demonstrated.)

It seems that, hitherto, most authors have accepted without comment that carcinomas occurring in e.v. had developed from original e.v. le-

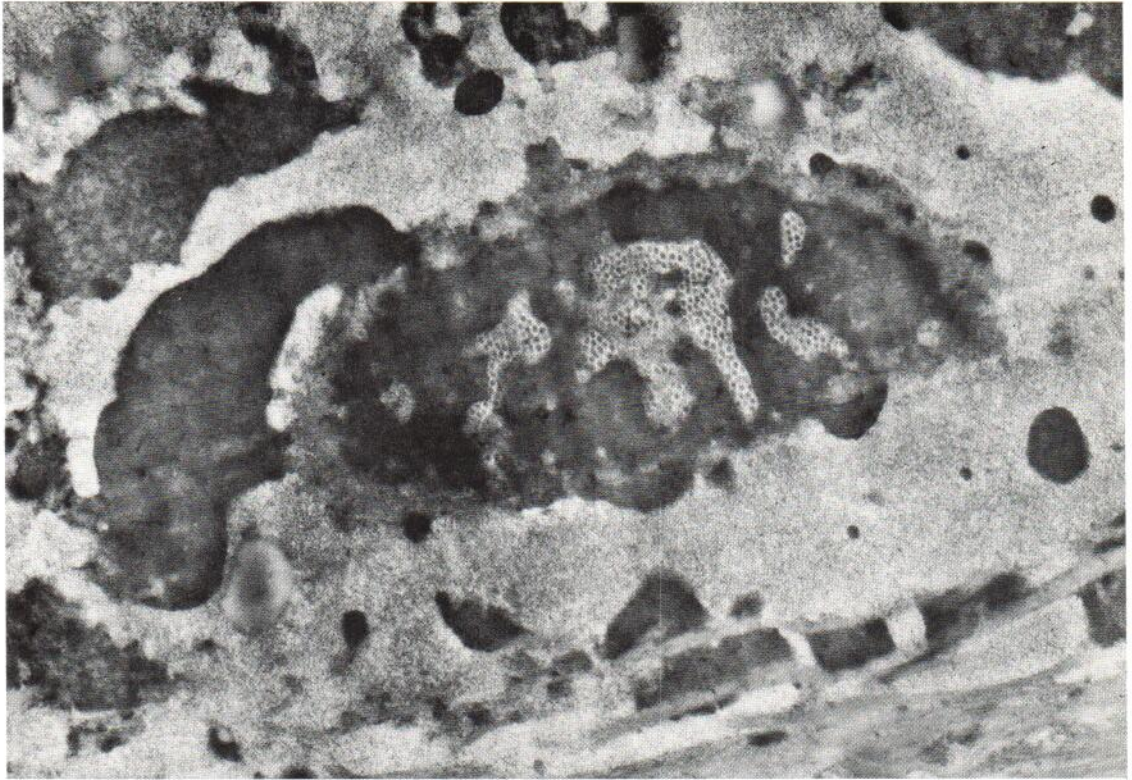


Fig. 6. E.M. micrograph of virus-containing cell (nucleus) situated in the lower layers of the stratum corneum from

an area showing intra-epidermal malignant changes (biopsy *d*).  $\times 22,500$ .

sions. Our findings of extensive solar elastosis in areas in which carcinoma is frequently seen in e.v., stimulated us to seek more direct evidence of its origin, especially as in a previous article we suggested that the role of a virus in carcinogenesis in man might be studied in this cutaneous condition. In 2 cases, an invasive bowenoid carcinoma and an incipient intra-epidermal carcinoma of the Bowen type, evidence could be advanced in favour of the idea that malignant degeneration may occur at least in part of the original lesions in e.v. exposed to the sun. At the same time—also in view of the distinct solar elastosis found in all biopsies—it is conceivable that autotochthonous actinic malignant changes may occur simultaneously with such lesions.

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