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ON THE HISTOMORPHOLOGY AND ORIGIN OF MALIGNANT CUTANEOUS CHANGES IN EPIDERMODYSPLASIA VERRUCIFORMIS

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Abstract. The results are reported of a histological examination by means of serial or multiple sections of 7 malignant lesions of varying type found in a patient with epidermodysplasia verruciformis. A number of more or less conspicuous histological features encountered in the various biopsies are recorded; Acantholysis was fairly common. Two of the biopsies showed the picture of an adenoid squamous cell carcinoma, while a keratosis-senilis-like lesion, if not removed, would also probably have developed into this type of carcinoma. Areas of atypically proliferating epithelium showing Bowenoid features were seen in a number of the examined biopsies. Adjacent to rudimentary pilar structures, distortion and degenerative alterations of hair follicles were found. Unususal cells observed in some of the biopsies are described and discussed. All biopsies showed actinic elastosis in the upper layers of the dermis. No conclusive evidence of a relationship between the malignant changes and the original E.V. lesions could be demonstrated from a histological point of view. This is in contradistinction to the opinion of other authors. Microscopic examination of clinically unaffected skin from the forehead showed, besides atrophy and pigmentation of the basal layer of the epidermis, the occurrence of scattered melanophages and actinic elastosis in the dermis. Serial section of this biopsy additionally revealed at one place a downward proliferation of epithelium suspected of being malignant. Since knowledge of the origin of malignant changes in E. V. must be considered to be still incomplete, more attention should be paid in the author's opinion to the unusual actinic damage found at a relatively early age in the exposed skin of these patients.

One of the more remarkable features of epidermodysplasia verruciformis (E.V.) is the development at a relatively young age of malignant changes on the exposed skin. It is generally assumed that these are derived from "original" (virus-induced) E.V. lesions. In 1969 we suggested that epidermodysplasia verruciformis might be one of the rare conditions in human pathology in which a virus may be involved in carcinogenesis. In a subsequent article (1970), however, we reported our suspicion that, in addition to malignant transformation of E.V. lesions, solar carcinomatous changes might occur independently. In view of this the possibility of skin carcinomas developing from "original" E.V. lesions needs further proof.

The purpose of the present paper is to study the histopathology of malignant changes occurring in E.V. on a larger scale. At the same time, attention has been paid to the question whether the malignant skin alterations in E.V. show histological features which might indicate that they are the result of a malignant transformation of "original" lesions in this cutaneous condition. A characteristic histological feature of the latter is the presence of nests of clear (vacuolated) cells, which locally give the epidermis a "basket-weave" pattern.

MATERIAL AND TECHNIQUES

The patient (male) was at the time of the first examination (1964) 22 years of age and was institutionalized elsewhere because of imbecility. The skin picture was typical, and a virus could then be demonstrated for the first time (5). In 1968 malignant changes were observed on the forehead. Since then, precarcinomas and carcinomas developed on the face. In addition to biopsies and excisions of 7 suspect lesions, a biopsy of clinically unaffected skin (forehead) was used for microscopical examination. Formalin- or Bouin-fixed paraffin sections were stained with hematoxylin and eosin or by means of the Verhoeff technique. Serial or multiple sections of all biopsies were examined.

RESULTS OF HISTOPATHOLOGICAL EXAMINATION

Biopsy (a) (oval, slighly scaling greyish lesions from the forehead)

Most sections showed two types of histological changes (Fig. 1). Plaque-like acanthotic areas were found, consist-

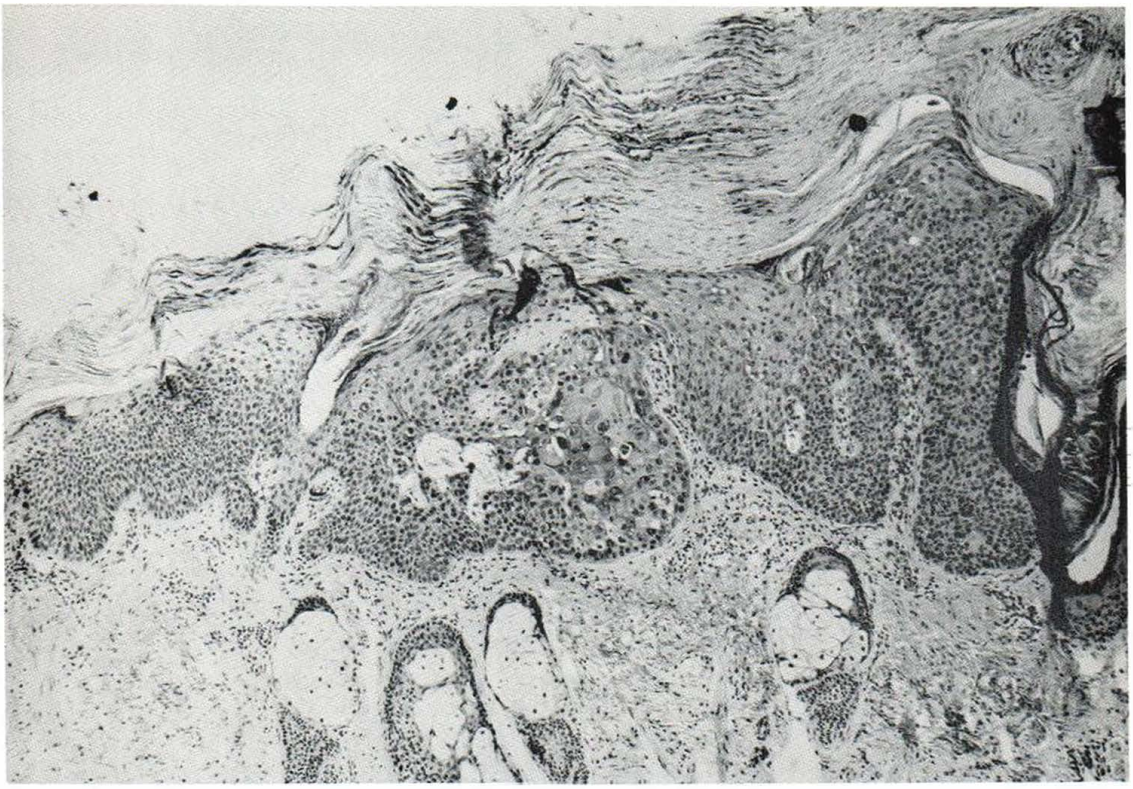

Fig. 1. From biopsy (*a*). (*Left*) proliferation of small dark cells. (*Right*) proliferating squamous epithelium with disordered arrangement and some atypia. (*Centre*) an area showing dyskeratosis. H. and E., × 70.

ing of small dark basophilic cells showing intercellular bridges. Pigment was often seen in the basal layer and elsewhere in the thickened epidermis. Other areas showed a more or less pronounced papillomatosis associated with verrucose acanthosis. Here the epidermis often showed loss of normal architecture, some of the cells being atypical. Occasionally a small horn pearl was found. Areas of dyskeratosis (see also Fig. 1) were also observed in these parts. The adjacent proliferating epidermal cells showed a disordered arrangement with polymorphous nuclei, incipient horn pearls and whorl-like arrangement of prickle cells. In a few areas deep in the acanthotic dysplastic epithelium there were minute cystic foci which contained a few acantholytic cells. The cystic foci seemed to be developing a glandular space. The cells of the stratum granulosum lying over the dyskeratotic areas were darkly stained and often extremely elongated, giving them a band-like appearance. The latter structures were also seen in the overlying (thickened) horny layer (parakeratosis). In addition discrete mostly round nests of faintly stained prickle cells were seen within the thickened epidermis (Fig. 2 A) which were obviously distinct from the adjacent compressed Malpighian cells. They were cuboidal in shape or, less frequently, elongated; the latter were more often found at the periphery of the cell nests. In serial sections these cells showed a tendency to keratinization (often incomplete). In some of these cell nests either at the periphery or in the centre there were one or two large swollen cells with considerable amounts of violet homogeneous cytoplasm and large rather pale nuclei. In a number of sections similar cells were also found elsewhere in the epidermis. They occurred singly, in small groups, or even in a tubular arrangement (Fig. 2 B). They were devoid of intercellular bridges. Some showed an enveloping membrane. Similar swollen cells were seen in areas of dyskeratosis and more especially at their periphery, where they often showed partial keratinization increasing towards the centre of such areas.

In the sections, hair follicles were seen to be in various stages of development. They showed distortion and degenerative changes. Thus a follicle was filled with degenerated cells and no hair could be demonstrated (Fig. 3 A). Serial sections sometimes revealed keratotic material in the centre of these intrafollicular masses of degenerated cells. Occasionally the ostium of a hair follicle was surrounded by several layers of large, darkly stained fusiform cells with abundant, occasionally foamy cytoplasm and large vesicular nuclei (Fig. 3 B). Only the upper layers of these cells seemed devoid of intercellular bridges. They were seen to extend for a short distance at both sides of the ostium between the granular layer and the underlying rete Malpighii. Several rudimentary pilar structures were found. The upper layers of the dermis showed actinic elastosis.

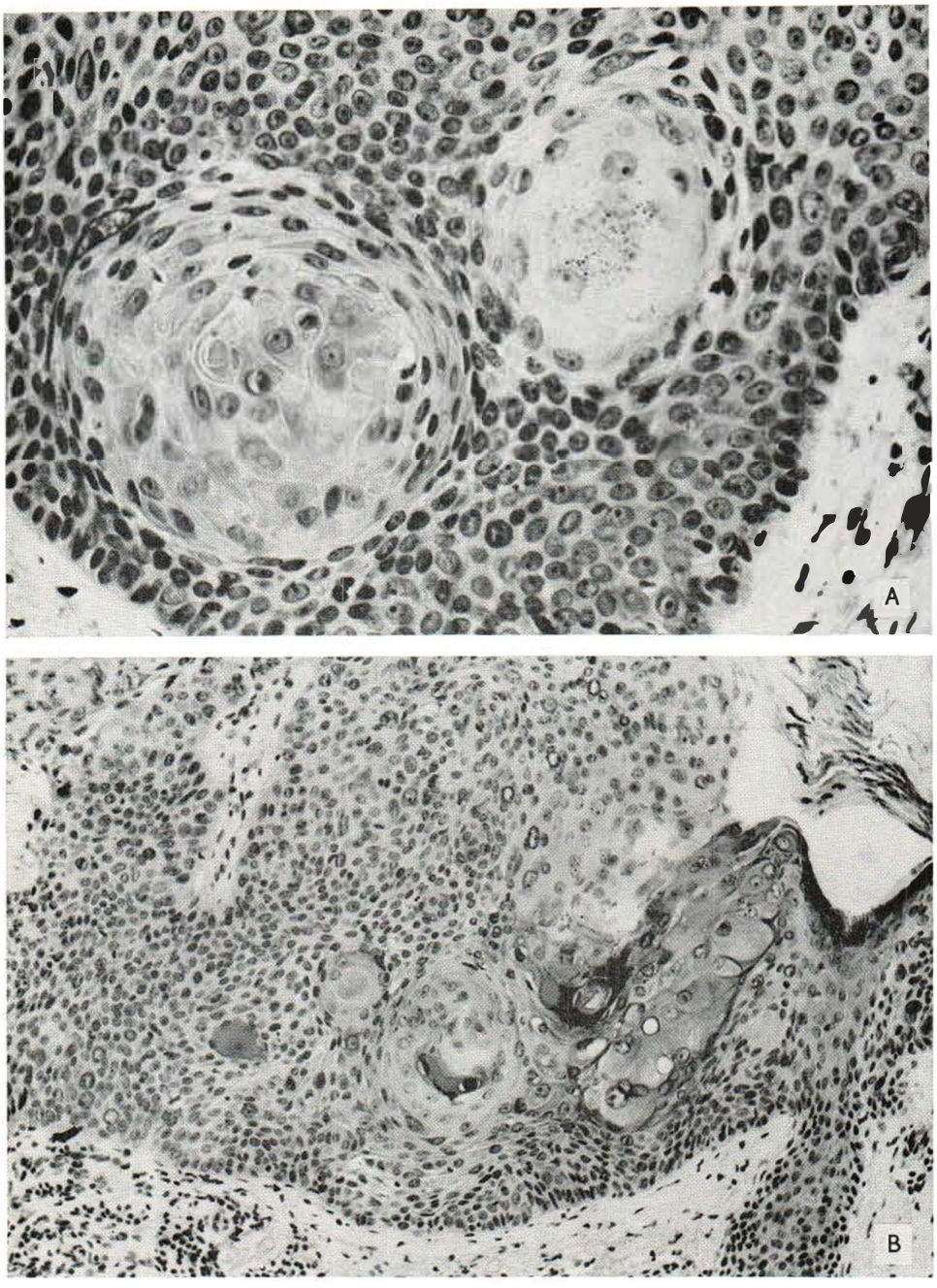

Fig. 2. From biopsy (*a*). (A) Sharply demarcated nests of pale cells. There is almost complete keratinization in the right upper specimen: to the left is a larger nest in which the pale cuboidal cells are clearly shown. In the centre of the larger specimen there is incipient individual cell keratinization. (B) Large cells with homogeneous bluish cytoplasm and rather large nuclei. There are no intercellular bridges. Incipient membrane formation is seen in places. In the centre (lower part) a nest of pale cells shows a similar cell with large amounts of bluish cytoplasm. H. and E., × 150.

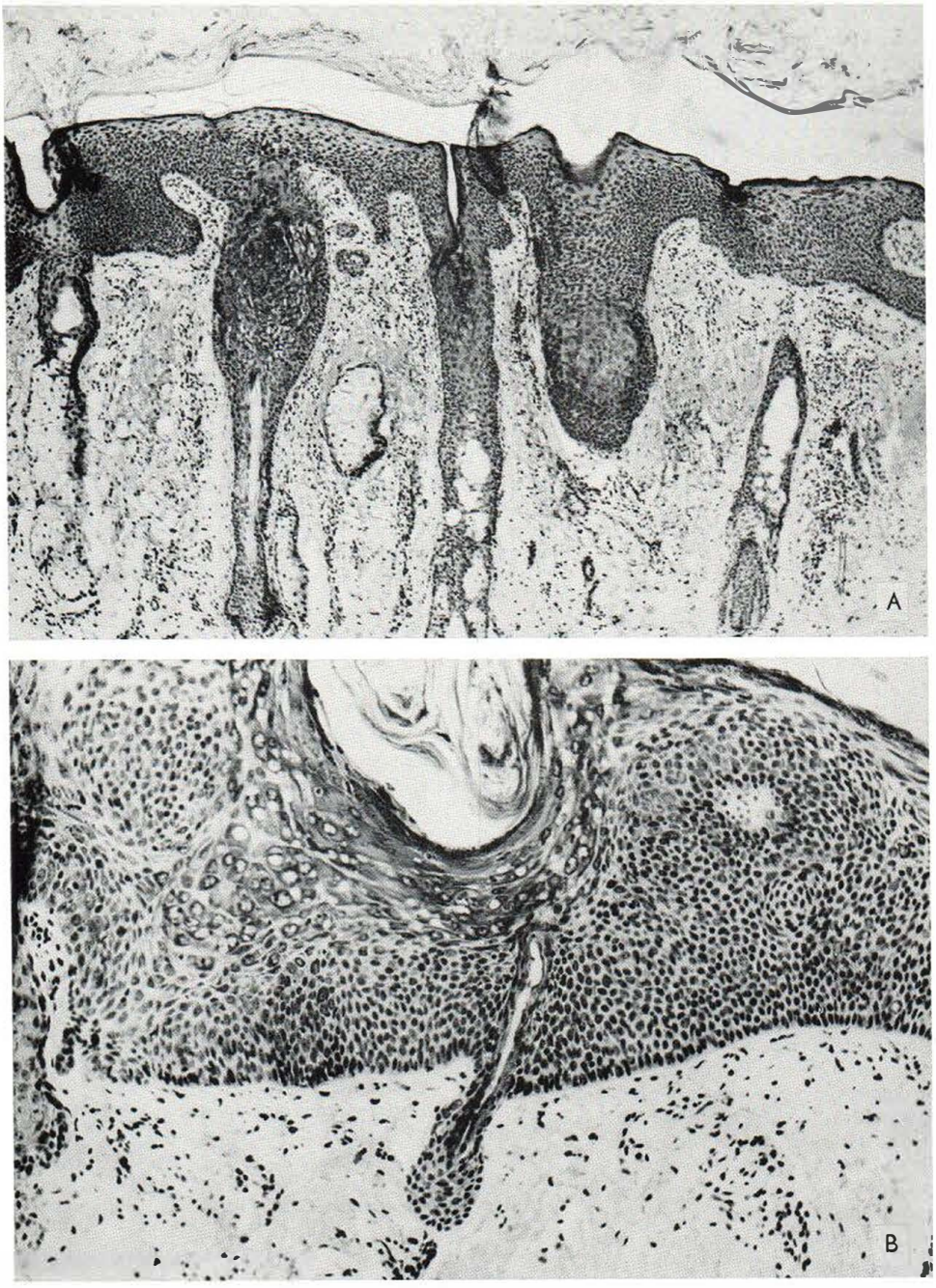

Fig. 3. From biopsy (*a*). (A) Alterations of hair follicles showing bud-like swelling in the upper third, caused by accumulation of degenerated cells. (× 60). (B) Several layers of large fusiform dark cells with vesicular nuclei surround the orifice of a hair follicle. Similar cells were found in biopsy (*b*). H. and E., × 150.

Biopsy (b)

The horny layer displayed hyper- and parakeratosis; the epidermis showed moderate papillomatosis, often associated with verrucose acanthosis. The cells of the stratum Malpighii presented a disordered arrangement; several were atypical. Buds of proliferating atypical cells were seen extending downwards at the dermo-epidermal junction, occasionally surrounding the upper part of a hair

follicle. Within the atypically proliferating epidermis small horn pearls—often with partially keratinized cells—and individual cell keratinization were observed locally. From an examination of serial sections it appeared that in the upper part of these bud-like proliferations occasional degenerative processes occurred with swelling and fusion of the cells, resulting in amorphous basophilic masses. Here too were found small groups of large rounded cells with abundant, occasionally foamy, basophilic cytoplasm, sometimes surrounded by membrane-like structures. They appeared not unlike the large cells which were found lining the ostia of a number of hair follicles in biopsy (*a*). Ostia of hair follicles surrounded by similar cells were also seen in the biopsy under discussion. A number of hair follicles showed distortion and degenerative changes. Rudimentary and hypoplastic hair follicles were observed in several sections. In the dermis a moderately dense inflammatory infiltrate and distinct actinic elastosis were found.

Biopsy (c)

The histologic examination showed a Bowenoid appearance. Multiple sections were examined. Oedema of the upper layers of the epidermis was noted in all sections; among the epithelial cells a number of oval or fusiform specimens with basophilic (occasionally foamy) cytoplasm and pale-staining nuclei of varying size were seen. Some resembled the large basophilic cells observed around the follicular ostia in the preceding biopsies. The intra-epidermal sweat ducts were distended. In the dermis a chronic inflammatory reaction was observed. In the upper layers of the connective tissue, foci of actinic elastosis were present.

Biopsy (d)

In the first sections of the series there was hyperkeratosis and parakeratosis. In most of these sections the epidermis was slightly thickened but seemed to be otherwise normal, though at one place one or two layers of atypical prickle cells were seen at the dermo-epidermal junction. In the dermis, several bizarre nests of atypical prickle cells were found. Some of them showed in the centre horn cysts or horn pearls, the latter containing in most cases partially keratinized cells. In one or two of these horn pearls, large elongated cells with lightly stained vacuolated cytoplasm were found at the periphery. In subsequent sections the epidermis became more and more involved in the pathologic process and finger-like projections of atypical prickle cells penetrated into the dermis. Here the horny layer invaginated in places deep into the epidermis. In the upper layers of the dermis a moderately dense chronic inflammatory infiltration was present. There was also actinic elastosis.

Biopsy (e)

Some of the histologic changes of this biopsy have already been described in a previous article (6). The picture of an invasive carcinoma with Bowenoid features was found. There was abundant keratosis with local accumulations over the tumor of dyskeratotic cells having distorted shrunken nuclei. Basophilic Bowenoid areas were separated by strands of swollen, lightly-stained cells with granular cytoplasm surrounded by thin membrane-like structures. The swollen cells showed downwards an increasing tendency to (malignant) dyskeratosis. Examination of serial sections in the present investigation revealed that similar cells also occurred within the Bowenoid proliferations (Fig. 4 A). Here were observed areas of cells with abundant greyish (occasionally foamy) cytoplasm, showing very large pale irregular nuclei with prominent nucleoli. At the periphery some of these cells showed intercellular bridges. The majority, however, were surrounded by thin enveloping membranes. The pale swollen cells appeared to participate in the carcinomatous processes. At the end of the series the central tumour had diminished in size and the adjacent epidermis showed bud-like proliferations of atypical prickle cells, sometimes associated with acantholysis.

In the upper part of the dermis there was a dense chronic inflammatory infiltration. Here were found several nests of atypical prickle cells frequently showing individual keratinization or a foamy cytoplasm. Occasional cell nests were seen with one or two layers of elongated large foamy cells at their periphery and keratinized cells or horny masses in the centre. The upper layer of the dermis showed actinic elastosis in places.

Biopsy (f) (from a tumour on the upper lip, the size of a bean)

Histologically, an invasive prickle cell carcinoma of the segregating type (adenoid squamous cell carcinoma) was found. At the bottom, several nests of atypical prickle cells were seen showing acantholysis leading to tubular and alveolar lumina. The cells lining these lumina often showed individual cell keratinization. Sometimes the lumina were filled with completely or partially keratinized cellular elements. In the dermis a chronic infiltrate and locally moderate actinic elastosis were seen.

Excision (g) (suspect lesion on the upper lip)

The epidermis showed hyperkeratosis and parakeratosis; there was atrophy of the epidermis in places. Locally, large bud-like proliferations composed of mostly atypical epithelium were observed. They appeared to be attached to the undersurface of the epidermis. Here the overlying epidermis often showed cells with a disordered arrangement and with large irregular hyperchromatic nuclei. The major part of the atypical downward proliferations consisted of swollen pale cells presenting a disordered arrangement with rather large vesicular nuclei differing in size and staining properties (Fig. 4 B), while the intercellular bridges appeared to be diminished in number. There was distinct intercellular oedema. The basal layer of these proliferations was largely intact, but in places it seemed to have disappeared. Next to the bud-like proliferations a few elongated strands of atypical epithelium were seen penetrating into the dermis. At both sides of the carcinomatous focus the rete ridges of the epidermis were absent, or diminished in size. In particular, no E.V. changes were observed in the long stretches of epidermis not affected by the carcinomatous process.

The dermis showed a rather marked inflammatory reaction. The behaviour of the hair follicles was unusual. In some sections starting from the papilla they penetrated

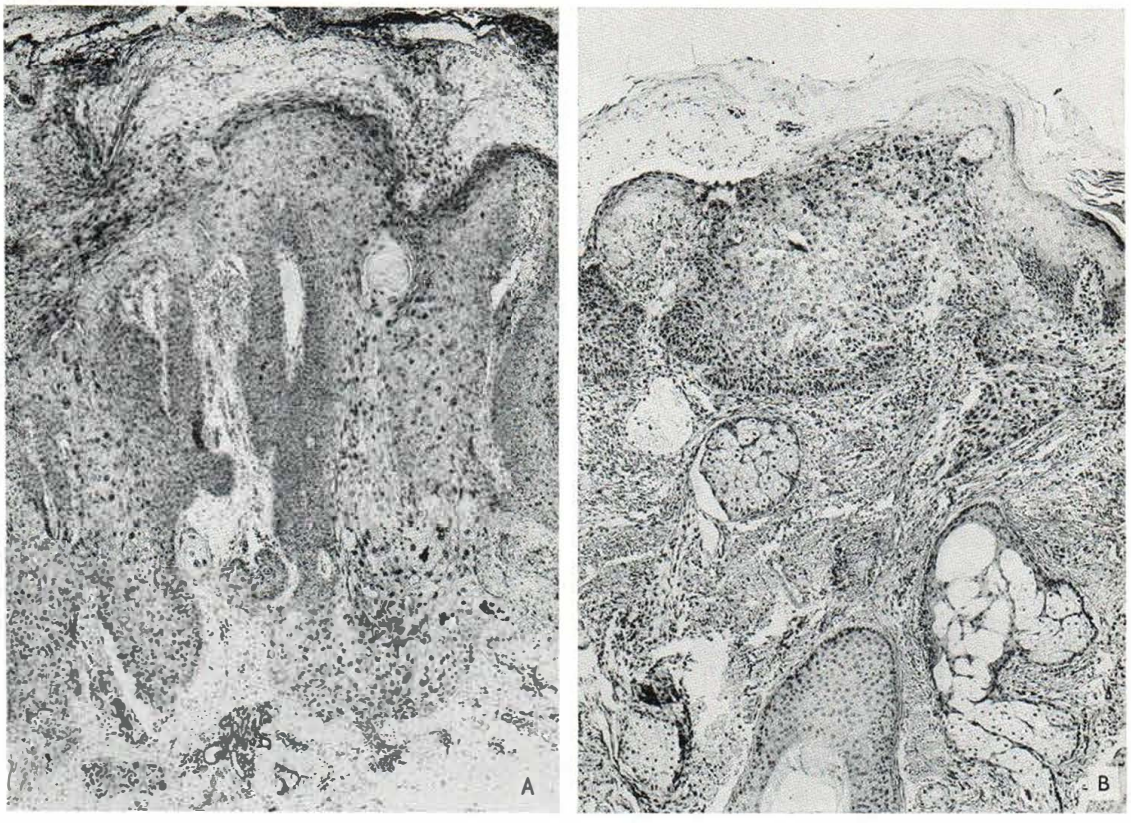

Fig. 4. From biopsy (*e*). (A) Next to dark fields of atypical proliferating epithelium showing Bowenoid features there are large areas composed of irregular, swollen cells with large polymorphous nuclei and enveloped by thin membranes. These cells show a tendency to individual cell keratinization. H. and E., × 34. (B) From biopsy (g). Bud-like atypical proliferation of epidermis. In the upper layers of the epidermis there are atypical cells with large, darkly stained nuclei. The major part of the carcinomatous focus consists of atypical swollen cells; the number of intercellular bridges is reduced. H. and E., × 50. Elsewhere this tumour showed the histological picture of an adenoid squamous cell carcinoma.

into the dermis in a horizontal direction, often turning upward towards the epidermis after a short distance. Occasional large round or elongated keratin-filled cysts were found lined with epidermis. In places, a few small structures of a similar character were observed in the adjacent tissue. The presence of hairs in their centre could not be established. In a number of sections foci of actinic elastosis were seen. Sections from another area of the excision showed a quite different picture. Here, histological examination revealed an adenoid squamous cell carcinoma.

Biopsy (h)

Clinically unaffected skin from the forehead showed the following changes: There was slight hyperkeratosis. The epidermis was thinned and the rete ridges obliterated. The basal layers showed pigment in places. In the dermis there was some chronic infiltrate, partly perivascularly, with a few melanophores. In the upper layers of the dermis the small blood vessels were enlarged. There were foci of actinic elastosis. Serial sections revealed at one place an irregular epithelial proliferation suspect of incipient carcinoma in situ (Fig. 5) at both sides of a lanugo hair.

COMMENT

In the seven biopsies of the lesions various histological pictures were found, all showing a more or less pronounced degree of malignancy. Histological features common to the investigated biopsies in varying degree were: a tendency to degenerative changes and (atypical) keratinization. Distortion and degenerative alterations of hair follicles were found among others in biopsies (*a*), (*b*) and (*g*). Acantholysis was fairly common. Hypoplastic and rudimentary pilar structures were observed several times. In addition, areas of atypically proliferating epithelium showing Bowenoid features were seen in a number of the biopsies

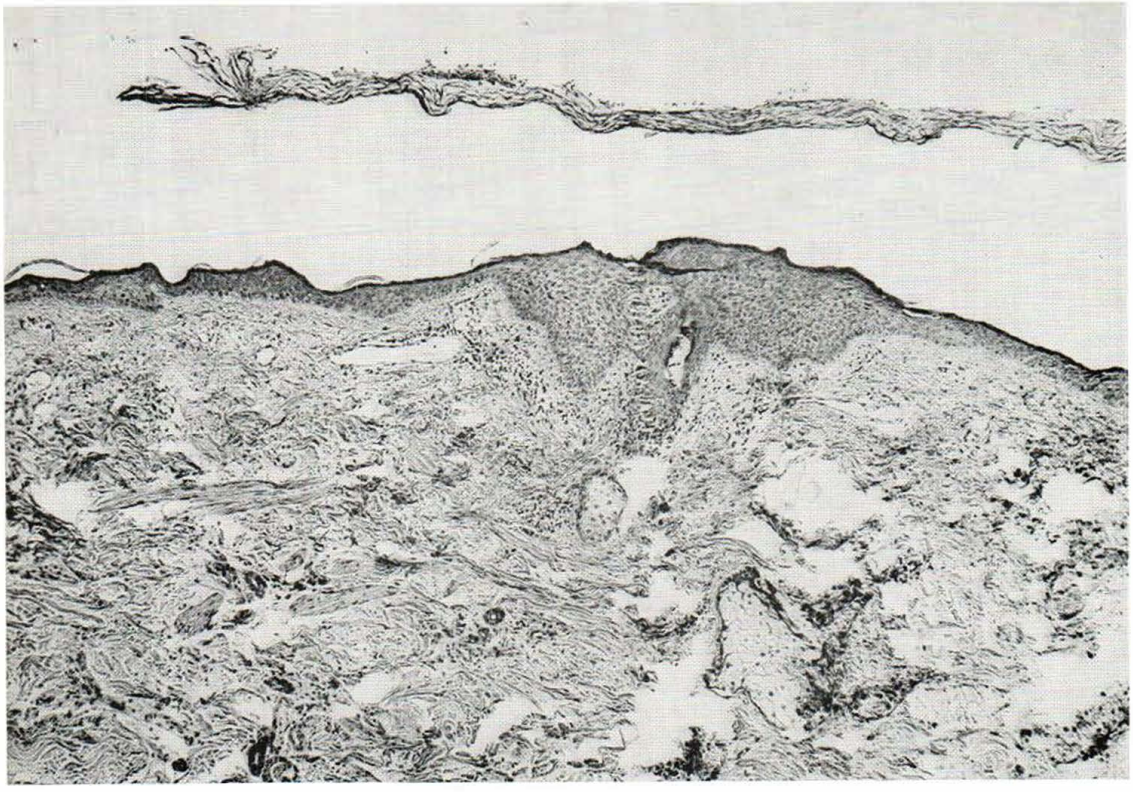

Fig. 5. From biopsy (h) Clinically unaffected skin from the forehead. There is atrophy of the epidermis associated with other signs of actinic damage. Local atypically proliferating epithelium with rests of a small hair follicle in its centre. H. and E., × 70.

(*b*), (*c*), (*e*). All biopsies showed a more or less extensive "actinic" elastosis of the upper layers of the dermis.

Since it is generally assumed that the malignant changes in E.V. are due to malignant transformation of the "original" lesions of this cutaneous affection, special attention was paid, in examining the precarcinomas and carcinomas, to the possible presence of histological features considered to be characteristic of this cutaneous condition. Among the histological changes of "E.V. lesions" the occurrence of nests of swollen "clear" cells is the most characteristic.

One of the aims of this paper was an attempt to determine whether epidermal cells could be demonstrated in the pre-malignant and malignant cutaneous changes examined, which might be considered to be equivalent to the "clear" cells of the original E.V. lesions. Especially in biopsy (*a*) our attention was drawn to the presence of rather unusual cells with large amounts of cytoplasm. Histologically this lesion appeared to be a small epithelial growth of the keratoma senile type. Locally, large areas of dyskeratosis were noted. The unusual cellular elements of the epidermis occurred in the form of round to oval cells with abundant homogeneous bluish cytoplasm (Fig. 2 A). The rather pale staining nuclei of these cells were round, often eccentrically situated and larger than those of normal prickle cells. The cells in question were found singly, in small groups, or in what might be called tubular arrangement. They showed a tendency to fuse and were occasionally enveloped by a membrane. Intercellular bridges were lacking.

The origin of these cells is not clear, though it was of interest that they were sometimes found (Fig. 2 B) in discrete round nests of pale-staining prickle cells which were clearly distinct from the adjacent compressed Malpighian cells. In serial section these cell nests showed a tendency to atypical or individual cell keratinization. It is

conceivable that the large bluish cells play a role in individual cell keratinization, since they were also observed at the periphery of an area of dyskeratosis (biopsy *a*).

The large, swollen purplish-blue cells mentioned above differed in our opinion from the "clear" cells occurring in epidermodysplasia verruciformis. Thus their bluish homogeneous cytoplasm only occasionally contained one or two vacuoles; there was no pyknosis, and keratin granules were absent. In contrast, the "clear" cells in E.V. are distinctly vacuolated, show irregular pyknotic nuclei and, especially in the upper layers, large amounts of keratohyalin. Dr Helwig (Armed Forces, Institute of Pathology, Washington D.C.) who was so kind as to review a number of sections of this biopsy, believed that in view of acantholytic foci situated deep in the epidermis, this lesion, if not removed, would have developed into an adenoid squamous cell carcinoma.

Large fusiform darkly-stained basophilic cells were found in both biopsies (*a*) and (*b*). Several layers of these cells surrounded the orifices of one or more hair follicles (Fig. 3 B) extending into the adjacent rete Malpighii. Their cytoplasm, occasionally foamy, sometimes showed several small vacuoles; their nuclei often had a vesicular appearance. They probably originated from the epithelium of hair follicles. By their location and their morphological and staining properties they were obviously different from the "clear" cells characteristic of epidermodysplasia verruciformis.

The carcinomas observed in biopsies (*e*) and (*g*) were rather unusual. Histological examination by means of serial sectioning of biopsy (*e*) revealed areas of swollen cells embedded in atypically proliferating epithelium showing Bowenoid features. The large to very large swollen cells showed abundant, greyish, finely granular cytoplasm and pale, large to monstrous irregularly-shaped nuclei with prominent nucleoli. They were surrounded by thin membranes and obviously participated in the carcinomatous process. Especially in the lower parts of the tumour they showed a conspicuous tendency to individual cell keratinization. At the periphery of the areas of swollen cells some specimens still showed a number of intercellular bridges. The majority, however, appeared to be surrounded by enveloping membranes, the nature of which could not be established. In original E.V. lesions transitional cells still showing a number of intercellular bridges also occur at the periphery of the intra-epidermal nests of "clear" cells, which for the major part, however, are surrounded by membrane-like structures. Nevertheless, it is unlikely in our opinion, that the carcinoma under discussion represents the result of malignant transformation of an "original" E.V. lesion. We are more inclined to consider it to be a so-called Bowenoid carcinoma; showing numerous irregular, hydropic cells and an unusual degree of individual keratinization.

The histology of part of the carcinoma found in biopsy (*g*) showed large bud-like proliferations of atypical and disorderly arranged epithelial cells; the centres of these carcinomatous foci showed pronounced intercellular oedema. No histological changes concurrent with those of E.V. could be demonstrated in the long stretches of the locally atrophic epidermis on both sides of the carcinomatous focus. Sections from another area of the excision revealed the picture of an adenoid squamous cell carcinoma.

In biopsy (*e*), rather large oval cells with basophilic occasionally foamy cytoplasm and pale-staining nuclei of varying size were seen scattered through the oedematous upper layers of the intra-epidermal Bowenoid carcinoma found here. At the time we believed them to be related to the "clear" cells of E.V. In contradistinction to that opinion however, we believe now—based on recent experience — that no special significance can be attached to these cells.

Finally biopsies (*d*) and (*f*) showed squamous cell carcinomas, the latter of the segregating type. No peculiarities worth mentioning were observed.

To conclude, no convincing evidence of a relationship between the malignant changes and the original E.V. lesions could be demonstrated from a histological point of view. This contrasts with other authors. Thus Jablonska et al. (1) described transitional states of E.V. lesions to Bowen's disease and invasive Bowen's carcinoma. With one possible exception published elsewhere (8), no such pictures were encountered in the present histological investigations.

One of the more interesting findings, however, in the investigations reported in this paper was the occurrence of more or less pronounced actinic elastosis in the upper layers of the dermis in all biopsies examined.

DISCUSSION

It is the common opinion that the malignant changes in E.V., which are known to develop at a relatively early age on exposed areas of the skin, are derived from the "original" (virus-induced) lesions in this cutaneous condition. To our knowledge, Montgomery (3) is the only author who believes that the malignant degeneration of skin lesions in E.V. is probably overemphasized. At the time (6) we disagreed with this author. In a second article (7), however, we came to the conclusion that in addition to carcinomas developing from "original" E.V. lesions, actinic malignant changes probably occur independently in E.V. From the latter observation the question arises as to whether malignant changes in E.V. may actually develop from the skin lesions in this condition. The histology of the various malignant changes reported in this paper suggest that the evidence of malignant transformation of E.V. lesions is so far incomplete. At the same time, these histological investigations emphasize once more the importance of actinic damage in promoting the development of malignant changes in E.V. Therefore more attention should be paid, in our opinion, to the actinic damage observed at a relatively early age in the exposed skin of these patients. A further argument underscoring the significance of the actinic skin damage in the development of malignant changes in E.V. was finally found on examination of clinically unaffected skin of the forehead. Here, apart from atrophy and pigmentation of the basal layer of the epidermis and the occurrence of scattered melanophages and of actinic elastosis in the dermis, serial sections revealed local downward proliferation of epithelium suspected of being malignant. Whether the virus and/or an inherited terrain also play a role in the carcinogenesis in E.V. is still an open question. At the time we believed (8) we had demonstrated the virus in relatively normal cells in an incipient intraepidermal Bowenoid carcinoma. No virus could be demonstrated in fully developed carcinoma. This experiment has not been confirmed up till now. No conclusions can be drawn from the experiments by Okamata et al. (4) who found wart-like virus particles in an early squamous cell carcinoma in a case of E.V. associated with Rhabdomyosarcoma by negatively staining the cancer extract. Localization of virus-containing cells in malignant tissue is not possible in this way.

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