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# Involucrin expression in adnexal skin tumours

## An immunohistological study

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**Summary.** The expression of involucrin was studied in a group of skin neoplasms, mostly of adnexal origin. As happens with other types of epithelial tumours, involucrin was detected in the most differentiated areas (presenting a squamoid or ductal differentiation). No reactivity was observed in non-epithelial skin tumours. These results suggest that involucrin is a specific marker for epithelial and adnexal differentiation of skin tumours and may thus be a useful aid in histopathologic diagnosis and classification of neoplasms.

**Key words:** Involucrin – Adnexal skin tumours

Involucrin (INV) is a cytoplasmic protein synthesized in human squamous epithelial cells; it represents a soluble precursor of the cross-linked envelope of the human horny layer and is therefore a marker of normal keratinocyte differentiation and maturation (Rice and Green 1979; Banks-Schlegel and Green 1981). On normal human skin, INV is present in the cytoplasm of the cells of the upper spinous and the granular layer, the inner root sheath, the infundibulum and the sebaceous duct and the acrosyringium (Murphy et al. 1984).

The expression of INV has been found to be altered in some cutaneous epithelial neoplasms, suggesting an abnormal maturation pathway in these conditions (Said et al. 1984; Murphy et al. 1984; Lacour et al. 1985; Walts et al. 1985). The purpose of the present work was to study INV expression further in a wider variety of benign and malignant epithelial (mostly adnexal) and in non-epithelial skin tumours.

## Material and methods

*Lesions studied.* A total of 45 tumours (Table 1), retrieved from the files of the Histopathology Laboratory (Dr C. Hermier) of the Clinic of Dermatology, Hôp. E. Herriot, were included

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**Table 1.** Reactivity pattern of the tumours studied

| | Number of cases | Reactivity |
|---|---|---|
| *A. Epithelial skin tumours* | | |
| Metastatic carcinomas | 2 | 0 |
| Neuroendocrine (Merkel-cell) carcinoma (NC) | 2 | 0 |
| Extramammary Paget's disease (EPD) | 3 | 2 cases: 0<br>1 case: weakly + |
| Hidroacanthoma simplex (HS) | 1 | 0 |
| Eccrine syringofibroadenoma (ES) | 1 | ++ |
| Eccrine poroma (EP) | 2 | + |
| Syringoma (S) | 4 | ++ |
| Chondroid syringoma (CS) | 1 | + |
| Nodular hidradenoma (NH) | 3 | 2 cases: +<br>1 case: 0 |
| Eccrine spiradenoma (ES) | 2 | 1 case: +<br>1 case: 0 |
| Cylindroma (CD) | 1 | 0 |
| Naevus sebaceous (NS) | 2 | + |
| Apocrine hidrocystoma (AH) | 3 | 0 |
| Trichilemmoma (TL) | 2 | 0 |
| Trichofolliculoma (TF) | 1 | + |
| Trichoepithelioma (TE) | 3 | + |
| Mummified epidermal cyst (MEC) | 1 | + |
| Pilomatricoma (PM) | 2 | 1 case: +<br>1 case: 0 |
| *B. Non-epithelial skin tumours* | | |
| Naevocellular naevus | 2 | 0 |
| Malignant melanoma (MM), nodular | 1 | 0 |
| Dermatofibrosarcoma protuberans (DFP) | 2 | 0 |
| Neurofibroma (NF) | 2 | 0 |
| Stewart-Treves syndrome (STS) | 1 | 0 |
| Malignant haemangioendothelioma of the scalp (MHS) | 1 | 0 |

in a retrospective study. They had been collected during the last 3 years, fixed in Bouin's solution, dehydrated and embedded in paraffin. In parallel, samples of non-diseased skin were studied in the same way as controls.

*Antibody.* A polyclonal antibody raised in rabbits against human INV as described elsewhere (Rice and Green 1979) was kindly provided by Dr. Green.

*Immunoperoxidase techniques.* An avidin-biotin-peroxidase (ABP) technique was employed in the following way: 3 μm-thick sections were deparaffinized in xylene and toluene and rehydrated in alcohol. The following steps were then performed: a) incubation with a 1% solution of $H_2O_2$ in PBS (15 min) for blocking of endogenous peroxidase, b) rapid washing in PBS, c) incubation with normal swine serum (Dako, Denmark, 20% in PBS) (15 min) to reduce non-specific labelling, d) incubation with anti-involucrin antibody (diluted at 1:400 in 20% swine serum in PBS, 37°, 45 min), e) washing in PBS (15 min), f) incubation with biotinylated goat anti-rabbit antibody (Vector, Burlingame, USA) (37°, 45 min), g) washing as in (e), h) incubation with avidin-biotin-peroxidase complex (Vector, Burlingame, USA) (37°, 45 min), i) washing as in (e). Sections were then incubated with a solution containing 5 mg of 3-amino-9-

ethylcarbazole (Sigma, St. Louis, USA) in 200 µl of N-N-dimethylformamide (Riedel de Haën, Hannover), 100 µl of hydrogen peroxide and 10 ml of acetate-buffer (pH 5.2), to visualize the site of antibody binding. After a final washing, the sections were counterstained with Mayer's haematoxylin and mounted with a medium containing gelatin (3 g) in glycerin (25 ml) and distilled water (75 ml). Control slides were included in each reaction, by omitting the first antibody.

## Results

All *control slides* were consistently negative.

### *A. Normal human skin*

On normal human skin immunoreactivity was detected in the cytoplasm of the keratinocytes of the upper third of the epidermis (granular layer and upper spinous layers), the stratum corneum being negative. A positive staining was also detected in cells lining acrosyringeal ducts; in hair follicles, immunostaining was seen in the inner root sheath, the infundibulum and the sebaceous duct. The secretory portion of both eccrine and apocrine glands was negative.

### *B. Epithelial tumours*

In general, a positive immunostaining was observed in the epidermis overlying all epithelial tumours; most – but not all – tumoural proliferations were stained, but the proportion of positive cells varied according to the case studied:

*Extramammary Paget's disease.* In two cases, Paget's cells were completely negative and could thus easily be identified among the surrounding labelled keratinocytes (Fig. 1). In one case, some Paget's cells that seemed to float in pseudo-acantholytic cavities were lightly stained.

*Hidroacanthoma simplex.* Cells forming the characteristic intraepidermal nests were completely negative (Fig. 2). Interestingly, normal epidermal cells surrounding the nests, even those seen in a basal location, were often positive.

*Eccrine syringofibroadenoma.* In this case, cells lining acrosyringeal structures inside the tumour were positive.

*Eccrine poroma.* Only small groups of 2–20 cells were seen positive; they tended to form squamoid whorls, with or without an apparent lumen (Fig. 3). Positive cells represented less than 1% of the total.

*Syringomas.* These showed a strong positivity of the cells lining dermal ductal structures (Fig. 4).

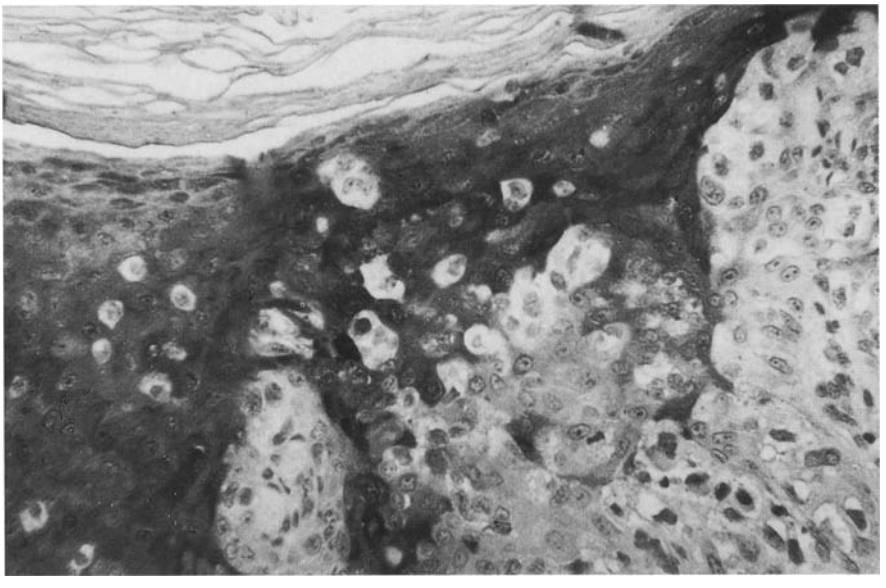

**Fig. 1.** Extramammary Paget's disease. Note absence of immunolabelling on intraepidermal Paget's cells (ABP, ×250)

*Chondroid syringoma.* Strongly positive cells were seen in areas of squamoid differentiation (Fig. 5).

*Nodular hidradenoma.* In two cases, positive cells were seen in areas of ductal differentiation (Fig. 6). One case was completely negative.

*Eccrine spiradenoma.* In one case, cells lining ductal structures (seen mainly at the periphery of the tumour) were slightly stained (Fig. 7). However, involucrine-positive cells constituted less than 1% of the total population. The second case proved negative.

*Naevus sebaceous.* Labelling was similar to that observed on normal skin, save that at places more epidermal layers were stained. Apocrine glandular structures were negative (Fig. 8).

*Trichilemmoma.* A strong labelling was seen in the epidermis overlying the tumoural lobules; however the clear-cells forming this tumour were negative (Fig. 9).

*Trichoepithelioma, trichofolliculoma.* A strong labelling was seen on the inner row of cells lining areas of pilar (keratinizing) differentiation (Figs. 10, 11).

*Mummified epidermal cyst.* A strong labelling was seen on the inner row of cells of the malpighian wall; the keratin content of the cyst (both the lamellate and the mummified) was negative (Fig. 12).

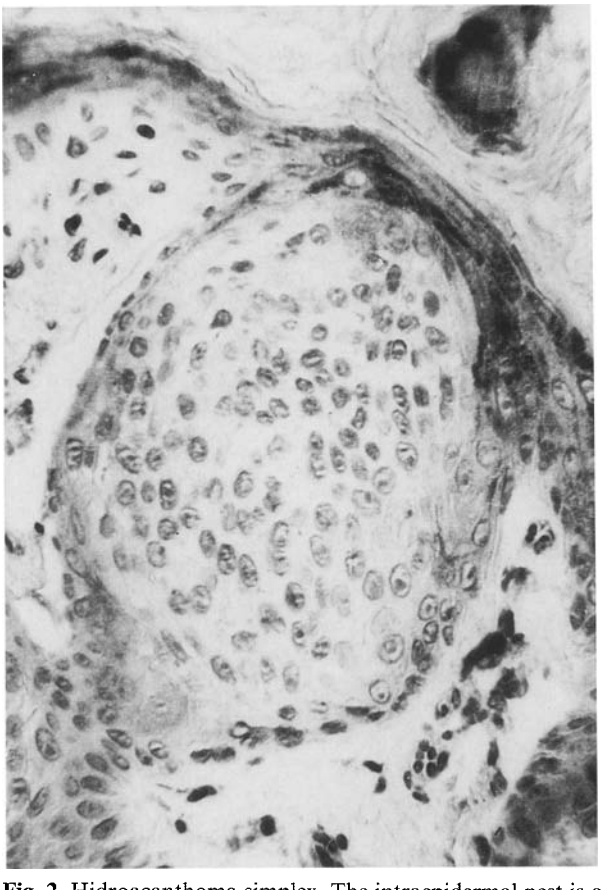

**Fig. 2.** Hidroacanthoma simplex. The intraepidermal nest is completely negative (ABP, × 250)

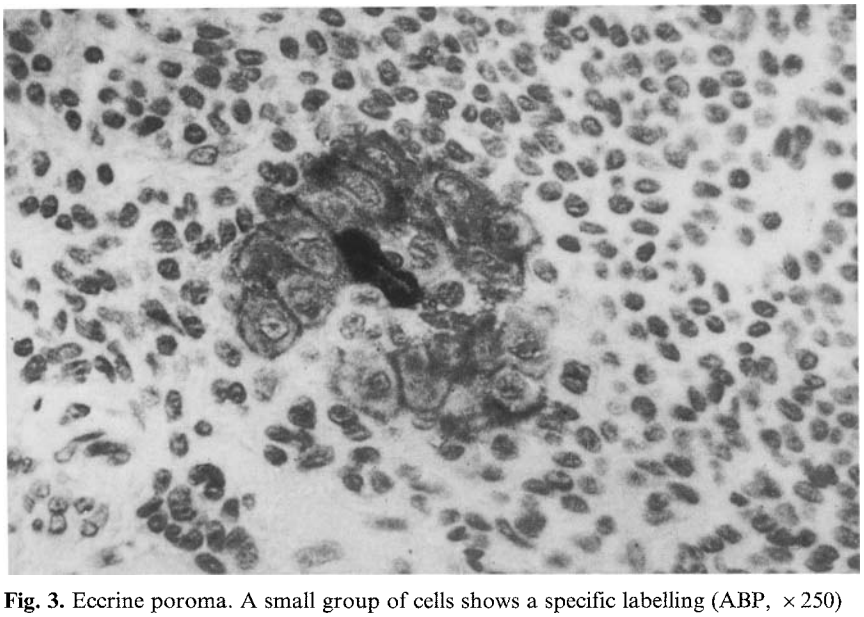

**Fig. 3.** Eccrine poroma. A small group of cells shows a specific labelling (ABP, × 250)

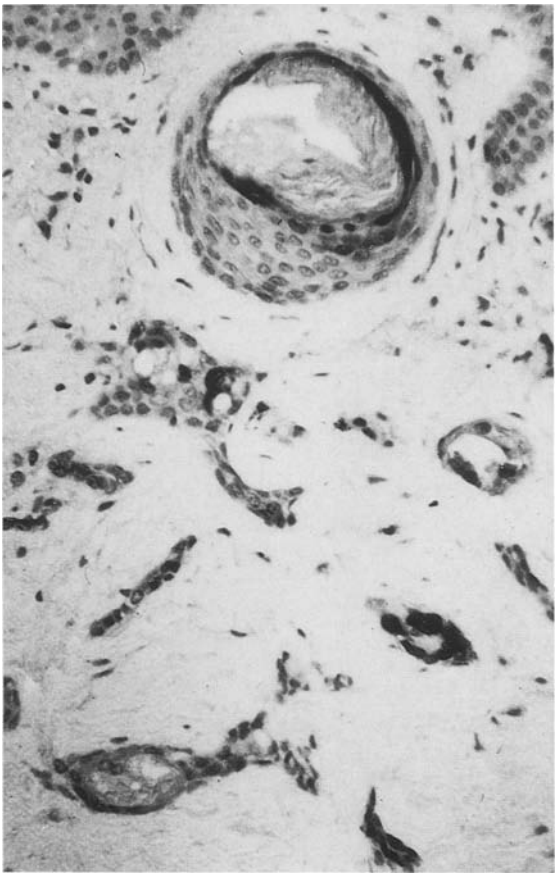

**Fig. 4.** Syringomas. A positive labelling of cells lining ductal structures is observed (ABP, × 250)

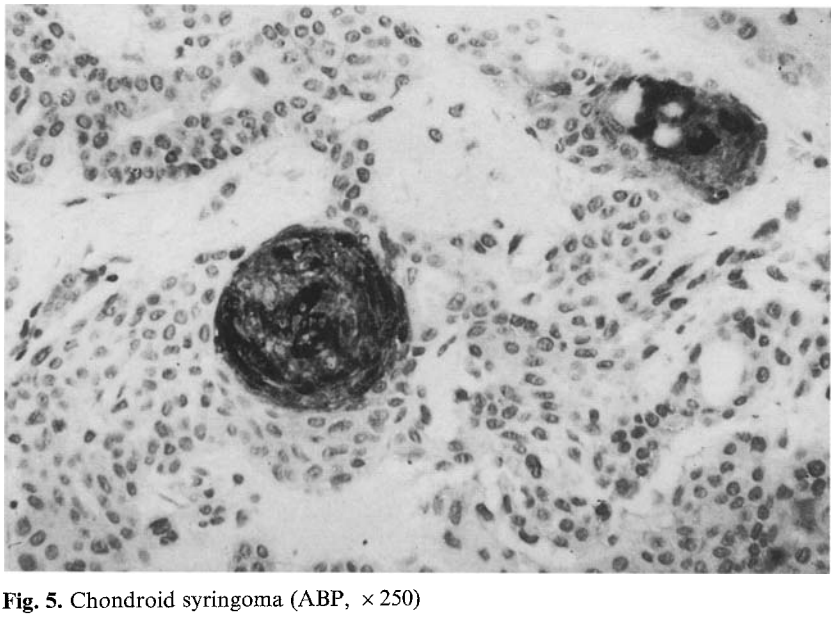

**Fig. 5.** Chondroid syringoma (ABP, × 250)

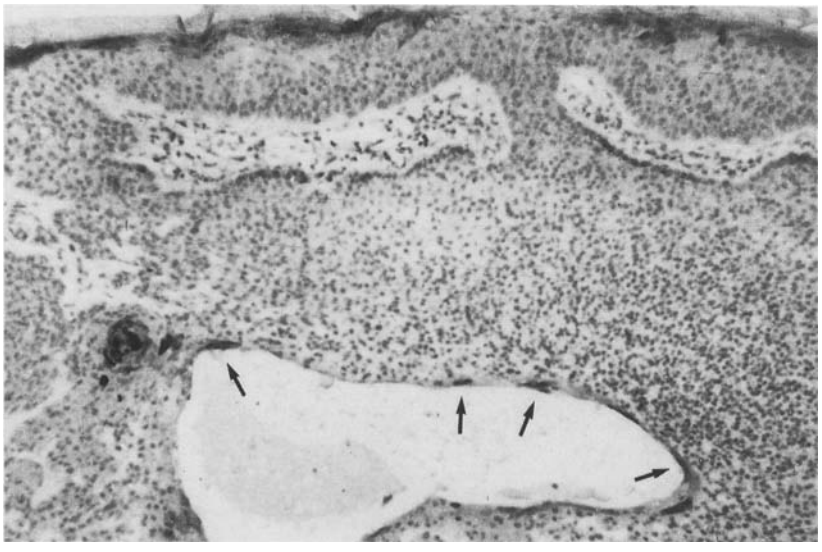

**Fig. 6.** Nodular hidradenoma. A positive labelling is seen in an area of ductal differentiation (left) and on scattered cells linging a cystic space (*arrows*) (ABP, × 100)

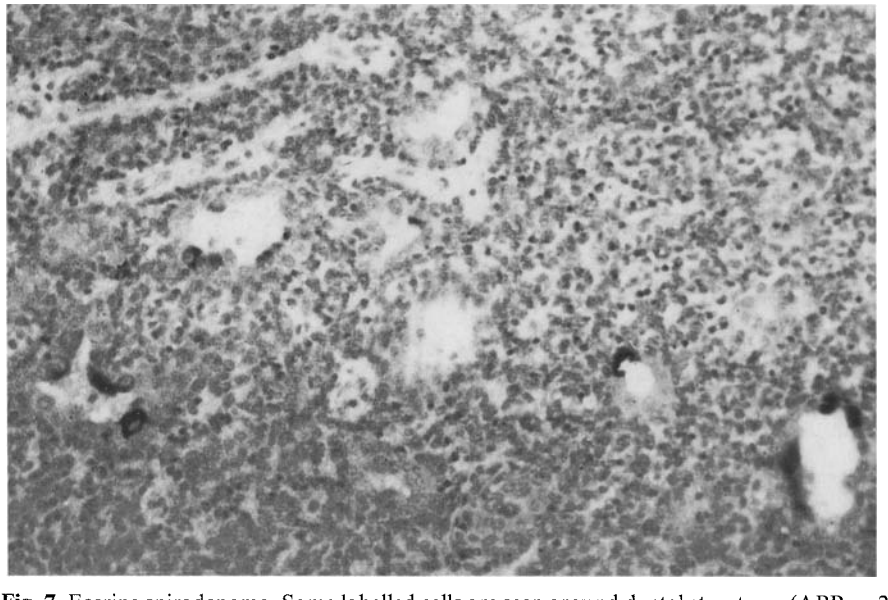

**Fig. 7.** Eccrine spiradenoma. Some labelled cells are seen around ductal structures (ABP, × 250)

*Pilomatricoma.* In one case, a strong labelling was seen on the "transitional" cells, i.e. those located between the basophilic and the ghost cells (Fig. 13). The second case consisted only of mummified areas that were negative.

*Neuroendocrine skin carcinomas, apocrine hidrocystoma, cylindroma, metastatic carcinomas to the skin:* These tumours proved completely negative.

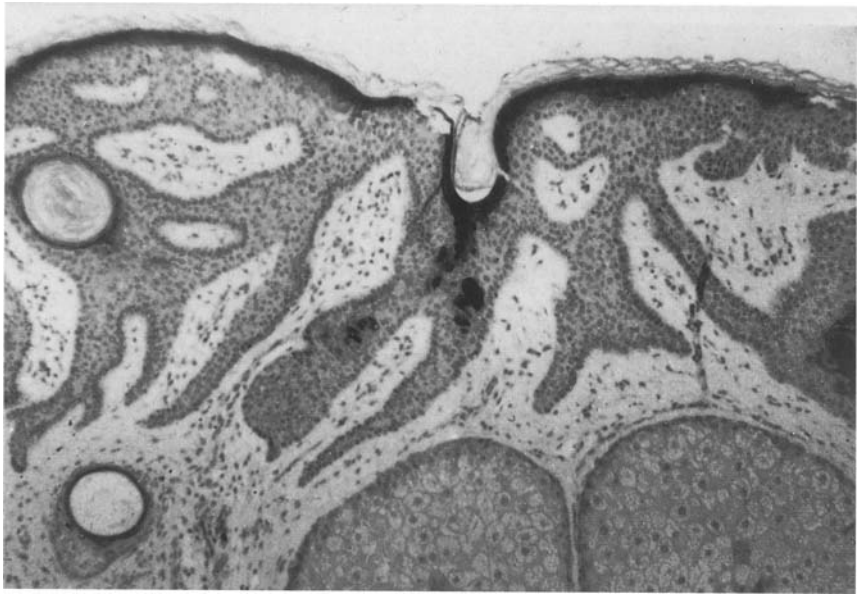

**Fig. 8.** Naevus sebaceous (ABP, ×100)

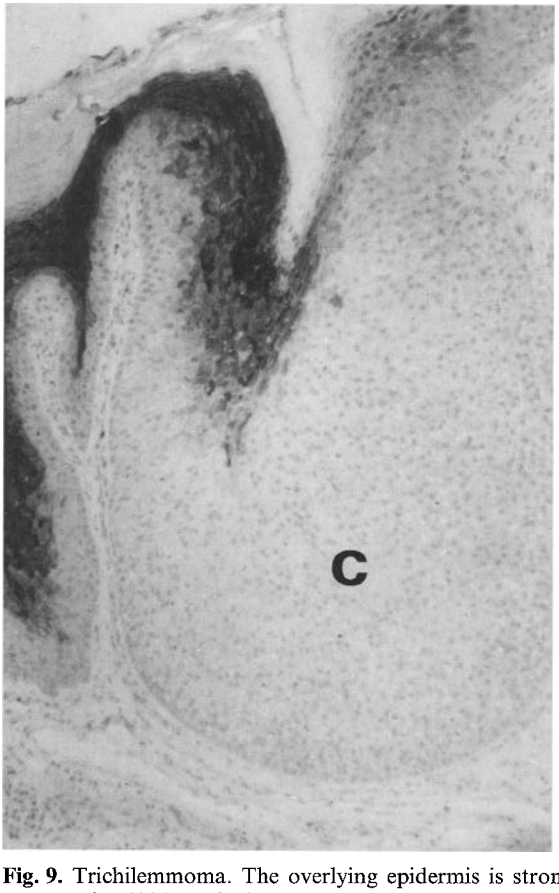


**Fig. 9.** Trichilemmoma. The overlying epidermis is strongly labelled, while the clear cells (*c*) are negative (ABP, ×250)

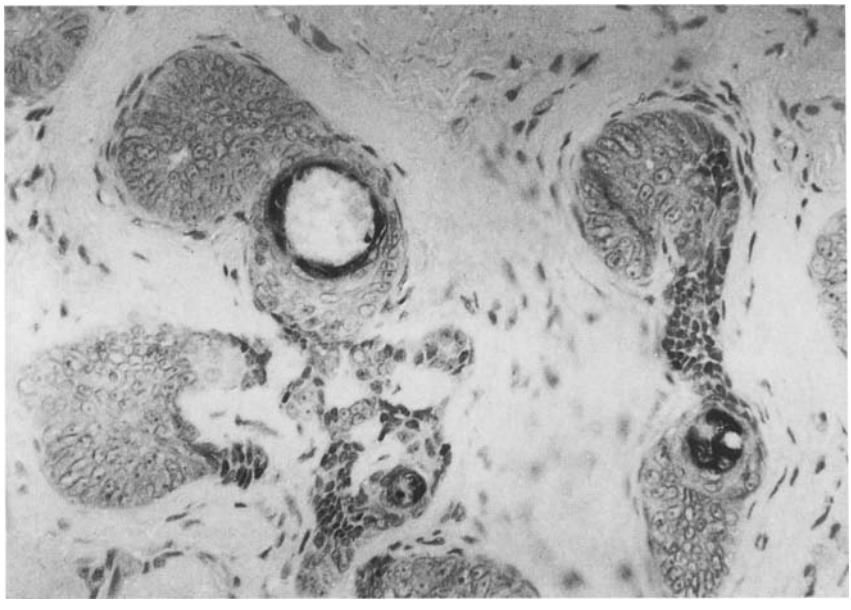

**Fig. 10.** Trichoepithelioma. A strong labelling is seen in cells lining areas of pilar differentiation (ABP, × 250)

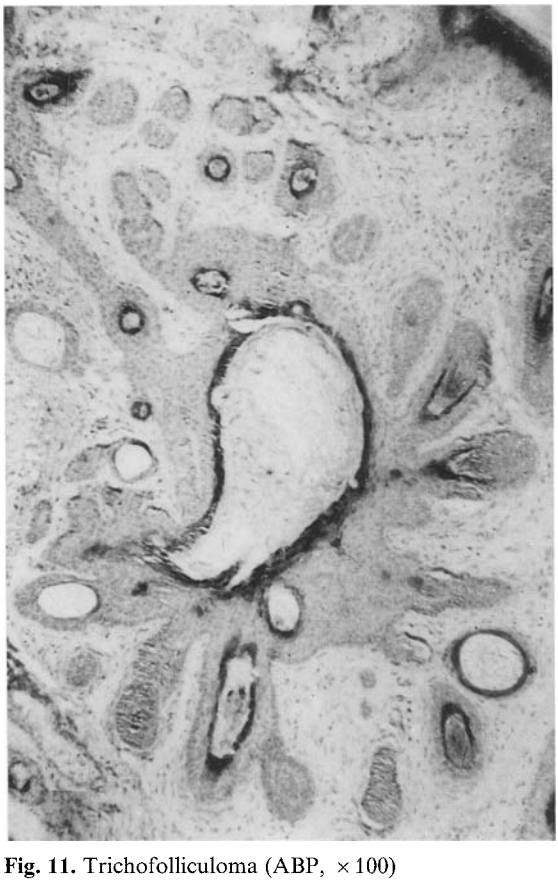

**Fig. 11.** Trichofolliculoma (ABP, × 100)

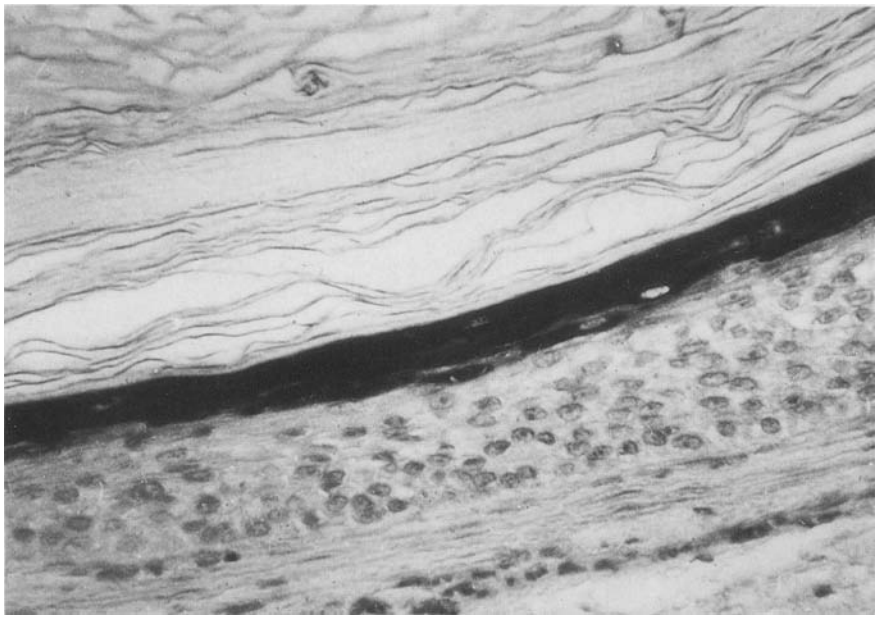

**Fig. 12.** Mummified epidermal cyst showing a strong labelling of cells of the inner row of the malpighian wall (ABP, × 250)

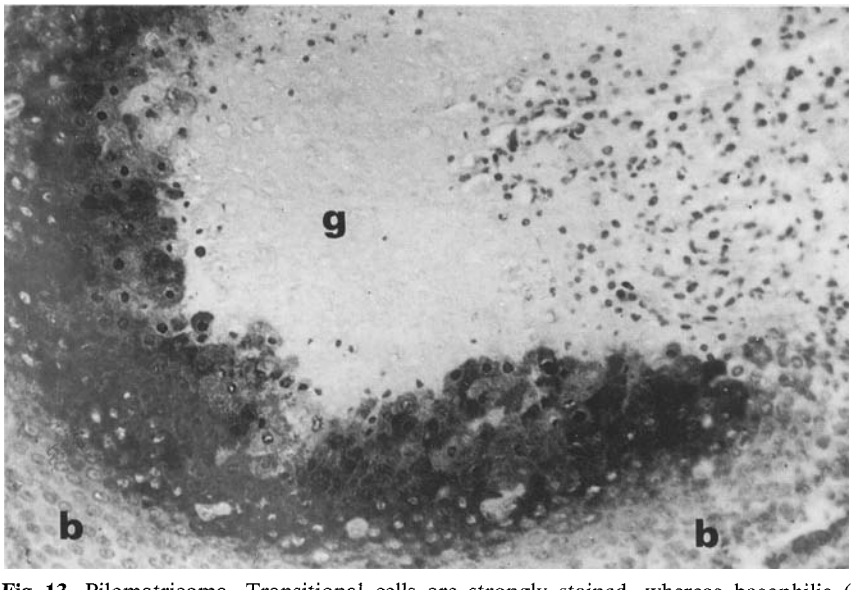


**Fig. 13.** Pilomatricoma. Transitional cells are strongly stained, whereas basophilic (*b*) and ghost (*g*) cells are negative (ABP, × 250)

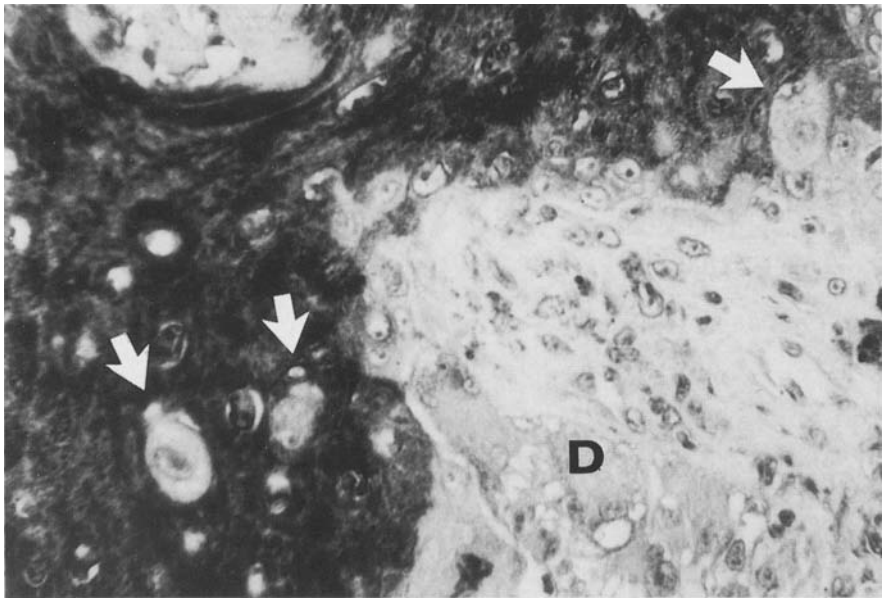


**Fig. 14.** Malignant melanoma. Note absence of specific immunolabelling of both dermal (*D*) and intraepidermal (*arrows*) malignant melanocytes

*C. Non-epithelial skin tumours*

In all cases studied, no labelling was seen in the tumour cells; the contrast was particularly sharp in the case of malignant melanoma (MM), where unstained malignant melanocytes invading the epidermis could be easily distinguished from adjacent, labelled keratinocytes (Fig. 14). The epidermis overlying the tumours was labelled in all cases, but the immunostaining assumed a different pattern from that of normal epidermis at times: thus, labelling was sometimes weak in the epidermis overlying the tumours (NF, DFP); in the case of MM the epidermis adjacent to the nodular melanocytic growth was weakly stained, but that overlying the tumour was strongly positive. In some instances (DFP, MHS) nuclear staining was observed. In the two cases of angiosarcoma (STS and MHS), immunostaining was seen on virtually all suprabasal epidermal layers, with some occasional isolated basal cells being positive.

## Discussion

From the results of our study and from those reported in the literature, we feel that the following points merit discussion:

A. INV distribution in epithelial tumours is not very widespread but seems to be limited to cells undergoing squamous or ductal differentiation; therefore INV cannot be used for the immunohistochemical exclusion of the

diagnosis of carcinoma. The negative results obtained in our cases of metastatic carcinoma (which were strongly labelled by anti-keratin antibodies) clearly confirm this fact. However, INV represents probably the most specific marker of epithelial differentiation and as such it is useful in the immunohistochemical confirmation of the epithelial origin of a tumour, just as narrow-specificity monoclonal anti-keratin antibodies are. In this respect, the lack of reactivity in the Stewart-Treves syndrome, whose epithelial vs endothelial derivation has long been debated, is in agreement with a vascular origin, as has already been illustrated by using other immunohistochemical markers (Miettinen et al. 1983; Kanitakis et al. 1986). In parallel, the contrast of the immunostaining pattern observed between positive vs negative cells may be used to identify such cells as (malignant) melanocytes or Paget's cells when these are located in the upper epidermal layers.

B. INV expression on the epidermis overlying mesenchymal tumours was found to be altered, i.e. generally stronger or more diffuse when compared to that of normal epidermis, as has already been observed in both lichen planus and psoriasis (Murphy et al. 1984; Bernard et al. 1985). This suggests a trouble of normal keratinocyte differentiation, most probably induced by the underlying (dermal) proliferative cellular population, and representing another example of a mesenchymal-epithelial interrelationship whose mechanism remains to be clarified.

C. In our study we observed some rare individually labelled cells within the basal layers, raising the question of whether these could represent Merkel cells. These cells are nowadays considered to be of epithelial origin, since they express cytokeratins and desmosomal proteins (Moll et al. 1984; Ortonne and Darmon 1985) and therefore the possibility exists that they also express INV. In the two cases of NCS we studied, no INV reactivity was noticed; it should be borne in mind, however, that NCS cells display some different immunohistochemical characteristics from normal Merkel cells (Gould et al. 1985) so that more studies are needed to elucidate this problem.

D. With respect to adnexal skin tumours, the staining pattern observed on individual cells was generally uniform, i.e. positive cells exhibited a homogeneous intracellular staining. The labelling was as a rule clear-cut, insofar as cells were either strongly positive or totally negative. On the whole, INV expression seen on adnexal tumours paralleled that observed in the normal counterparts of the cutaneous adnexa: sweat-gland tumours of eccrine-secretory differentiation (CS, NH, ES) were negative or only focally positive in areas of ductal differentiation. Of interest is the fact that Paget's cells were negative even when they were present in the upper epidermal layers; this finding is in keeping with their glandular-secretory derivation and shows that these cells maintain a proper differentiation which is expressed by a particular immunohistochemical phenotype. In one case, Paget's cells floating in pseudo-acantholytic intraepidermal cavities were lightly stained; this might be due to an abnormal maturation pathway, secondary

to the loss of cellular contact. In contrast, in glandular tumours of eccrine-excretory differentiation, the staining was generally stronger, the best example being observed in the cases of S, which reproduced the intense INV expression of the sweat-duct and the acrosyringium. Cells composing the intraepidermal nests of HS were negative; however, no ductal differentiation (as evidenced by a lumen formation) was seen inside this tumour.

Tumours showing apocrine differentiation (NS, AH) were negative, in line with the lack of INV reactivity on normal human apocrine glands (Murphy et al. 1984).

On tumours of pilar differentiation, the staining pattern again reproduced that seen on normal hair follicles (where only the inner root sheath and the infundibulum are positive). Thus, the clear cells of TL (derived from the outer root sheath or trichilemma) were negative. On TF and TE, positivity was seen on the inner row of cells lining follicular structures, prior to terminal keratinization. Interestingly, in PM positive cells were seen located between the basophilic and the shadow ones; this pattern is also reminiscent of the normal hair follicle (with basophilic cells corresponding to hair-matrix ones, transitional cells to those of the inner root sheath and mummified areas to keratin of the hair-stem), and further supports the pilar differentiation of PM.

In conclusion, INV appears as a highly specific and sensitive marker for squamous differentiation of epithelial cells. In skin tumours, it is not yet settled whether INV staining pattern may be helpful in differentiating benign from malignant proliferations (Murphy et al. 1984), as has been postulated for urothelial lesions (Walts et al. 1985). However, immunohistological studies with antibodies to INV, which offer the advantage of giving reliable and reproducible results on routinely-processed tissue specimens, may certainly aid in a more precise diagnosis of epithelial tumours and in the understanding of their differentiation.

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