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Human eccrine sweat gland. Expression of neuroglandular antigens and coexpression of intermediate filaments

Uwe Wollina
Departments of Dermatology at the University of Köln, and the University of Jena, F.R.G.

Summary. Acrosyringium, duct and secretory epithelium as well as myoepithelial cells of human eccrine sweat glands have been characterized by different immunostaining patterns with mono- and polyclonal antibodies to a wide spectrum of tissue antigens. Using monoclonal antibodies to neuron-specific enolase (NSE) and melanoma-associated antigens (LS 59, HMB-45, NKI/C-3) the expression of neuroectodermal antigens in secretory coils was demonstrated. Myoepithelial cells were double-stained with polyclonal vimentin and monoclonal CAM 5.2 (against keratins nos. 8, 18, 19) antibodies.

Key words: Human eccrine sweat, Immunohistology, Neuroectodermal antigens, Neuroglandular antigens, Intermediate filaments, Coexpression

Introduction

Current opinion holds that eccrine sweat glands are ectodermal derivates (Sato et al., 1989). On the other hand, both the analogies in immunostaining patterns of normal eccrine and neuroectodermal epithelia (Wollina, 1990) and the presence of neuroendocrine differentiation in sweat gland tumors and vice versa (Gould et al., 1988; Heenn et al., 1990) raises doubts about a true epithelial nature of eccrine glands.

Recent immunohistochemical and lectinhistochemical investigations with a limited number of «markers» like lectins (Oriol, 1987; Saida et al., 1989), antibodies to blood group antigens (Oriol, 1987), S-100 protein (Cochran and Wen, 1985), epithelial membrane antigen (EMA) (Cordell et al., 1985), milk fat globule antigen (MAM) (Tsubara et al., 1987), carcinoembryonic antigen (CEA) (Penneys et al., 1981), and keratins (Cotton, 1986) provided evidence for characteristic, different staining properties of each type of sweat gland epithelium. For the practical proposal of tumour classification a rather wide spectrum of tissue antigens is recommendable.

The present paper deals with a comprehensive immunohistochemical approach in eccrine sweat gland differentiation, including some functional aspects.

Offprint requests to: Dr. sc. med. Uwe Wollina, Universitäts-Hautklinik, Erfurter Straße 35, Jena, O-6900, Federal Republic of Germany

Materials and methods

Tissues

Human skin biopsy specimens from various body sites were obtained from the tissue bank of the Department of Dermatology, University of Jena. The material was snap frozen in liquid nitrogen and stored at -196° C. Additionally, routine processed skin biopsies were included after they had been deparaffinized and rehydrated.

Antibodies

See Table 1. Peroxidase-conjugated rabbit antimouse, peroxidase-conjugated swine antirabbit, mouse bridging antibody and mouse monoclonal APAAP complex were purchased from DAKO, Hamburg, F.R.G. In double staining procedures goat bridging antibody from Tago Inc. was employed.

Staining procedures

Frozen sections were cut at 4 µm and fixed in cooled acetone for 10 min. Formalin-fixed material was cut at 5 µm, deparaffinized and rehydrated.

The unlabelled peroxidase technique (POX) was performed according to Sternberger et al. (1970). Endogenous peroxidase activity was blocked by a mixture of glucose/glucose oxidase in phosphate-

buffered saline - TRIS buffer. Peroxidase activity was visualized using either 3,3'-diaminobenzidine (DAB) and hydrogen peroxide or 3-amino-9-ethylcarbazole (Graham Jr., et al., 1965). Sections were counterstained with hemalaun.

The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was performed according to Schaumburg-Lever (1987) with either naphthol-AS-biphosphate or Fast Blue BB. The latter was preferred in double staining experiments.

Double-staining procedures used POX (chromogen: 3-amino-9-ethylcarbazole, AEC) in conjunction with APAAP (chromogen: Fast Blue BB), which gives a bright reddish and blue contrast. Counterstaining with hemalaun was avoided. For further details see Mason and Sammons (1978).

Controls with substitution of primary antibodies by buffer gave negative signals.

Results

The reactivity of mono- and polyclonal antibodies with sweat glands is summarized in Table 2. The different cell types disclosed specific staining patterns without significant interindividual variability.

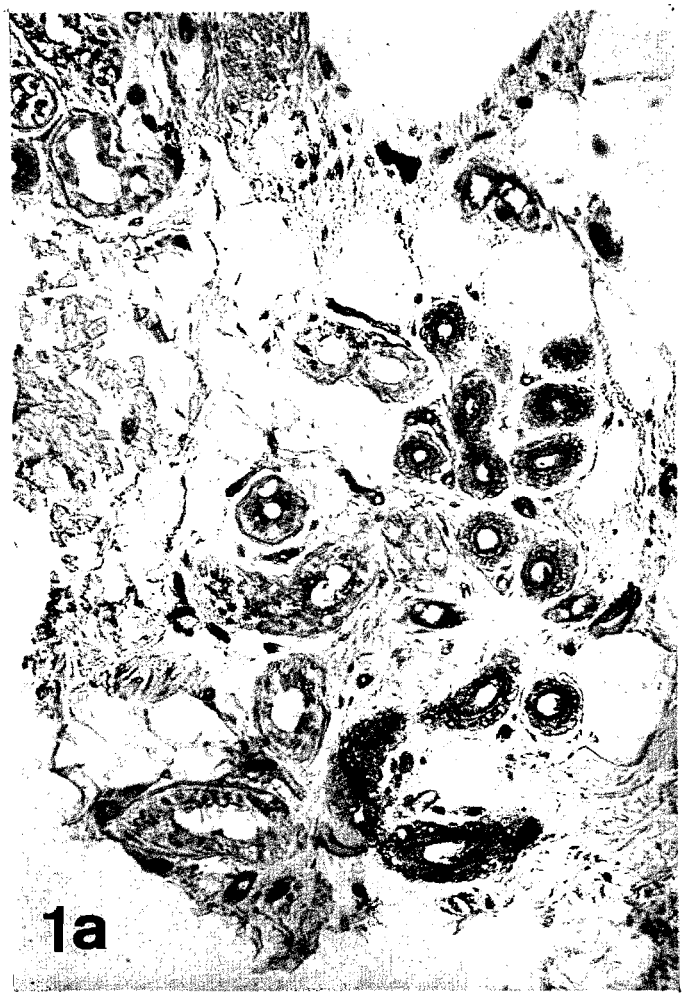
Duct epithelium

The inner cells of both acrosyringium and dermal duct expressed glandular antigens, such as CEA, EMA, and S-100. They were decorated with antibodies against epidermal growth factor (EGF-) receptor and calmodulin. Only the acrosyringium expressed filaggrin (Fig. 1).

The outer duct cells were labelled with polyclonal anti-human keratin and monoclonal anti-keratin KL 4. A weaker immunoreactivity was noted for CEA and S-100.

Secretory coil

The secretory epithelium was decorated by polyclonal antihuman keratin and monoclonals KL 4, CAM 5.2, CK 2, and K 8.12. It expressed CEA and



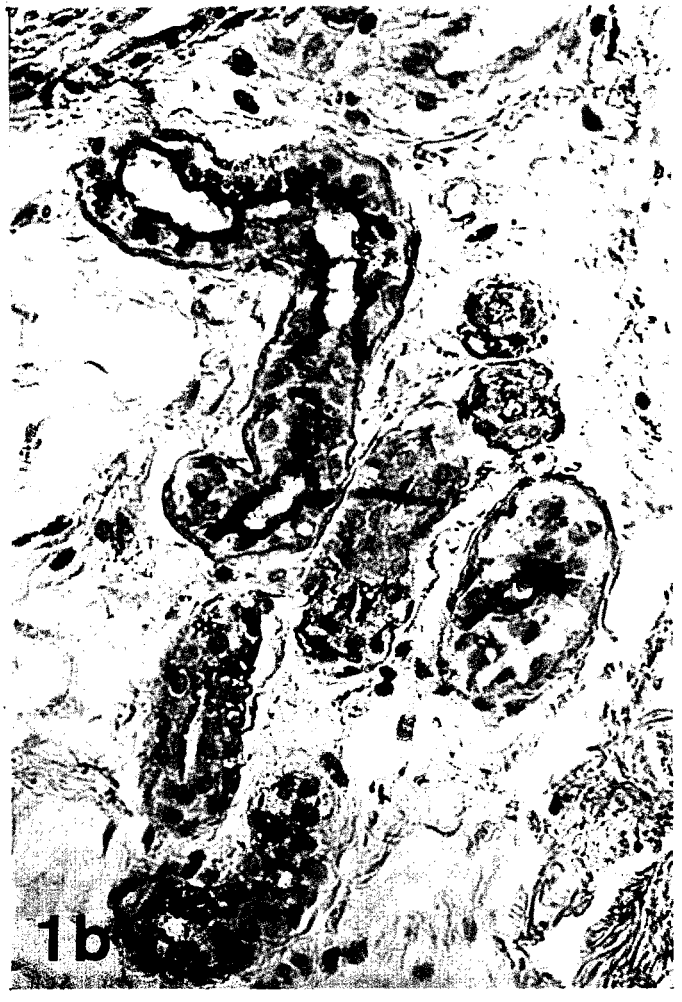
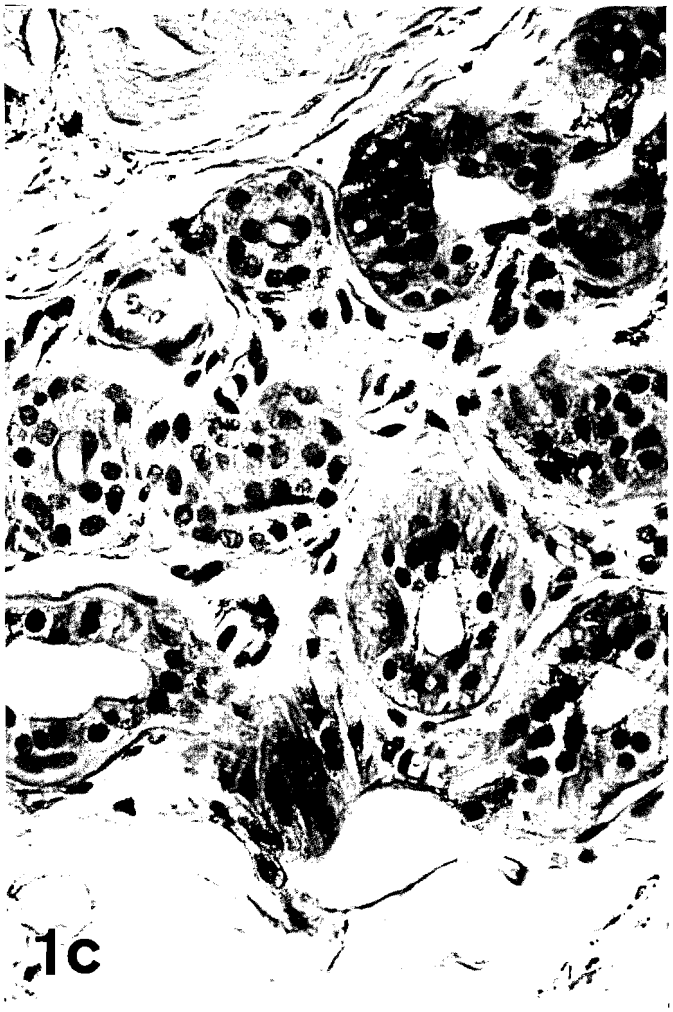


Fig. 1. Immunostaining of eccrine sweat glands with antibodies against glandular antigens. **a.** Staining with antibody 29.11 against the EGF-receptor (POX with AEC), × 100. **b.** Staining with monoclonal antibody EMA against epithelial membrane antigen (POX with AEC), × 250. **c.** Staining with polyclonal antibody against S-100 protein (POX with DAB) × 250



— less intensively than ducts — S-100. Only secretory cells were decorated with antibodies to melanoma-associated antigens, i.e. LS 59, HMB-45, and rather faintly NKI/C-3. Additionally, NSE was localized within secretory coils. The immunoreactivity to these anti-neuroectodermal antibodies was most pronounced along the luminal surface (Fig. 2).

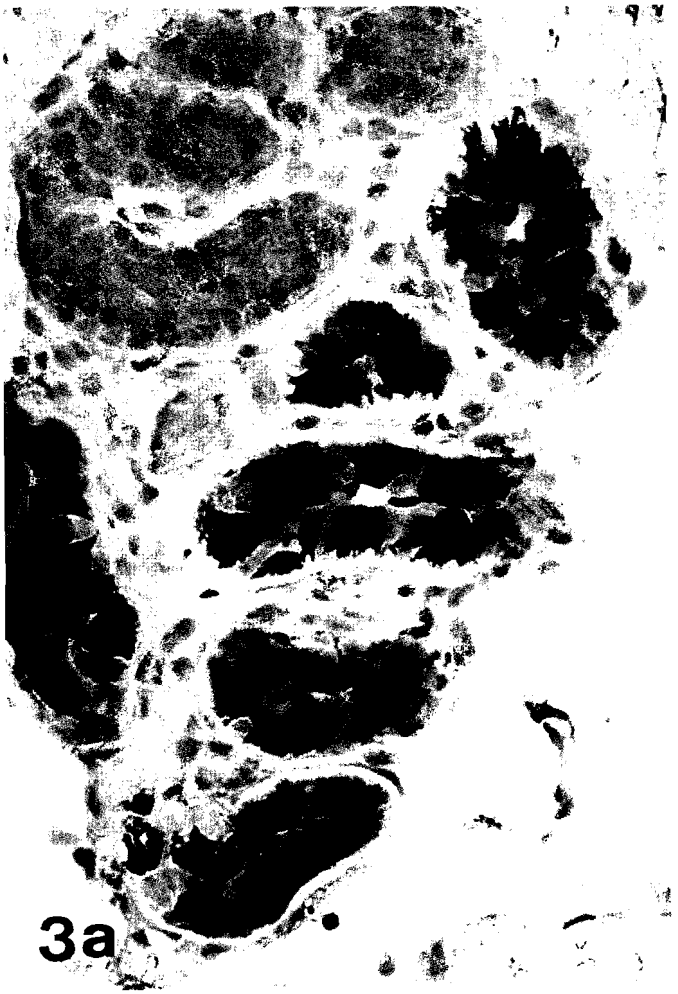
Myoepithelial cells

Myoepithelial cells were labelled by polyclonal antihuman keratin, S-100 and CAM 5.2. Occasionally, vimentin-positive myoepithelial cells were observed. To evaluate a possible coexpression of keratin and vimentin, double staining with polyclonal anti-vimentin was combined with CAM 5.2 to glandular keratins. Cells coexpressing both types of intermediate filaments were identified. On the other hand, there was no evidence for the expression of either neurofilament or glial fibrillary acidic protein (GFAP) (Fig. 3).

Basement membranes and surrounding connective tissue

Basement membranes were stained with antibodies

Fig. 2. Immunostaining of eccrine sweat glands with antibodies against neuroectodermal antigens. **a.** Staining with HMB-45 (APAAP with naphthol-AS-biphosphate), × 250. **b.** Staining with LS 59 (APAAP with Fast Blue), × 250. Note the weak luminar labelling in **b.**



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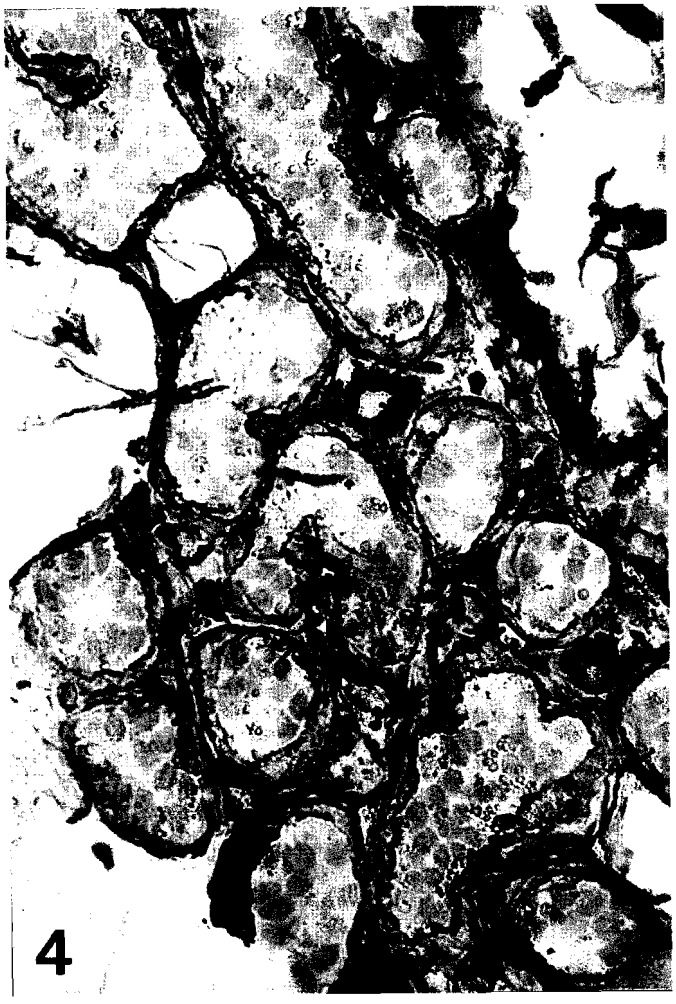

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Fig. 3. Immunostaining of eccrine sweat glands with antibodies to intermediate filaments. **a.** Staining with monoclonal anti-keratin K 8.12 (POX with AEC), × 250. **b.** Staining with monoclonal Vim 9 (1) against vimentin (POX with DAB), × 250 note labelling of myoepithelial cells and fibroblasts. **c.** Double staining of eccrine epithelium with polyclonal anti-vimentin (POX with AEC) and monoclonal anti-keratin CAM 5.2 (APAAP with Fast Blue BB), note double labelling of cells. × 250

Fig. 4. Immunostaining of eccrine sweat glands with monoclonal antibody against collagen type IV. × 250

to collagen type IV and (weakly) fibronectin. Vimentin-positive fibroblasts were found in the adjacent connective tissue. They were loosely distributed without any condensation along sweat glands (Fig. 4).

Discussion

The present study provides evidence for distinct immunostaining properties of each type of eccrine sweat gland epithelium with a wide spectrum of tissue antigens.

Keratins of the glandular type, i.e. keratins nos. 8, 18, 19, were localized within outer duct and secretory cells (Figs. 1, 2). In a recent immunofluorescence study at our laboratory, keratin 19 could not be identified with monoclonal antibody A53-B/A2 (Kasper et al., 1987; Wollina et al., 1991). K 8.12 against keratins 13 and 16 (Huszar et al., 1986) stained secretory cells selectively which were negative for Ki67. This indicates a limited value of the anti-keratin K 8.12 as a marker of proliferation in eccrine epithelia (De Mare et al., 1989).

Table 1. List of antibodies. Abbreviations: p, polyclonal; m, monoclonal; A, POX with DAB; B, POX with AEC; C, APAAP with naphthol-AS-biphosphate; D, APAAP with Fast Blue BB.

Antibody	Type	Specifity	Origin	Technique and working dilution
Antihuman keratin	p (rabbit)	keratins§	Dakopatts	1 : 100, B
RPN 1161	m IgG2a	keratin 1, 2	Amersham-Buchler	1 : 5, B + D
KL 4	m IgG1	keratins§	Immunotech	1 : 100, B
K 8.12	m IgG1	keratin 13, 16	Bio-Yeda	1 : 100, B + C
RKSE 60	m IgG1	keratin 10	Euro-Diagnostics	1 : 50, C
CK 2	m IgG1	keratin 18	Boehringer	1 : 5, C
CAM 5.2	m IgG1	keratin 8, 18, 19	Becton Dickinson	1 : 2, B + D
Vimentin	p (rabbit)	vimentin	Euro-Diagnostics	1 : 50, B + C
Vim 9(1)	m IgG1	vimentin	Monosan	1 : 10, C
NE (14)	m IgG1	neurofilament	Boehringer	1 : 2, D
G-A-5	m IgG1	GFAP	Biochrom	1 : 5, B
Filaggrin	m IgG1	filaggrin	Paesel	1 : 50, B + C
CEA	p (rabbit)	carcinoembryonic antigen	Dakopatts	1 : 100, A
S-100	p (rabbit)	S-100 protein	Dakopatts	1 : 500, B
EMA	m IgG2a	epithelial membrane antigen	Dakopatts	1 : 50, B
NSE	p (rabbit)	neuron specific enolase	Dakopatts	1 : 25 - 1 : 50, B
Phe-5	m IgG1	chromogranin A	Ortho-Diagnostics	1 : 2, C
LK2H10	m IgG_k	chromogranin A	Camon	1 : 1, B
LS 59	m IgG2a	neuroglandular antigen	Dr. Jerry, Calgary (Canada)	1 : 100, C + D
HMB-45	m IgG1	melanoma-associated antigen	Enzo	1 : 200, B / 1 : 1000, C + D
NKI/C-3	m IgG1	melanoma-associated antigen	Monosan	1 : 20, B + C + D
Ki67	m IgG1	proliferation-associated antigen	Dakopatts	1 : 5, B + C
ACAM	p (rabbit)	calmodulin	Dr. Wenz, Jena (FRG)	1 : 30, B + C
BF 8	m IgG2a	calmodulin	Dr. Jablonski, Canberra (Australia)	1 : 1, B
29.11	m IgG1	EGF receptor	Sigma	1 : 2000, B + C + D
ICAM-1	m IgG1	CD-54 antigen	Immunotech	1 : 50, B
FVIIIrAg	p (rabbit)	v.-Willebrandt factor	Dakopatts	1 : 100, B
$Alpha_1$-ACT	p (rabbit)	alpha-1-antichymo-trypsin	Dakopatts	1 : 500, B
Lysozyme	p (rabbit)	lysozyme	Dakopatts	1 : 400, B
Collagen IV	m IgG1	collagen type IV	Biogenesis	1 : 50, B + D
Fibronectin	p (rabbit)	fibronectin	Dakopatts	1 : 100, A + B
Laminin	p (rabbit)	laminin	EY-Laboratories	1 : 5, B

§) wide spectrum antibodies.

Table 2. Immunostaining patterns of eccrine sweat glands with mono- and polyclonal antibodies. Score: -, no staining; (+), weak staining; +, moderate to strong staining.

Antibodies	Myoepithelial cells	Secretory cells	Dermal duct cells inner	Dermal duct cells outer	Acrosyringium inner	Acrosyringium outer
Antihuman keratin	+	+	-	+	-	+
RPN 1161	-	-	-	-	-	-
KL 4	-	+	-	-	-	-
K 8.12	-	+	-	-	-	-
RKSE 60	-	-	-	-	-	-
K CK 2	-	+	-	-	-	-
CAM 5.2	+	+	-	-	-	-
Vimentin	+	-	-	-	-	-
Vim 9(1)	+	-	-	-	-	-
NE 14	-	-	-	-	-	-
G-A-5	-	-	-	-	-	-
Filaggrin	-	-	-	-	(+)	+
CEA	-	+	+	(+)	+	(+)
S-100	+	+	+	(+)	+	(+)
EMA	-	+	+	-	+	-
NSE	-	(+)	-	-	-	-
Phe-5	-	-	-	-	-	-
LK2H10	-	-	-	-	-	-
LS 59	-	(+)	-	-	-	-
HMB-45	-	(+)	+	-	-	-
NKI/C-3	-	(+)	-	-	-	-
Ki67	-	-	-	-	-	-
ACAM	-	-	-	-	-	-
BF 8	-	-	-	-	-	-
29.11	-	-	+	-	+	-
ICAM-1	-	-	-	-	-	-
FVIIIrAg	-	-	-	-	-	-
Alpha$_1$-ACT	-	-	-	-	-	-
Lysozyme	-	-	-	-	-	-

Footnote: Collagen type IV, fibronectin, and laminin were localized within the basement membranes.

Our findings of keratin expression in sweat ducts and coils are in general agreement with other reports (Cotton, 1986; Kurokawa et al., 1988; Noda et al., 1987, 1988; Wollina et al., 1991).

Myoepithelial cells were labelled with CAM 5.2 (present paper) and A51-B/H4 (Wollina et al., 1991) covering a spectrum of keratins, nos. 5, 8, 18, and 19. On the other hand, they were negative for CK2 (Debus et al., 1982) and A53-B/A2 (Kasper et al., 1987; Heidenbluth and Conrad, 1990), antibodies which are monospecified for keratin 18 and 19, respectively.

Antibodies to vimentin (Ramaekers et al., 1983) gave a positive staining of myoepithelial cells and adjacent fibroblasts in the connective tissue. Otherwise neither GFAP (Debus et al., 1983) nor neurofilament (Moll et al., 1986) could be identified in eccrine glands.

Vimentin was recently identified in myoepithelial cells of rat (Warburton et al., 1989) and human (Guelstein et al., 1988), and human prostate epithelium (Leong et al., 1988). Dual staining experiments in rat lactating breast suggested a coexpression of vimentin and keratin (Warburton et al., 1989). Double staining in pleomorphic adenomas indicated a coexpression of keratin 14 and vimentin in myoepithelial tumour cells (Thrane et al., 1990). To the best of my knowledge, the present report is the first on physiological coexpression of vimentin and keratin in sweat gland myoepithelial cells.

The secretory coils disclosed an unexpected immunoreactivity with monoclonal antibodies against certain melanoma-associated antigens and NSE (Fig. 2). The glycoproteins recognized by LS 59 (Sikora et al., 1987), HMB-45 (Smoller et al., 1989), NKI/C-3 (Vennegor et al., 1985), and NSE (Bishop et al., 1988) are thought to be of neuroectodermal origin. This supports a recent line of thinking (Gould et al., 1988; Heenen et al., 1990; Wollina, 1990; Wollina et al., 1990), suggesting a close kinship of eccrine and neuroendocrine cells. This view would imply a mesectodermal origin of eccrine sweat glands to be possible.

The secretory coils, but not the ducts, were negative for prolactin-like material (Walker et al., 1989). The present paper deals with a similar distribution of both calmodulin (Wollina et al., 1990) and EGF-receptors (Nanney et al., 1984). All of these staining patterns seem to be related to the osmoregulator function of the duct epithelium.

In conclusion, immunostaining of human eccrine sweat glands yields information which aids not only the sophisticated classification of adnexal tumours but also the understanding of their biology and function.

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