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Study of the distribution of glycosidic residues in eccrine sweat glands, with special reference to the content of sialic acid

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SUMMARY

We used a group of lectins (Con-A, WGA, SBA, DBA, RCA-1, UEA-1), enzymes (neuraminidase digestion) and conventional histochemical techniques (periodic acid-Schiff reaction and reduction-saponification-Schiff reaction) in order to detect the presence of glycoproteins rich in sialic and neuraminic acids in the human eccrine sweat glands. Using both identification systems, our results showed an abundant secretion, rich in C8Oxi-acylated sialic acid.

INTRODUCTION

Various studies have been performed at the optical (Ookusa *et al.*, 1983; Wollina *et al.*, 1989) and ultrastructural levels (Metzler *et al.*, 1990; Schaumburg-Lever, 1990; Schaumburg-Lever *et al.*, 1991) to determine, using lectins, the sugar composition in eccrine sweat glands. Lectins are a family of glycoproteins, or proteins that bind non-covalently to cellular carbohydrates (Goldstein and Hayes, 1978; Monsigny *et al.*, 1980; Sharon and Lis, 1989, 1993). For that reason they are an effective tool for detecting glycidic residues not only in human skin and mucosa but in their adnexa (Bell and Skerrow, 1984; Katsuyama *et al.*, 1985; Ono and Katsuyama, 1985; Hazen-Martin *et al.*, 1986; Spicer and Schulte, 1988; Agawa and Jass, 1990). Lectin methods allow the cell types present in eccrine sweat glands and their content, to be differentiated.

We chose six lectins (Con-A, WGA, SBA, DBA, RCA-1, UEA-1) that allowed us to infer the majority of the glycidic residues present in human sweat glands. In order to expose the penultimate N-acylglucosamine residue blocked by sialic acid, an enzymatic pretreatment with neuraminidase was made before incubation with WGA-HRP. We used also a group of conventional histochemical techniques (oxidation with periodic acid followed by the Schiff-PAS reaction (McManus and Mowry, 1960) and reduction-saponification-PAS (Reid *et al.*, 1988) to identify the nature of the sialic acids from the degranulation of dark cells.

The aim of the present study was to determine the glycoconjugate sugar residues in normal eccrine sweat glands paying special attention to the content in sialic acids, which to the best of our knowledge, has not been reported before. For this purpose, the

use of conventional techniques, lectins and enzyme pretreatment could yield information about the exact kind of sialic acid from their O-acylated chains.

MATERIAL AND METHODS

The human skin was obtained from the abdomen of twelve healthy adults (four males and eight females). The samples were fixed in Bouin for 4-6 hours and dehydrated in graded alcohol, then cleared in xylene and embedded in semisynthetic paraffin (Histowax, melting point 57°C). The samples were routinely processed and 6 mm thick paraffin sections were obtained. The sections were kept in a moist chamber at 37°C for 20-30 minutes. Next, we removed the paraffin beginning the process with three steps in xylene 5 min each, one more in a 1:1 mix of xylene-alcohol, continuous steps in degraded alcohols and then in distilled water for five minutes. The staining for the morphological study was performed with modified haematoxylin of Harris-VOF of Gutierrez (Gutierrez, 1963).

The battery of lectins used is listed in TableI: Con-A HRP (Sigma, L-6397); DBA-HRP (Sigma, L-4258), RCA-HRP (Sigma, L-2633), SBA-HRP (Kem-en-tec, 2400), WGA-HRP (Kem-en-tec, 2440), UEA-1 (Kem-en-tec, 2480). The direct method for horseradish peroxidase was used. The lectin procedure was the following: removal of the paraffin and hydration in degraded alcohol, inhibition of the endogenous peroxidase and pseudoperoxidase activity with 3% hydrogen peroxide in distilled water, rinsed three times in PBS for 5 minutes, incubated in lectins with variable rank of 20-100 mg/ml of PBS at 4°C for 48 h, rinsed twice in PBS for 5 minutes each and developed with 3,3' diaminobenzidine (DAB, Sigma D-5905) in a concentration of 3-6 mg/l 0 ml of PBS, pH 7.2, in the presence of 20µl/10ml of 3% hydrogen peroxide, at room temperature for 20-30 min. Con-A was incubated with Ca++ and Mn++ cations in 1 mg/ml.

The controls for the lectin staining included: exposure of the specimens to substrate without lectin; samples were incubated using lectins that had been preincubated with the corresponding haptene sugar inhibitors, used at a concentration of 0.2 M, for 30 min at room temperature.

Neuraminidase enzyme was type VI from *Clostridium perfringens* (Sigma, N-3001). Incubation with the enzyme was carried out in some sample prior to WGA staining. Sialic acid was removed by pretreatring the sections in a solution of sodium acetate buffer 0.25 M, pH 5.5, containing 10 unit/80 ml sialidase, 5.0 mM $CaCl_2$ and 154 mM NaCl, at 37°C for 18h (Uehara *et al.*, 1985). Controls containing the sialidase buffer without the enzyme were also prepared.

The periodic acid-Schiff reaction (PAS) (McManus and Mowry, 1960) and reduction-saponification-PAS reaction (RSP) (Reid *et al.*, 1988) were also done to identify O-acylated glycoproteins. In order to detect neuraminic acid, RSP was used (sodium borohydride reduction, potassium hydroxide saponification and PAS-reaction method).

The periodic acid-Schiff reaction was the following: removal of the paraffin and hydration; oxidation with periodic acid 1 g/100ml for 10 min at room temperature, rinsed in distilled water for 5 min; stained in Schiff solution in dark chamber for 30 min; rinsed in 1% potassium metabisulfite with 5% HCl 1N three times for 5 min; washed in distilled water. The procedure for RSP was the following: removal from the paraffin and hydration; reduction with $NaBH_4$ 25 mg in a solution of Na_2HPO_4

Table I

Lectins	Abbreviation	Specific sugar
Canavalia ensiformis	Con A	αman>αglu>GlcNac
Ricinus communis	RCA-I	βGal>αGal>>GalNac
Dolichos biflorus	DBA	GalNacα1,3GalNac
Triticum vulgaris	WGA	GlcNac>Neu5Ac
Glycine max	SBA	α-β GalNAc
Ulex europaeus	UEA-I	αL-fuc

1g/100 ml of distilled water with shaking for 10-12 minutes; rinsed in distilled water; immersion in the potassium hydroxide solution 0.56 g/100 ml ethanol 80° for 30-60 minutes; rinsed in ethanol 80° and later in distilled water and then treated for the PAS reaction (Reid *et al.*, 1988).

RESULTS

Sections stained with haematoxylin-VOF of Gutierrez showed a normal preserved skin. No staining was present in sections exposed both to substrate without lectin and to lectin preincubated with specific haptena. No staining was found in sections containing sialidase buffer without neuraminidase enzyme.

Con-A

Stronger reactivity was found in the excretory tubule than in the secretory segment. In acini, a strong granular staining in the dark cells (Fig. 2) was noticed. When Con-A was used at a very low concentration , a stronger reactivity in the excretory tubule than in secretory segment was seen. In the acini, two kinds of cells were shown. The intensity of the staining was stronger in dark cells than in light cells, mainly in the cytoplasm, with granular staining. The light cells showed uniform weak intensity in the cytoplasm and in the cell membrane.

RCA-1

The excretory tubule showed middle intensity with stronger affinity in the luminal part of the cells. In the secretory segment, the difference between both cell groups was minimum. There was only weak evidence of a stronger intensity in granulation of the dark cells (Fig. 3)

DBA

Cells in the excretory tubule showed a moderate reactivity, mainly in the cytoplasm. In the acini, we observed strong affinity inside the dark cells and moderate intensity in the cytoplasm and the membranes of the light cells (Fig. 4).

UEA-1

Intense staining was present in cytoplasm and membranes of the cells in the excretory tubule. In the acini, dark cells showed strong granulation in cytoplasm. The light cells did not stain (Fig. 5).

WGA

The cell membrane of the excretory tubule showed moderate staining. In acini, the difference between both types of cells is clear; the dark cells showed strong positivity in the cytoplasm and glycocalyx, while the light cells showed very weak reactivity (Fig. 6).

SBA

Negative, in every kind of cell.

PAS

The excretory tubule was negative. Glandular acini were weakly positive in both cell groups (Fig. 7).

RSP

Positivity was found in the dark cells only, showing a granular part inside the cytoplasm (Fig. 8).

Neuraminidase-WGA

Only a very weak affinity in Neuraminidase-WGA sections in the secretory segment of the light cells was obtained.

Table II
Table to identify the nature of the sialic acid

	WGA	Neuram-WGA	PAS	RSP
Neuraminic acid	positive	negative	positive	negative
Sialic acid O-acyl, C-7	positive	positive	positive	positive
Sialic acid O-acyl, C-8	positive	positive	negative	positive
Sialic acid O-acyl, C-9	positive	positive	positive	negative

DISCUSSION

This study has shown the existence of a different and specific label for each kind of cell in the eccrine glands. Some of our findings agree with previous reports, although those studies did not determine the exact kind of sialic acid present (Ookusa *et al.*, 1983; Bell and Sherrow, 1984; Wollina *et al.*, 1989; Schaumburg-Lever, 1990; Schaumburg-Lever *et al.*, 1991).

The α-glucose and α-mannose residues are very abundant in sweat eccrine glands, not only in the excretory tubule and the secretory segment, but also inside the granular part of the dark cells. The very low concentrated Con-A shows the existence of α-mannose inside that granulation, which also has β-galactose, α-N-acylgalactosamine and α-L-fucose. Sugars β-galactose, β-N-acylgalactosamine and N-acylglucosamine are also present both in the excretory tubule and the light within basal cells. In these cells, the amount of N-acylglucosamine is minimum. The presence of α-L-fucose is also important in the excretory tubule, although the light cells did not show it.

Bell and Sherrow (1984), Ookusa *et al.* (1983), Schaumburg-Lever (1990), Schaumburg-Lever *et al.* (1991) showed the existence of RCA- and DBA-positive granulation inside the light cells, and RCA positive granulation in the dark cells, which ties in with our results, although they do not coincide in the positivity to SBA. Those differences could be attributed to the different incubation time used by these authors. The similar labelling patterns of the dark cells are found by Hazen-Martin *et al.* (1986), Schaumburg-Lever (1990), Schaumburg-Lever *et al.* (1991) for all the lectins used. Although there are

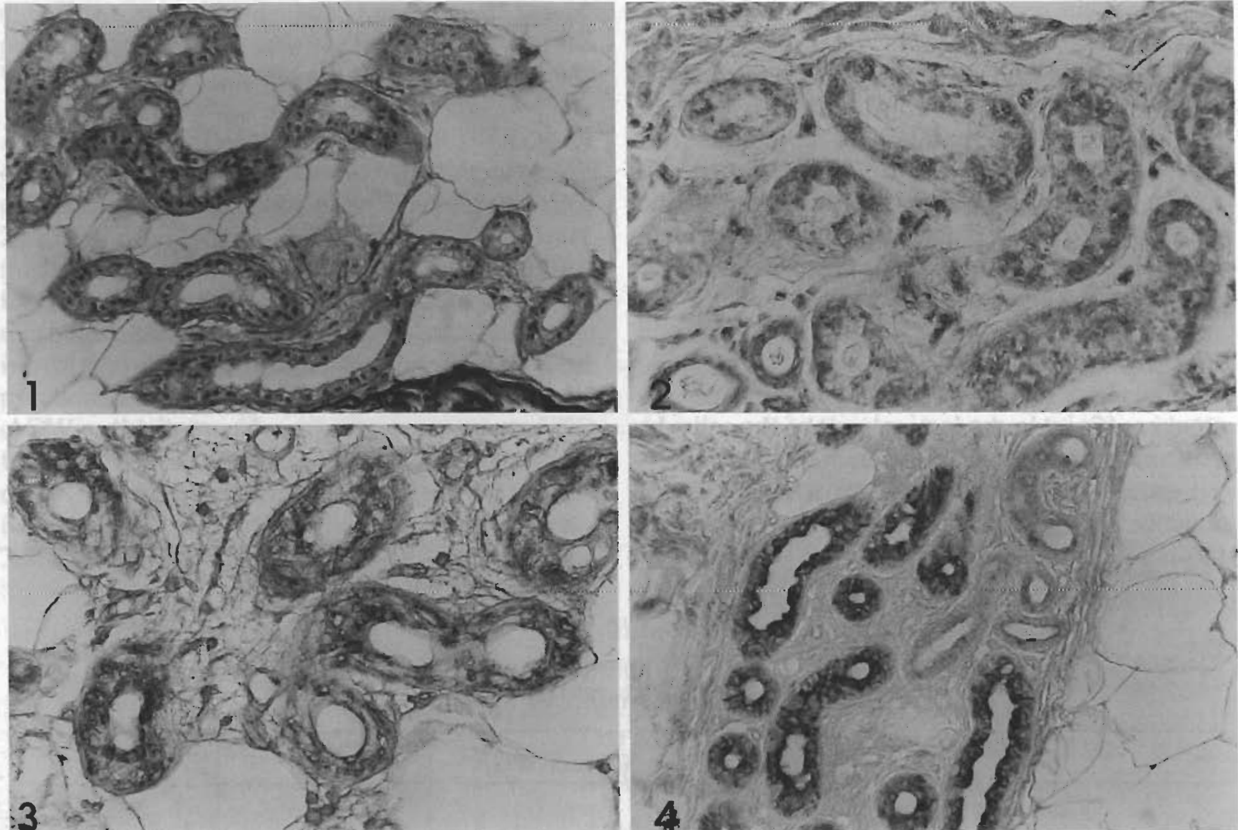
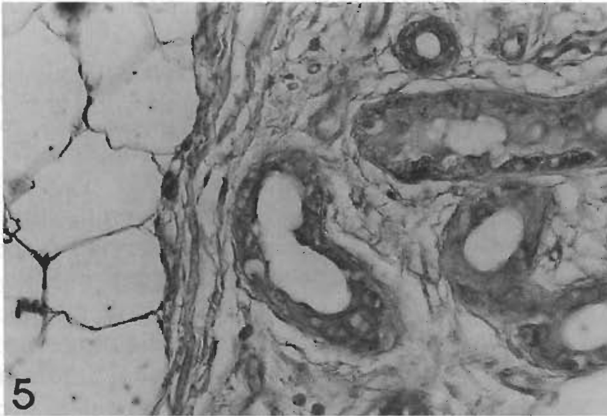


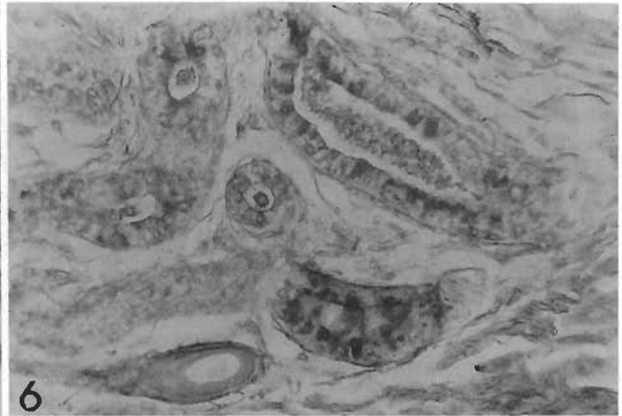
Fig. 1 - Eccrine sweat gland staining with Harris's modified haematoxilin Gutierrez's VOF. x250.
Fig. 2 - Eccrine sweat gland staining with Con-A-HRP, x400.
Fig. 3 - Eccrine sweat gland staining with RCA, x400.
Fig. 4 - Eccrine sweat gland staining with DBA, x250.

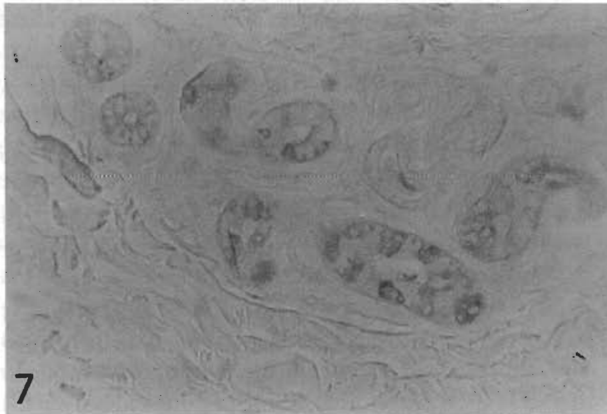
Table III
Lectin binding sites in the human sweat glands

LECTINS	SWEAT GLAND		
	Excretory tubule	Secretor segment	
		Light cell	Dark cell
Con-A	3	2	3
Con-A very low conc.	2	2	1
RCA-I	2	2	2
DBA	2	2	3
WGA	2	0 (1)	2
SBA	0	0	0
UEA-I	3	0	2
Neuram-WGA	0	1	0
PAS	0	1	1
RSP	0	0	2

The number shows the intensity of the staining: 0, negative reaction; 1, weak reaction; 2, moderate reaction; 3, strong reaction.







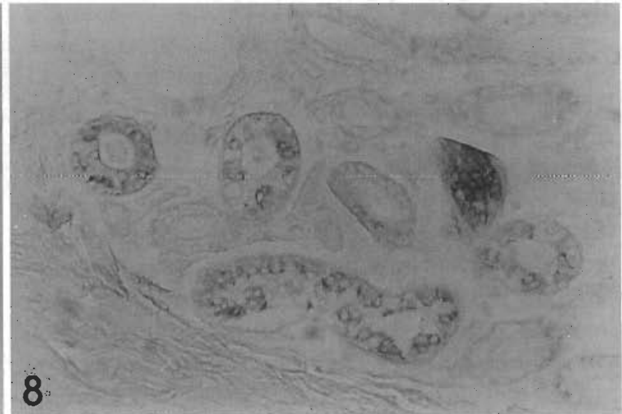


Fig. 5 - Eccrine sweat gland staining with UEA-I,x400.
Fig. 6 - Eccrine sweat gland staining with WGA, x250.
Fig. 7 - Eccrine sweat gland staining with PAS, x250.
Fig. 8 - Eccrine sweat gland staining with RSP, x250.

differences in the intensity of reaction with DBA, where Hazen-Martin *et al.* (1986) found some negative samples. These differences would be related to the use of avidin-biotin-complex by Schaumburg-Lever and coworkers (1991). Some authors have found differences in the lectin DBA, with negative samples from subjects with blood group 0 (Gheri *et al.*, 1993). They also showed positive granulation with UEA, RCA and WGA. The most interesting aspects in the study are the positivity to WGA and the histochemical estimation of the type of sialic acid, which is why we have followed the criterion of identification shown in Table II. The PAS and RSP reactions have been able to determine that the positivity obtained with WGA inside the granulation of the dark cells is due to the presence of C8Oxi-acylated sialic acid. On the other hand, positivity obtained in the rest of the gland, not removed by neuraminidase, is due probably to the existence of N-acylglucosamine.

This is corroborated by the results obtained with neuraminidase-WGA after extracting the neuraminic acid. The labeling obtained is negative or weakly positive in every structure of the sweat glands. Thus, we can say that the most abundant penultimate glycidic residue is not N-acylglucosamine, which would be exposed to the lectin WGA binding after extracting the neuraminic acid.

We think the indirect determination of the content in sialic and neuraminic acids can be reaffirmed with the use of these associated techniques, which have low cost and that are routinely used in a large number of laboratories, in order to study the pathological alterations of the gland.

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Glycoconjugates during the annual sexual cycle in lizard epididymal ductuli efferentes: a histochemical study

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SUMMARY

The epididymal ductuli efferentes of the lizard *Podarcis sicula campestris* De Betta are lined with simple, columnar, nonciliated and ciliated cells. The use of lectin histochemistry has provided information about changes of sugars associated with glycoconjugates of epithelial cells and intraluminal spermatozoa which are conveyed from the longitudinal canal to the cranial region of the ductus epididymis. Epithelial cells exhibited residues of α-D-mannose, N-acetylglucosamine, and β-D-galactose-(1-4)-N-acetylglucosamine as revealed with lectins Con A, WGA and RCA_{120}, respectively, throughout the sexual cycle. An increase of RCA_{120} staining was observed on microvilli of nonciliated cells and in the cytoplasm of ciliated cells during the reproductive period. However, during the following refractory period, when the organ was in regression, there was a decreased staining with Con A on microvilli and the absorbent surface of nonciliated cells, with WGA in nonciliated cells and the cytoplasm of ciliated cells, and with RCA_{120} on microvilli and the cytoplasm of both cell types. Terminal α-D-galactose residues were increasingly stained from autumn up to the reproductive period with BS I-B_4 on microvilli, the absorbent surface and cilia, whereas they were entirely lacking during the refractory period. UEA I revealed α-L-fucose residues on the absorbent surface of nonciliated cells during the abortive and reproductive periods, increasing in the latter period when cilia also expressed this sugar. Terminal α/β-D-N-acetylgalactosamine was evidenced with SBA on the absorbent surface of nonciliated cells during the reproductive period. The terminal β-D-galactose-(1-3)-N-acetylgalactosamine dimer was never found with PNA, whereas O- and N-linked sialoglycoconjugates were present only during the reproductive period. The spermatozoa head exhibited N-linked glycans with high-mannose content, and β-D-galactose-(1-4)-N-acetylglucosamine as well as O- and N-linked sialoglycoconjugates throughout the year. During the reproductive period, oligosaccharides with α-D-mannose residues increased, and oligosaccharides with terminal α-D-galactose, α-L-fucose and sialic acid-N-acetylgalactosamine dimer were also present. Unlike spermatozoa of seminiferous tubules, the spermatozoa head of the lizard epididymal ductuli efferentes exhibited seasonal variability in the lectin binding pattern which may be related to time-dependent changes in the glycoconjugate profiles of epithelial cells.