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Endocrine cells in ectocervical epithelium

An immunohistochemical and ultrastructural analysis

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Summary. A systematic study of endocrine cells in the ectocervix was carried out using histochemical, immunohistochemical and ultrastructural techniques. Serotonin and calcitonin immunoreactive cells were demonstrated in this site. Serotonin and calcitonin immunoreactivities were coexpressed in the same endocrine cell. These distinctive cells were encountered in two main morphological varieties of ectocervical epithelium. Normal-appearing stratified squamous epithelium contained only very rare serotonin and calcitonin cells. In contrast, endocrine cells were fairly abundant in a specific epithelium termed "transitional-like". This type of epithelium was not only confined to the transformation zone but could also extend onto the portio as far as the vaginal cut margin. In some cases, transitional-like epithelium bore morphological resemblance to urothelium. In other cases, it could be regarded as basal cell hyperplasia or immature squamous metaplasia. Of interest, serotonin and calcitonin cells have been well-documented as normal inhabitants of some other non-squamous epithelia, such as urothelium or pseudostratified columnar epithelium. Therefore, it is suggested that certain ectocervical epithelia show some similarities to urothelium, in respect of their morphological appearance and endocrine profile. Further investigations using more objective and specific markers of urothelial cells are needed to assess the exact degree of homology connecting all these types of epithelium.

Key words: Ectocervix – Serotonin cells – Calcitonin cells – Ultrastructure – Transitional epithelium

Introduction

Argyrophil cells in the ectocervix were first recognized by Tateishi in 1975. Recently, serotonin-storing cells have been described in several morphological varieties of "portio epithelium". Certain of these epithelia were suspected of having some similarities to the transitional (urothelial) epithelium of the urinary tract. However, transitional epithelium of the urinary tract contains various endocrine cell types; in particular, serotonin and calcitonin immunoreactive cells.

A systematic study of endocrine cells in ectocervical epithelium was carried out on our recent surgical specimens, using the argyrophilic Grimelius procedure as well as immunohistochemical and ultrastructural techniques. The purpose of this study was to analyse the different morphological varieties of endocrine cell-containing ectocervical epithelia and to reappraise the degree of analogy with transitional epithelium.

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Materials and methods

210 ectocervices formed the basis of this report. This material was obtained from recent hysterectomy specimens.

All tissue samples were fixed in aqueous Bouin's fluid and then embedded in paraffin. Argyrophilic cells were detected by a modified Grimelius reaction.

Tissues containing argyrophilic cells were investigated immunohistochemically. Immunoperoxidase stains were performed on deparaffinized sections with rabbit antisera to ACTH, bombesin, calcitonin, gastrin, glucagon, motilin, neurotensin, human pancreatic polypeptide, serotonin and somatostatin, with guinea pig antiserum to insulin and with mouse monoclonal antibody to free α-human chorionic gonadotropin (α-hCG). Antisera to ACTH, gastrin, insulin, neurotensin, pancreatic polypeptide, serotonin and somatostatin were generous gifts from M.P. Dubois (Station de Physiologie de la Reproduction, Institut National de Recherche Agronomique, F-37380 Nouzilly, France). Anticalcitonin serum was kindly furnished by D. Guilloteau (Hôpital Bretonneau, Tours, France), antiglucagon serum by R. Assan (Diabetes department, Hôpital Bichat, Paris, France), antimotilin serum by J.A. Chayvialle (INSERM U 45, Hôpital E. Herriot, Lyon, France). Antibombesin immune serum was purchased from Amersham Laboratory. α-hCG monoclonal antibody was obtained from Bioart Laboratory. In order to investigate the relative proportion of argyrophilic cells to immunoreactive cells, combined techniques were

Table 1. Clinicopathologic features in eleven grimelius-positive cases

Case	Histological and immunohistochemical findings		Age (years)	Pathologic diagnosis
	"Portio" epithelium	Transformation zone		
1	Transitional-like: Sero$^+$, Cal$^+$	Transitional-like: Sero$^+$, Cal$^-$	72	Mucinous cystadenoma of ovary
2	Transitional-like: Sero$^+$, Cal$^+$	Transitional-like: Sero$^+$, Cal$^-$	70	Serous cystadenocarcinoma of ovary
3	Transitional-like: Sero$^+$, Cal$^+$	Transitional-like: Sero$^+$, Cal$^+$	54	Serous papillary cystadenocarcinoma of ovary
4	Transitional-like: Sero$^+$, Cal$^+$ Squamous: Sero$^+$, Cal$^+$	Transitional-like: Sero$^+$, Cal$^+$	69	Uterine prolapse
5	Transitional-like: Sero$^+$, Cal$^+$ Squamous: Sero$^-$, Cal$^-$	Transitional-like: Sero$^+$, Cal$^+$	36	Carcinoma in situ of cervix
6	Transitional-like: Sero$^+$, Cal$^-$ Squamous: Sero$^+$, Cal$^-$	Transitional-like: Sero$^+$, Cal$^-$	58	Endometrial adenocarcinoma
7	Squamous: Sero$^+$, Cal$^+$	Transitional-like: Sero$^+$, Cal$^+$	77	Atypical endometrial hyperplasia
8	Squamous: Sero$^+$, Cal$^-$	Transitional-like: Sero$^+$, Cal$^-$	60	Serous cystadenoma of ovary
9	Squamous: Sero$^-$, Cal$^-$	Transitional-like: Sero$^+$, Cal$^-$	69	Mucinous cystadenoma of ovary
10	Squamous: Sero$^-$, Cal$^-$	Transitional-like: Sero$^+$, Cal$^-$	60	Undifferentiated carcinoma of ovary
11	Squamous: Sero$^+$, Cal$^-$	Reserve cell hyperplasia: Sero$^-$, Cal$^-$	34	Uterine leiomyoma

Abbreviations: Sero$^+$ = Presence of serotonin immunoreactivity; Cal$^+$ = Presence of calcitonin immunoreactivity; Sero$^-$ = No serotonin immunoreactivity; Cal$^-$ = No calcitonin immunoreactivity

applied sequentially to the same sections. A Grimelius reaction was carried out first; the silver deposits were then removed by treatment with 1% potassium cyanide. Subsequently, immunohistochemical techniques were performed.

In one case (case 5), fragments from vaginal apex (after hysterectomy) were fixed immediately in a mixture of 4% formaldehyde and 1% glutaraldehyde in phosphate buffer, for ultrastructural analysis.

Results

Argyrophil cells were identified in 11 out of 210 ectocervices. Immunohistochemical techniques, applied to the 11 ectocervices showing argyrophilia, demonstrated serotonin and calcitonin immunoreactive cells. All other immune sera gave negative results. Endocrine cells were encountered in 2 main morphological varieties of ectocervical epithelium. These distinctive cells could be detected in normal-appearing squamous epithelium as well as in a specific ectocervical epithelium termed "transitional-like". The term "transitional-like", used in the following text, refers to non-squamous epithelium containing endocrine cells.

A wide range of histological and immunohistochemical appearances was encountered from case to case (Table 1).

In 5 cases (cases 6, 8, 9, 10, 11), serotonin cells were the only endocrine cells visualized. Calcitonin (Figs. 1–3) and serotonin (Figs. 3–4) cells were both present in 6 specimens (cases 1, 2, 3, 4, 5, 7). In these cases serotonin cells significantly outnumbered calcitonin cells. Combined techniques demonstrated that all argyrophilic cells displayed serotonin immunoreactivity. However, only a small fraction of the argyrophil cell population proved to be calcitonin containing. It followed that calcitonin cells corresponded to a subpopulation of argyrophil, serotonin cells. This implied coexpression of calcitonin and serotonin within the same endocrine cell. Endocrine cells were more abundant in transitional-like epithelium than in normal-appearing squamous epithelium. Both types of epithelium could contain only serotonin immunoreactive cells or serotonin-, calcitonin-costoring cells.

In 3 cases (cases 1, 2, 3), native ectocervical epithelium was completely replaced by transitional-like epithelium. This epithelium overlaid a transformation zone (Fig. 1) and extended as far as the vaginal resection line, including the fornices (Fig. 2). In 3 cases (cases 4, 5, 6), the ectocervix was covered with areas of either transitional-like (Figs. 4–5) or normal squamous epithelium, in succession. In 4 cases (cases 7, 8, 9, 10), transitional-like epithelium was confined to the transformation zone. In case 5, hysterectomy was preceded, one

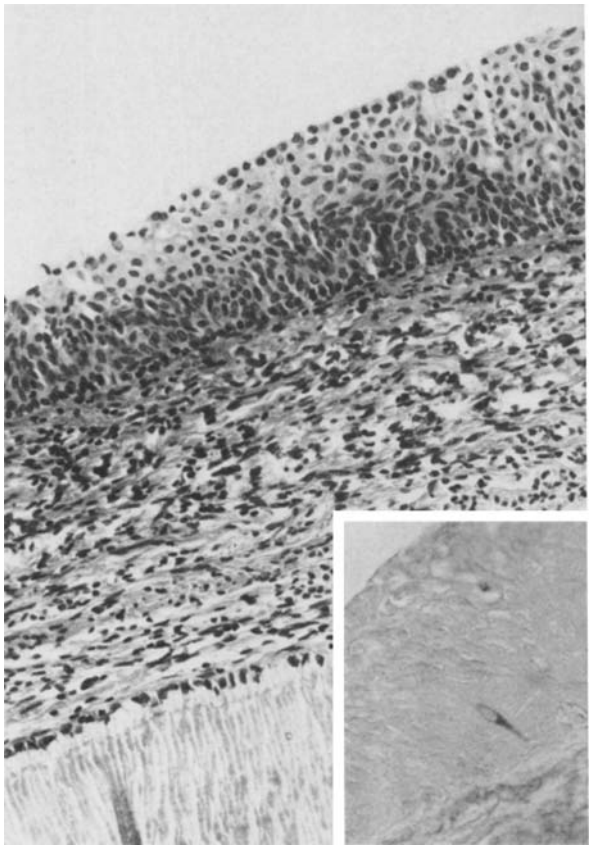

Fig. 1. *Case 1.* Transformation zone. The transformation zone is also covered by transitional-like epithelium. Large, somewhat squamoid or cuboidal cells are seen in focal areas of preservation of the superficial layers (HES × 140). *Inset:* An isolated and elongated calcitonin cell through the same lining (Anticalcitonin PAP × 170)

year before, by cervical conization showing no prior transitional-like epithelium. Transitional-like epithelium encompassed a spectrum of histological patterns. The majority of the cases might be regarded as immature squamous metaplasia (prosoplasia) or basal cell hyperplasia (Fig. 5). However, it must be mentioned that some specimens bore histological resemblance to the urothelium of the urinary tract (Figs. 1–2). The latter epithelium was composed of 5 to 12 layers of cells. A salient feature was the presence of ovoid coffee-bean grooved nuclei whose long axes were oriented perpendicularly to the basement membrane. Large and somewhat squamoid or cuboidal cells were seen in focal areas of preservation of the superficial layers. No sign of dysplasia was present. In places, this lining showed a gradual transformation into a fully mature squamous epithelium.

Electron microscopic examination was performed on cervico-vaginal epithelium from case 5 (Fig. 5). A single elongated endocrine cell was identified. This endocrine cell was admixed among other common epithelial cells. This distinctive cell contained tightly packed cytoplasmic neurosecretory-type granules. Granule diameters ranged from 75 nm to 200 nm, with a mean diameter of about 150 nm. The granules were round or more rarely elongated. The great majority contained an intensely osmiophilic, central or eccentric, core; this core was surrounded by lucent, poorly osmiophilic, material. This endocrine cell was bound to other epithelial cells by desmosomal attachments.

Most of our ectocervical specimens were negative for argyrophilic cells. These specimens comprised examples of normal squamous epithelium and immature squamous metaplastic lining.

Discussion

Argyrophil cells in ectocervical epithelium have been described by several authors (Tateishi et al. 1975; Albores-Saavedra et al. 1979; Satake et al. 1982; Bannatyne et al. 1983). Serotonin cells have been found in many morphological varieties of ectocervical epithelium. Argyrophil cells have not been noted in squamous type of dysplasia or carcinoma in situ (Fetissof et al. 1986a).

All cloacally derived tissues display the same endocrine profile. Serotonin, calcitonin, glycoprotein-hormones α-chain, somatostatin, and bombesin immunoreactive cells are an integral component of these tissues (Fetissof et al. 1983; di Sant' Agnese and de Mesy Jensen 1984; di Sant'Agnese et al. 1985; di Sant'Agnese 1986; Fetissof et al. 1986b). Calcitonin and serotonin immunoreactivities were found to be coexpressed in the same endocrine cell. Transitional epithelium from urethra, urinary bladder, anal transitional zone, and Bartholin's glands are particularly well-endowed with serotonin and calcitonin cells (Fetissof et al. 1984; Fetissof et al. 1985). Likewise, the transitional epithelium of Brenner tumors contains a quite similar spectrum of endocrine cells (Aguirre et al. 1986). As a matter of interest, bronchiolar and bronchial mucosa contains a very similar endocrine cell population (Becker et al. 1980). The similarity of the endocrine cell component from all these non-squamous epithelia (transitional, pseudostratified columnar) must be stressed.

Endocrine cells in ectocervical epithelium are uncommon. They were found in about 5% of our specimens. In particular it should be noted that argyrophil cells were detected in only a small fraction of the squamous metaplasias. Endocrine cell-containing ectocervical epithelia rarely corresponded to a normal-appearing squamous epitheli-

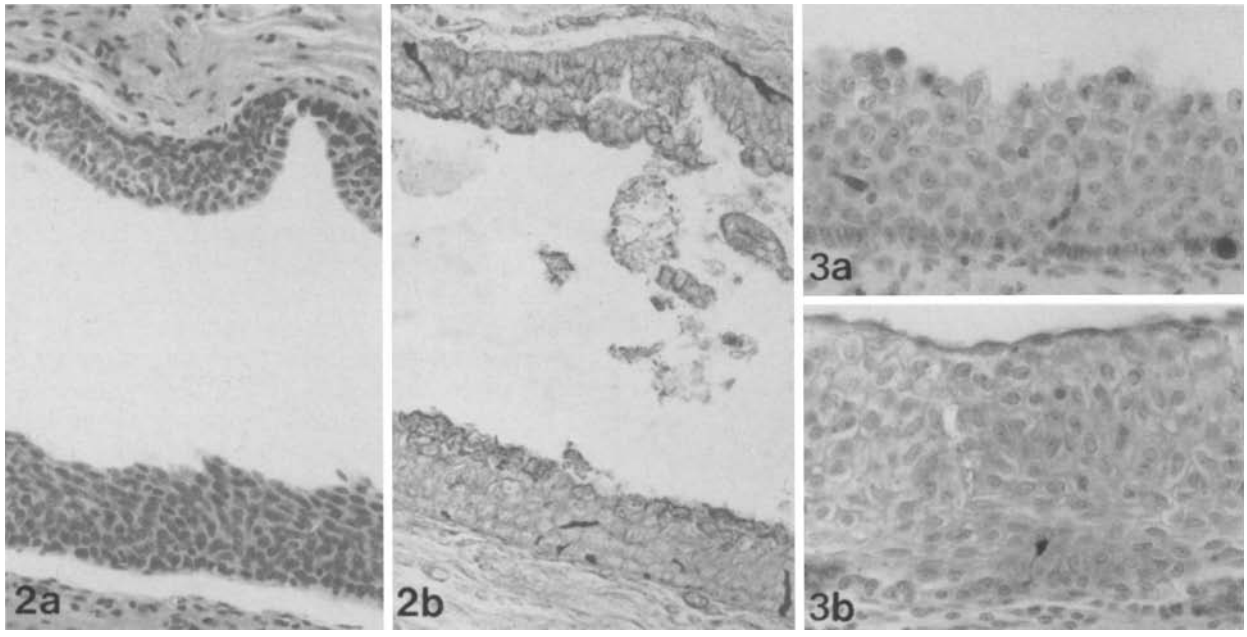

Fig. 2. *Case 2.* Portio epithelium. **a** Both sides of the fornix are covered by transitional-like epithelium. Note the resemblance (*at top*) to urothelium. No dysplasia is present (HES ×125). **b** Grimelius procedure easily detects dendritic argyrophil cells (Grimelius ×150)

Fig. 3. *Case 2.* Portio epithelium. This transitional-like epithelium contains abundant serotonin immunoreactive cells **a** and rather scarce calcitonin immunoreactive cells **b**. (**a:** Antiserotonin PAP; **b:** Anticalcitonin PAP; ×240)

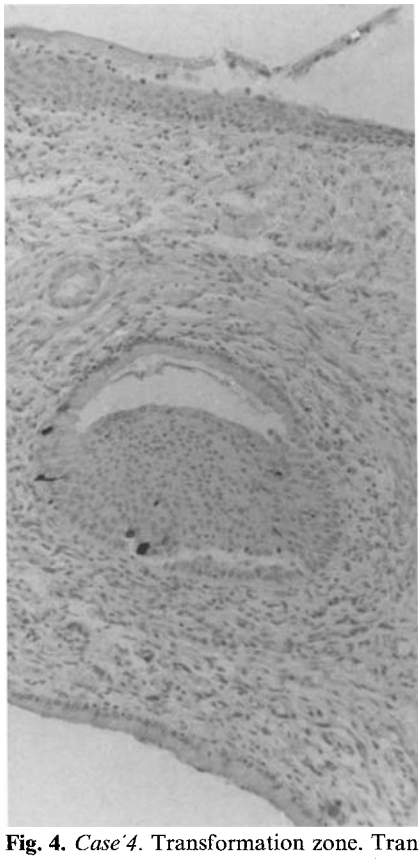

Fig. 4. *Case 4.* Transformation zone. Transitional-like epithelium extends into microcystic endocervical crypt. Serotonin cells are confined to the metaplastic epithelium. (Antiserotonin PAP ×85)

um; they must often corresponded to non-squamous epithelia termed "transitional-like" in this paper. Therefore, an abundance of endocrine cells was not independant of epithelium type. Some transitional-like epithelia might be regarded as immature squamous cell metaplasia (prosoplasia) or basal (reserve) cell hyperplasia. Others showed some morphological analogies to the urothelium of the urinary tract. It must be reinterated that these epithelia were not exclusively confined to the transformation zone. They occasionally involved the entire ectocervix, extending onto the portio far from the transformation zone. Some of them were obviously metaplastic in origin. These epithelia contained serotonin and calcitonin cells and thus reproduced an endocrine profile quite similar to that of other non-squamous epithelia such as urothelium and pseudostratified columnar epithelium.

Is it possible that some ectocervical epithelia could express a particular type of differentiation related to that of other non-squamous epithelia? The results of the present study might provide support for the relationship of certain ectocervical epithelia to urothelium with regard to the nature of their endocrine cell components and to their morphological appearance. Since these criteria (notably the latter) could be considered as rather subjective, the degree of analogy between these types of epithelium remains debatable. This matter, which

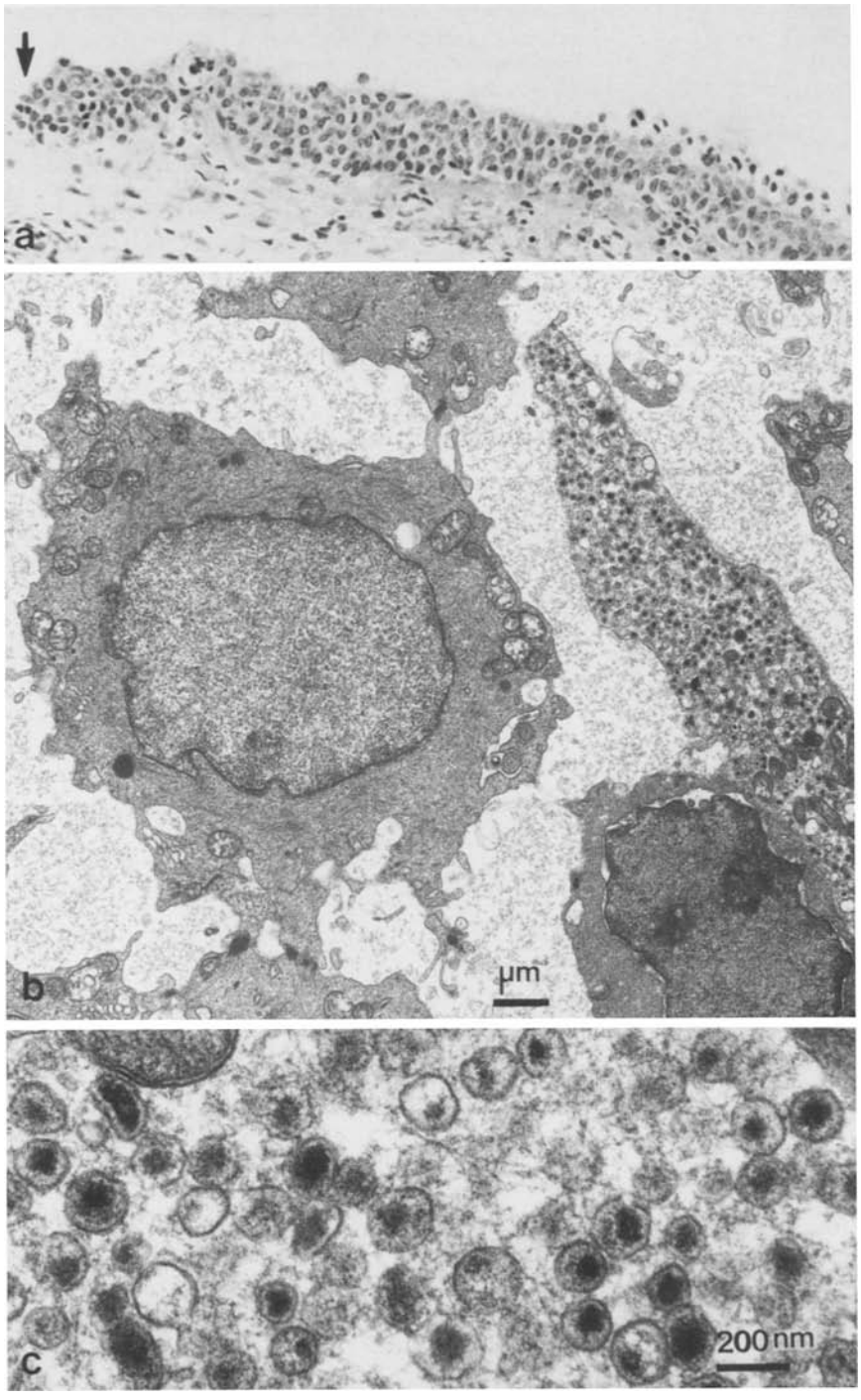

Fig. 5. *Case 5. Portio epithelium.* **a** General histological appearance of the epithelium on routine stained preparation. Note the cuboidal cells at the surface. Arrow indicates the vaginal resection line. Fragments for ultrastructural analysis were obtained from this area (HES ×130). **b** In this field, electron microscopic examination identified an endocrine cell process, admixed among common epithelial cells. This endocrine cell contains numerous small dense cytoplasmic granules (×7350). **c** Higher magnification showing the detail of neurosecretory-type granules (×50000)

clearly requires additional study, should stimulate further investigations using other characteristic or specific markers of urothelial cells.

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