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Endocrine differentiation in inflamed urinary bladder epithelium with metaplastic changes

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Summary. Several types of metaplasia can occur in human bladder epithelium under certain pathological conditions. We investigated 65 cases of cystitis, associated with different types of metaplasia, for the presence of endocrine cells, using histochemical and immunocytochemical methods. Tissues were obtained at cystoscopy and were routinely fixed in 10% buffered formalin. Endocrine cells were demonstrated, between the epithelial cells, in 40 out of 50 cases of cystitis glandularis or cystica. These cells were positive by the Grimelius' silver impregnation technique and were immunoreactive for protein gene product (PGP 9.5), a new general neuroendocrine marker, chromogranin and serotonin. No endocrine cells were detected in any of the specimens of normal epithelium nor those showing squamous metaplasia. Eighteen of these cases showed prominent nerve bundles in the subepithelial tissue, as revealed by PGP immunoreactivity.

Key words: Cystitis – Metaplasia – Endocrine cells – Urinary bladder

Introduction

The presence of endocrine cells in bladder epithelium has been reported only rarely (Fetissof et al. 1986a, b; Feyrter 1951) in humans, though other parts of the urinary system, particularly the prostate gland and urethra (Azzopardi and Evans 1971; Casanova et al. 1974; Di Sant'Agnese and De Mesy Jensen 1984a; Fetissof et al. 1983; Kazzaz 1974; Lendon et al. 1976) have been shown to contain such cells which are analogous to those of the gastrointestinal and pulmonary systems. A wide variety of polypeptide hormones and/or biogenic amines, usually serotonin (Di Sant'Agnese et al. 1985) have been described in this type of endocrine cell: recently, somatostatin, bombesin and calcitonin immunoreactivities have been described in the endocrine cells of normal human prostate gland (Di Sant'Agnese and De Mesy Jensen 1984b; Di Sant'Agnese et al. 1985; Festissof et al. 1986a).

Metaplasia of the urinary bladder epithelium (urothelium) is not an unusual phenomenon and is seen often in cases of chronic irritation, inflammation and exstrophy of the bladder (De La Pena et al. 1959). In the majority of cases, the epithelium undergoes metaplasia of squamous types while in a few it becomes mucus secreting (Weiner et al. 1979). A combination of the two types is not uncommon (Gordon 1963). A common bladder condition, cystitis glandularis-cystica, occurs in association with metaplastic changes of the transitional epithelium, as originally described by Von Limbeck (1887) and Von Brunn (1893).

Focal proliferation of the basal epithelial layer produces solid nests of cells, usually designated as Von Brunns' nests, which grow downwards. Later, glandular metaplasia develops within them and progressive accumulation of mucin results in formation of a cystic area. Finally, cysts lined by flattened epithelium develop (Mostofi 1954).

There have been a few reports describing the occurrence of endocrine-like cells in association with the metaplastic changes of the bladder and these were based on the presence of dense core secretory granules and positive silver impregnation (Newman and Antonakopoulos 1985; Ucci et al. 1985). We investigated retrospectively, using histochemical and immunocytochemical methods, 65 cases of cystitis associated with different types of metaplastic and proliferative changes for the pres-

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Table 1. Characteristics of antisera used

Antisera raised against	Raised in	Dilution[a]	Reference[b]
PGP 9.5	Rabbit	1:400	Thompson et al. 1983
Chromogranin[c]	Mouse	1:200	Lloyd and Wilson 1983
Bombesin	Rabbit	1:800	Wharton et al. 1978
Calcitonin	Rabbit	1:4000	Sikri et al. 1985
VIP	Rabbit	1:10000	Bishop et al. 1984
Serotonin	Rabbit	1:10000	Facer et al. 1979

[a] Optimal dilution for PAP immunostaining
[b] Reference in which full antiserum characteristics are given
[c] All antisera were polyclonal and raised in rabbits except antibodies to chromogranin which were mouse monoclonals

ence of endocrine cells in the epithelium and for any neural abnormalities.

Material and methods

Tissue obtained at cystoscopy from 65 cases of cystitis associated with various types of metaplasia were selected from the departmental files. Fifteen normal bladder biopsies were selected as controls. The tissues had been fixed in 10% buffered formalin embedded in paraffin wax and sectioned at 5 μm. Sections from each case were stained by haematoxylin and eosin, Grimelius' silver impregnation and by immunocytochemistry using several different antisera for neuroendocrine differentiation. Details of the primary antisera used are shown in Table 1.

Light microscopical immunocytochemistry. Sections were immunostained using the peroxidase anti-peroxidase (PAP) method (Sternberger et al. 1970). After de-waxing, endogenous peroxidase activity and nonspecific staining due to the bridging antisera were blocked by sequential incubation for 30 min in 0.03% hydrogen peroxide followed by a further 30 min in normal goat serum (dilution 1:30). Sections were immunostained for chromogranin, serotonin, PGP 9.5 and three peptides, bombesin, vasoactive intestinal peptide (VIP) and calcitonin. The primary antibodies were applied at appropriate dilutions (Table 1) and incubated for 16–20 h at 4° C in a moist atmosphere. After thorough rinsing in buffer, those sections incubated with a primary antibody from mouse (anti-chromogranin) received an excess (1:100 dilution) of goat anti-mouse immuno-globulin G, and those that had a rabbit primary antiserum (including peptide, PGP and serotonin antibodies) were incubated with an excess (1:200 dilution) of goat anti-rabbit immunoglobulin G. After further rinses in buffer, the sections were incubated with PAP complex. To localise mouse- and rabbit-derived reagents, mouse monoclonal PAP (clono-PAP, Sternberger-Meyer, dilution 1:200) and rabbit PAP (Miles Laboratories, Inc., Slough, UK; Elkhart Ind., dilution 1:500) were used, respectively. All incubations were for 30 min at room temperature.

Visualization of the PAP complex was achieved by the diamino-benzidine method of Graham and Karnovsky (1966).

Table 2. Histological features

	No.	Mean age (and range)	F/M
Chronic cystitis with squamous metaplasia	15	45 (27–63)	9/6
Chronic cystitis with Von Brunn's nests only	5	49 (35–63)	3/2
Cystitis glandularis	15	40 (32–48)	10/5
Cystitis glandularis with intestinal metaplasia	3	50 (49–61)	2/1
Cystitis cystica	27	43 (37–49)	18/9
Normal bladder	15	40 (28–52)	7/8

Table 3. Endocrine cells detected by histochemical or immunocytochemical methods

	Total cases with endocrine cells	Cg	PGP	5HT	Grim
Chronic cystitis with squamous metaplasia	0/15	0/15	0/15	0/15	0/15
Chronic cystitis with Von Brunn's nests only	5/5	5/5	4/5	3/5	5/5
Cystitis glandularis	13/15	13/15	9/15	8/15	10/15
Cystitis glandularis with intestinal metaplasia	3/3	3/3	0/3	2/3	3/3
Cystitis cystica	19/27	19/27	15/27	14/27	11/27
Normal bladder	0/15	0/15	0/15	0/15	0/15

No. of cases with endocrine cells/total No. of cases
Grim: Grimelius' silver impregnation; PGP: Protein gene product 9.5; 5HT: Serotonin; Cg: Chromogranin

When developed, the sections were dehydrated through graded alcohols to xylene, mounted in DPX, and examined under a transmitted light microscope. Photographs were taken using Technical Pan black and white film (speed 150 ASA, Kodak Ltd., UK).

Results

Conventional histology

All the diseased cases showed evidence of cystitis with one or more types of metaplastic changes (Table 2).

Histochemical and immunocytochemical results

Table 3 shows a summary of the results of histochemistry and immunocytochemistry. Chromo-

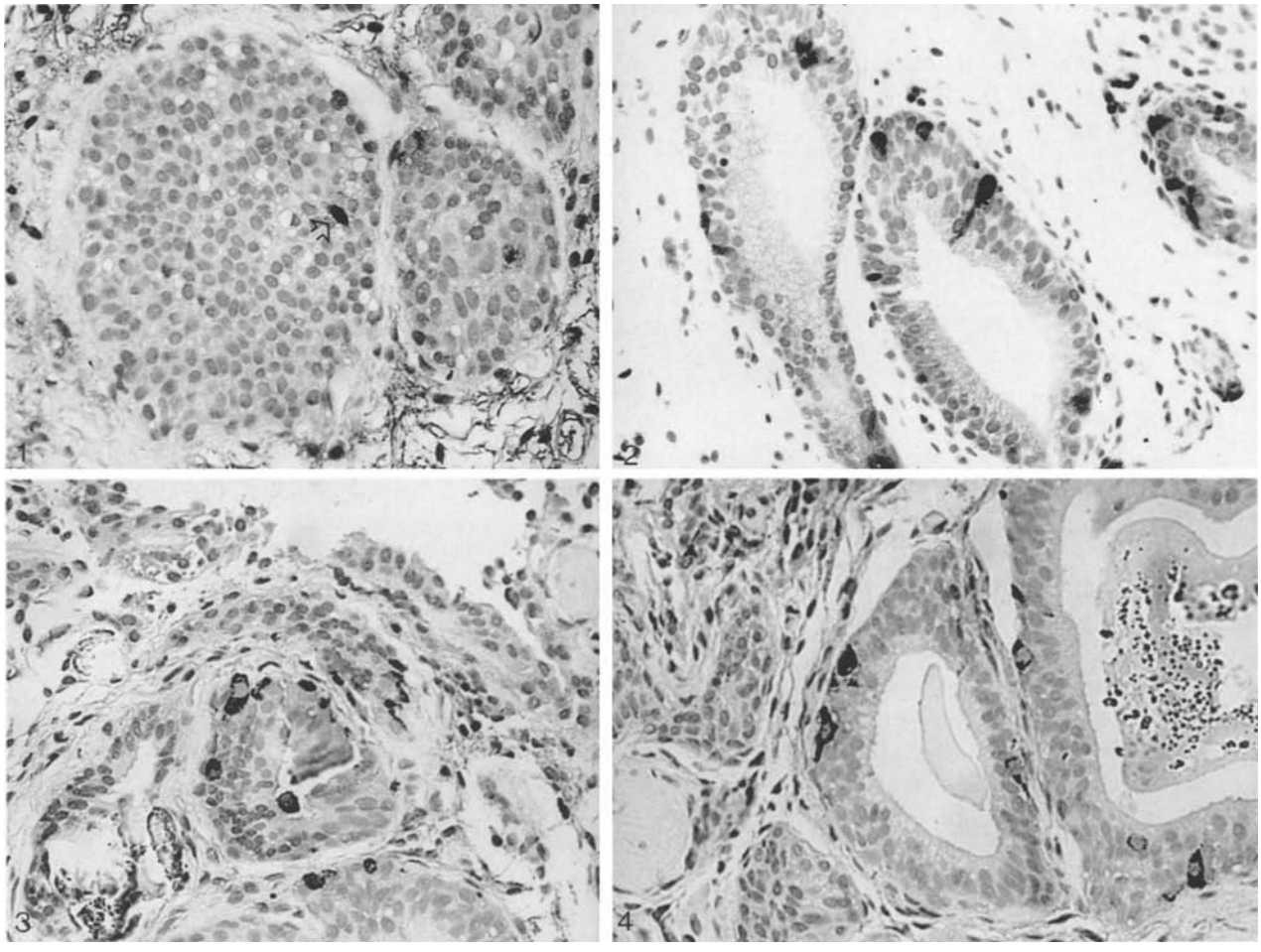

Fig. 1. Endocrine cells in Von Brunn's nest. Grimelius' silver impregnation. × 350

Fig. 2. Chromogranin-immunoreactive endocrine cells in cystitis cystica. PAP technique. × 350

Fig. 3. PGP 9.5-immunoreactive cells in cystitis glandularis. PAP technique. × 350

Fig. 4. Basally located endocrine cells in cystitis glandularis immunoreactive for serotonin. PAP technique. × 350

granin immunoreactivity proved to be the most effective marker for endocrine cells as all cases which demonstrated the cells by other means [PGP or serotonin immunoreactivity, positive silver impregnation] were not only positive for chromogranin but also contained larger numbers of immunoreactive cells. Out of 50 cases of cystitis glandularis or cystica, 40 contained chromogranin-immunoreactive endocrine cells. In chronic cystitis with Von Brunns' nests, endocrine cells visualised by chromogranin immunostaining were seen in Von Brunns' nests mainly located at the periphery but, in some cases, extending towards the centre of the nest (Fig. 1). In cystitis glandularis-cystica, the endocrine cells were located between the epithelial cells. The cells had a spindle or triangular shape and in some places were situated at the basement membrane under the usual epithelial cells (Figs. 2 and 3). The endocrine cells were immunoreactive for serotonin in 27 out of 50 of cases of cystitis glandularis-cystica (Fig. 4).

Using antibodies to PGP, prominent nerve bundles could be seen in the subepithelium of 18 of the 40 cases that showed endocrine differentiation (Fig. 5). These numerous, thick bundles of immunoreactive fibres often showed a close association with the epithelium. No peptide immunoreactivity could be found in either the endocrine cells nor the nerve bundles.

None of the cases of cystitis with squamous metaplasia or the endoscopic biopsies of the normal bladder showed endocrine cells.

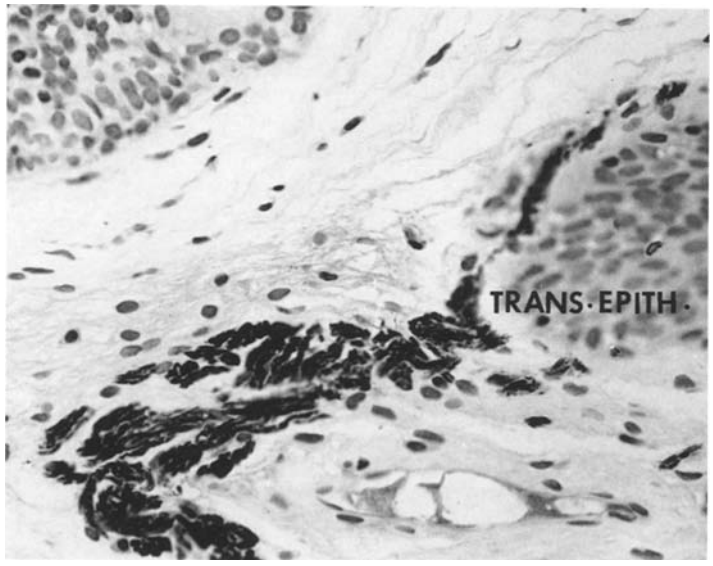

Fig. 5. Large nerve bundle in the subepithelium of a case of cystitis glandularis identified by PGP 9.5 immunoreactivity. PAP technique. ×280

Discussion

Our study shows two interesting findings, first is the presence of endocrine cells in Von Brunn's nests and among the epithelial population of a large number of cases of cystitis glandularis-cystica, the second is the presence of abnormally prominent nerve bundles in 40% (18 out of 50) of these cases. The changes were not seen in cases with squamous metaplasia nor in normal bladders.

The endocrine cells were demonstrated using both histochemical and immunocytochemical methods. Chromogranin represents a family of proteins whose presence in a cell is related to the secretory granules (Lloyd and Wilson 1983) and is considered to be a good general granular marker for endocrine cells (Facer et al. 1986). Another recently described general marker for neuroendocrine differentiation is PGP 9.5 a soluble brain protein with a molecular weight of 27000 Kd (Rode et al. 1985; Thompson et al. 1983). Using antisera to chromogranin and PGP 9.5, 40 out of 50 cases with cystitis glandularis-cystica showed endocrine cells between the epithelial cells. Immunostaining for chromogranin was found to be the best means for visualizing endocrine cells. The majority of the endocrine cells were positive by Grimelius' silver impregnation. Serotonin, a remarkably ubiquitous biogenic amine, is usually present in a proportion of the endocrine cells of gastrointestinal and respiratory tracts and has been reported in the endocrine cells of normal and hyperplastic human prostate gland (Di Sant'Agnese et al. 1985). It has also been shown to promote division of a wide variety of cell types, in both animals and plants (Mann 1967; Niaussat et al. 1958; Tutton 1974). Serotonin-containing cells have been reported in adenocarcinomas of the prostate, stomach and colon (Arends et al. 1986; Capella et al. 1981). The presence of serotonin immunoreactivity in 67% (27 out of 40) of cases of cystitis glandularis-cystica is an interesting observation, which merits further studies to evaluate the possible stimulatory effect of serotonin on bladder epithelium. Although none of the endocrine cells or nerves were found to show immunoreactivity for peptides in this study (vasoactive intestinal peptide, bombesin, calcitonin), this may be a result of the fixation methods used. It has been established that immunocytochemistry of small bioactive peptides requires specialised tissue fixation, particularly in nerves. A further study using appropriately fixed (Bouin's and benzoquinone solutions) tissue may be necessary to show the presence or absence of peptides.

There are some possible explanations for the origin of the large number of endocrine cells in cystitis glandularis-cystica. It may be that there is a very small population of endocrine cells in the epithelial lining of the normal bladder epithelium which cannot be detected easily by histochemistry or immunocytochemistry of routinely processed tissues. The endocrine cells, whether few or numerous, may contain too little product to be immunostained. The cells may undergo hyperplasia and/or accumulate their product following the stimulation associated with chronic inflammation and become distinguishable by our histochemical methods. Alternatively, a process encouraging endocrine differentiation of multipotential epithelial stem cells could be involved.

Adenocarcinoma of the urinary bladder represents a small percentage of bladder tumours and shows frequent association with cystitis cystica and glandular metaplasia (Ward 1971). Recently, endocrine cells have been described in transitional cell carcinoma of the urinary bladder (Collino et al. 1986) and there are increasing reports of primary neuroendocrine tumours of the bladder, mainly carcinoid and small cell carcinomas (Partanen and Asikainen 1985). An association of these tumour conditions with the endocrine cell changes we have reported, is thus quite possible. Using antibodies to PGP 9.5, an excellent neural marker (Gulbenkian et al. 1986), prominent thick bundles of nerves were detected in about half of the cases that showed endocrine cells. These prominent nerves could often be seen lying near to the epithelium. The trophic effect of nerves has been postulated for sometime (Burnstock 1981; Tutton and Barkla 1980) and, although it is not possible to discern in these morphological studies whether the nerve bundles are primary or secondary features, it is tempting to speculate that they contribute to the epithelial proliferation.

In conclusion, our study shows that readily demonstrable increases occur in both endocrine cells and nerves in the human bladder in association with inflammation and metaplastic epithelium. The presence of putative trophic factors may enhance the proliferative mechanisms of the epithelium.

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