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Calcitonin immunoreactive cells in prostate gland and cloacal derived tissues

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Summary. Calcitonin- and serotonin-storing cells have been immunocharacterized in prostate gland, urethra, urinary bladder and anal canal. In addition, a few hCG and somatostatin immunoreactive cells have been detected in prostate gland. All these cells were dispersed throughout the epithelial lining. In the anal canal, calcitonin cells were exclusively confined to the anal ducts and anal transitional zone epithelium. Calcitonin and serotonin cells were seen in some examples of prostatic adenocarcinoma. Combined techniques most often showed coexistence of calcitonin and serotonin immunoreactivities in the same endocrine cell. hCG immunoreactive cells corresponded to a subpopulation of serotonin-, calcitonin-storing cells. Calcitonin and serotonin cells were present in most organs which originated from the cloaca. In this territory, this distinctive endocrine pattern could be regarded as an excellent marker of cloacal derived tissues. These tissues constitute an additional site for extrathyroid C-cells. It is likely that calcitonin cells are a component of some prostatic adenocarcinomas.

Key words: Prostate – Anal canal – Urethra – Calcitonin cells – Prostatic adenocarcinomas

Introduction

Endocrine cells in cloacal derived tissues have received little attention. Since the first studies of Feyrter (1951), only a few reports have been concerned with these cells. Recent investigations, using ultrastructural and immunohistochemical techniques have produced a recrudescence of interest in this

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subject. It appears that this system of endocrine cells is more complex and noteworthy than initially thought (di Sant'Agnese et al. 1985).

In this paper, emphasis was laid on a peculiar type of endocrine cell, found in all tissues derived from cloaca.

Materials and methods

The material comprised 10 prostate glands, obtained from enucleated specimens of hyperplastic glands. Most tissue sections contained a fraction of normal prostate and often included fragments of prostatic urethra. Ten transurethral resections for adenocarcinomas were also collected.

Five cystectomy specimens, removed for transitional cell carcinoma, were investigated. Two were performed in female patients, providing opportunity to study female urethral mucosa and paraurethral glands. 2 penectomy specimens, for epidermoid carcinomas, were studied. In the latter samples, the cavernous portion of the urethra as well as paraurethral glands of Littre were represented.

Finally, we examined 5 abdomino-perineal resections for adenocarcinoma of the rectum. This material permitted an analysis of rectal type mucosa, anal transitional zone (ATZ), anal ducts, and pectinal folds. In addition, one 16-week-old fetus was processed. All tissue samples were fixed in aqueous Bouin's fluid and then embedded in paraffin. No frozen tissue has been used. Argyrophil and argentaffin cells were detected by a modified Grimelius and the 48-h Masson-Fontana techniques.

Immunohistochemical techniques were performed on deparaffinized sections. Indirect immunofluorescence or peroxidase-antiperoxidase (PAP) were used with rabbit antisera to ACTH, calcitonin, human chorionic gonadotropin (hCG and β-hCG), gastrin, glucagon/enteroglucagon, motilin, neurotensin, human pancreatic polypeptide, serotonin, somatostatin and thyroglobulin, with guinea pig antiserum to insulin and with sheep antiserum to calcitonin. Sheep anticalcitonin serum was kindly furnished by Dr. D. Guilloteau (Laboratoire de Biophysique médicale, Hôpital Bretonneau, Tours), antiglucagon serum by Dr. R. Assan (Diabetes department, Hôpital Bichat, Paris), antimotilin serum by Dr. J.A. Chayvialle (Inserm U45, Hôpital E. Herriot, Lyon). Antisera to ACTH, gastrin, insulin, neurotensin, serotonin and somatostatin were provided by Dr. M.P. Dubois (Station de Physiologie de la Reproduction, INRA, Nouzilly). All these antisera were used, with immunofluorescence technique, at a working dilution varying from 1/50 to 1/100. hCG- and β-hCG-antibodies were obtained from Ortho Diagnostic Systems (Raritan, NJ), USA. Rabbit anticalcitonin, antipancreatic polypeptide and antithyroglobulin sera were obtained from Biolyon Laboratory (69572 Dardilly, France). These antisera were used prediluted, with PAP procedure. Controls included the application of antiserum pretreated with the relevant antigen. In order to investigate the relative distribution of argyrophilic or argentaffin cells to immunoreactive cells, combined techniques were applied sequentially to the same sections. Silver impregnation techniques were carried out first, the silver deposits were then removed by treatment with 1% potassium cyanide. Subsequently, immunohistochemical techniques were performed.

Results

Calcitonin and serotonin immunoreactivities were both disclosed in prostate gland, urethra, urinary bladder, and anal canal. Sheep and rabbit anticalcitonin immune sera, used with immunofluorescence or PAP procedures, gave similar results. In addition, some hCG- and somatostatin-containing cells were observed in prostate gland. All other antisera gave negative results.

In all instances, prostate glands contained numerous serotonin- and calcitonin-storing cells. hCG immunoreactive cells, although found in all prostates, were less numerous; β-hCG immunoreactive cells were quite rare.

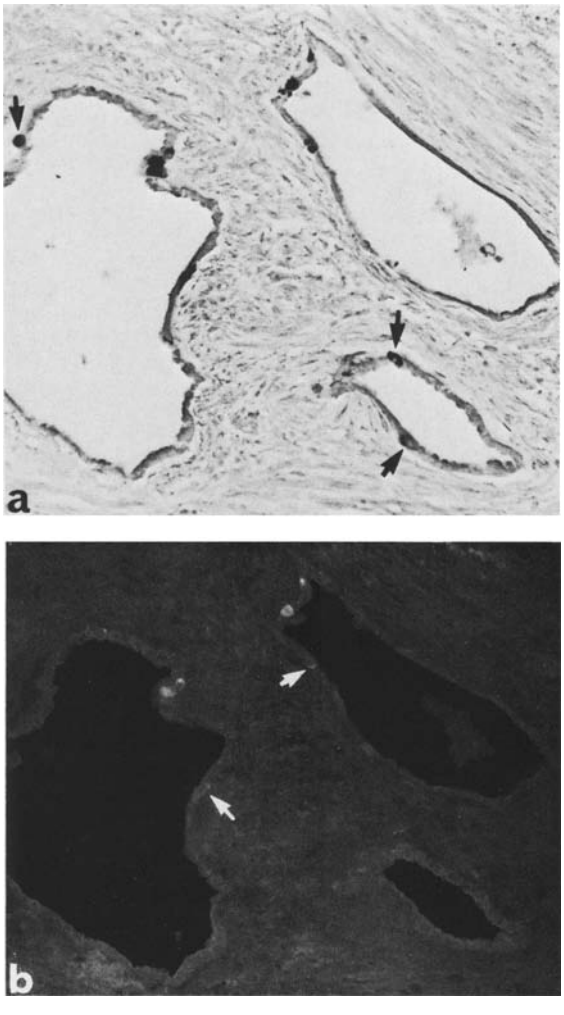

Fig. 1 a, b. Prostate gland. Same section sequentially stained by Grimelius (**a**) and sheep anticalcitonin immunofluorescence (**b**). All calcitonin-positive cells are argyrophilic. Inversely, note that some argyrophil cells (*black arrows*) are calcitonin-negative. In addition, some calcitonin cells exhibit only a weak immunostaining (*white arrows*). × 140

Only a few somatostatin cells were disclosed in 2 of 10 specimens. All these cells were dispersed throughout the epithelium. They were mostly found throughout the residual normal prostate. Most endocrine cells, including the calcitonin-storing, showed dendritic cytoplasmic processes extending among other cells or reaching the luminal surface. Combined techniques established that all calcitonin immunoreactive cells were argyrophilic. Conversely, the great majority of the argyrophilic cell population reacted unequivocally against anticalcitonin sera. Just a few argyrophilic cells proved to be calcitonin negative. In addition, it must be stressed that a small fraction of argyrophilic cells exhibited only a weak positivity for calcitonin immunostaining (Figs. 1, 2). Likewise, argentaffin cells corresponded, for the most part to calcitonin positive cells. All hCG immunoreactive cells were argyro-

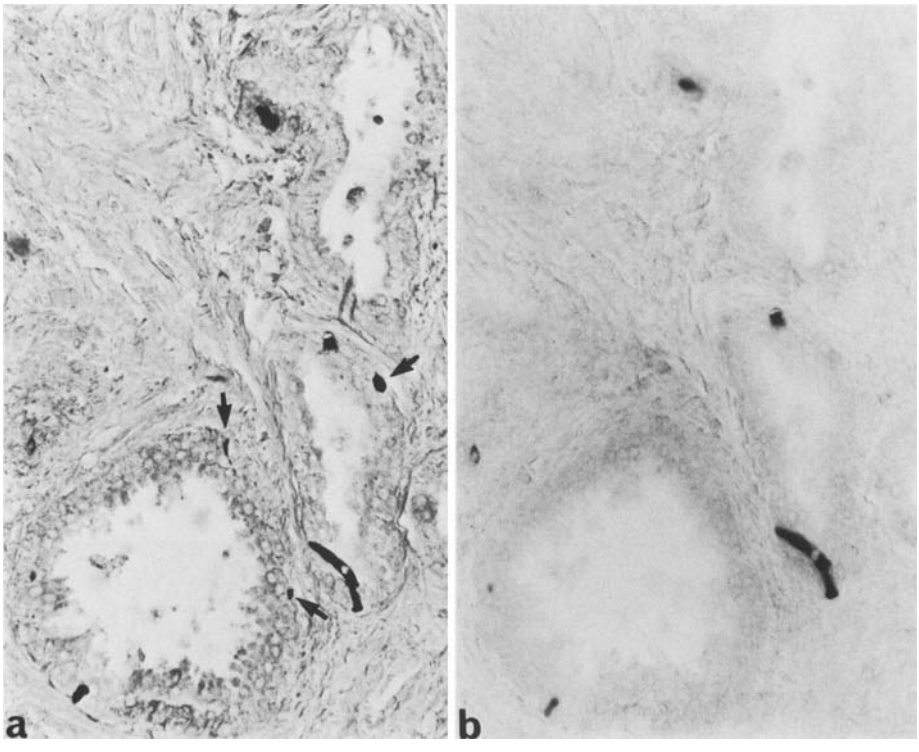

Fig. 2a, b. Prostate gland. Same section sequentially stained by Grimelius (**a**) and rabbit anticalcitonin PAP (**b**). Again, all calcitonin-positive cells are argyrophilic. Inversely, some argyrophil cells (*arrows*) appear calcitonin-negative. ×170

philic. Conversely, only a low proportion of the argyrophilic cells proved to be hCG-producing (Fig. 3).

Two out of ten prostatic adenocarcinomas contained abundant Grimelius positive cells. In both cases, immunohistochemical techniques permitted the recognition of calcitonin and serotonin cells (Fig. 4).

Urethra from both sexes, harbored invariably calcitonin and serotonin cells (Fig. 5). These cells were easily recognized throughout the transitional lining of urethral surface epithelium and lacunae of Morgagni. In males, they were identified in prostatic and cavernous portions of urethra, and in Littre's glands. In females, they were also distributed in paraurethral glands.

All specimens of bladder mucosa were found to contain some serotonin and calcitonin cells. These distinctive cells were essentially encountered among normal-appearing or slightly hyperplastic transitional epithelium, adjacent to transitional cell carcinomas. The carcinomas were unreactive for endocrine cells.

All examples of the anal canal showed a similar distribution of endocrine cells (Figs. 6, 7). Serotonin and calcitonin cells were found in the anal transi-

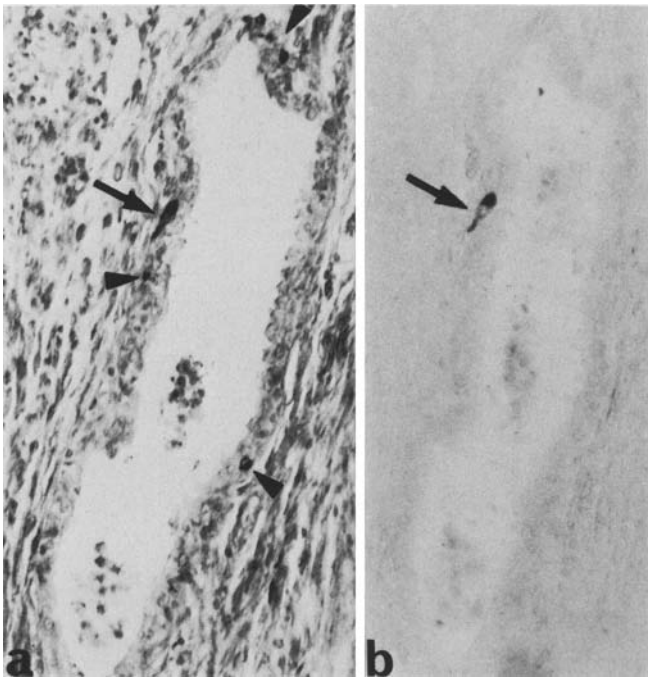

Fig. 3a, b. Prostate gland. Same section sequentially stained by Grimelius (**a**) and anti-hCG PAP (**b**). One gland contains a single hCG immunoreactive cell which appears argyrophilic (*arrow*). Inversely, many other argyrophil cells (*arrowheads*) are hCG-negative. × 160

tional zone epithelium (ATZ epithelium) and anal ducts. Colo-rectal type mucosa contained serotonin, somatostatin, pancreatic polypeptide and glucagon immunoreactive cells. Pectinal folds were devoid of endocrine cells. All these transitions were well-defined. Anal ducts were particularly rich in serotonin and calcitonin cells. Calcitonin immunoreactivity was completely lacking in rectal type mucosa and exclusively confined to ATZ. A similar pattern of endocrine cells was visualized in the fetal lining of anal canal; in addition, serotonin and calcitonin cells were identifiable in prostatic buds.

Discussion

Human prostatic endocrine cells were first mentioned by Pretl (1944). Feyrter (1951) recognized argyrophil and argentaffin cells in prostate gland, urethra, urinary bladder and Littre's glands. Prostatic endocrine cells were further studied by means of histochemical (Grasso 1954; Arrigoni et al. 1956; Azzopardi and Evans 1971; Kazzaz 1974; Pollice et al. 1979), Falck (Baumgarten et al. 1968; Aumüller et al. 1976) and ultrastructural techniques (Pages et al. 1975; Aumüller et al. 1976; Kano and Sato 1978; Capella et al. 1981). Likewise, urethral endocrine cells were subsequently investigated by means of histochemical (Grasso 1954), Falck (Lendon et al. 1976)

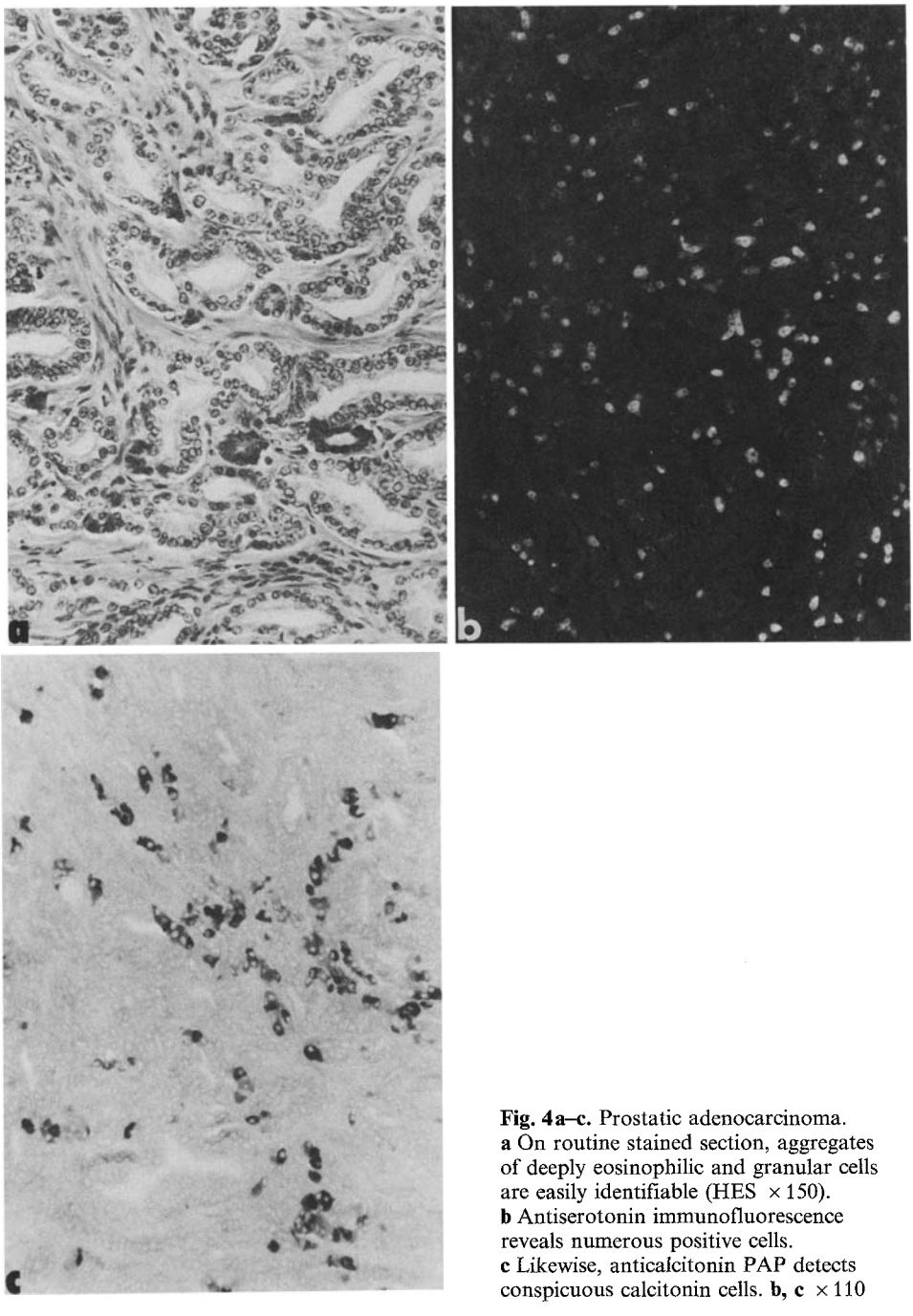

Fig. 4a–c. Prostatic adenocarcinoma.
a On routine stained section, aggregates of deeply eosinophilic and granular cells are easily identifiable (HES ×150).
b Antiserotonin immunofluorescence reveals numerous positive cells.
c Likewise, anticalcitonin PAP detects conspicuous calcitonin cells. **b, c** ×110

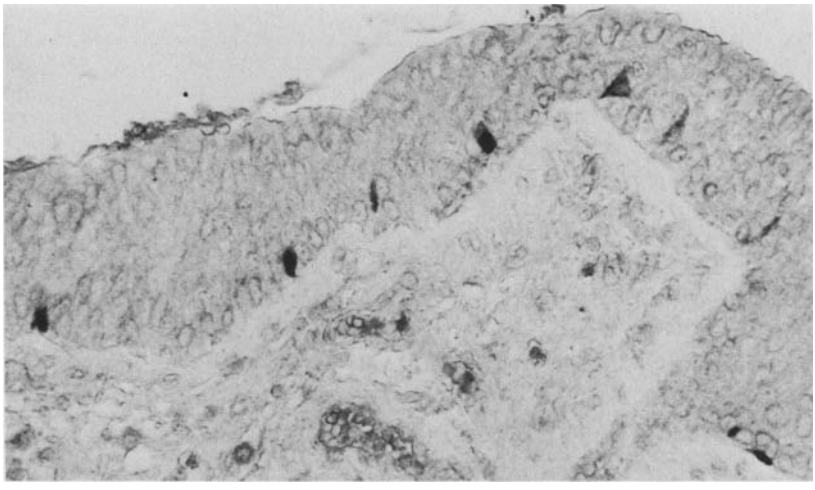

Fig. 5. Urethra. Calcitonin cells are dispersed through the surface transitional epithelium. (Anti-calcitonin PAP ×160)

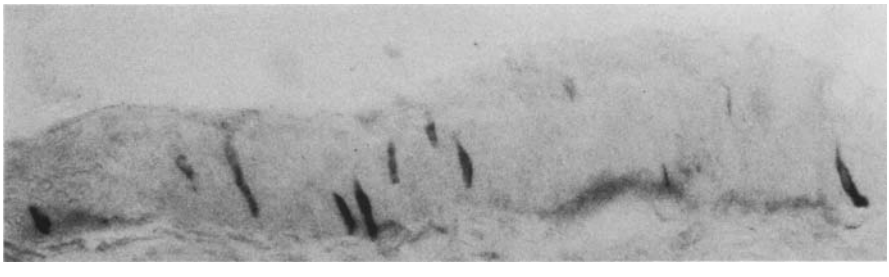

Fig. 6. Anal canal. Calcitonin cells are scattered among ATZ epithelium. (Anticalcitonin PAP ×150)

or ultrastructural techniques (Casanova et al. 1974; Lendon et al. 1976). Fenger and Lyon (1982) demonstrated enterochromaffin cells in ATZ epithelium and anal ducts from anal canal. In previous work, we identified serotonin-storing cells in prostate gland, urethra, urinary bladder and anal canal. All argyrophilic cells were immunocharacterized as serotonin-storing. Cells of endocrine lineage were quite exceptional in wolffian derivatives such as ureter and renal pelvis (Fetissof et al. 1983, 1984, 1986). More recently, di Sant'Agnese et al. (1984a, 1984b, 1985) conducted ultrastructural and immunohistochemical studies of prostatic and urethral endocrine cells. These authors reported somatostatin, serotonin and neuron-specific enolase immunoreactive cells; neuron-specific enolase immunoreactivity correlated closely with serotonin immunoreactivity.

Hormonal products such as ACTH (Ghali and Garcia 1984; Schron et al. 1984; Partanen and Asikainen 1985), ADH (Sacks et al. 1975; Kaye and Ross 1977) and serotonin (Sylora et al. 1975; Dauge et al. 1985; Ucci

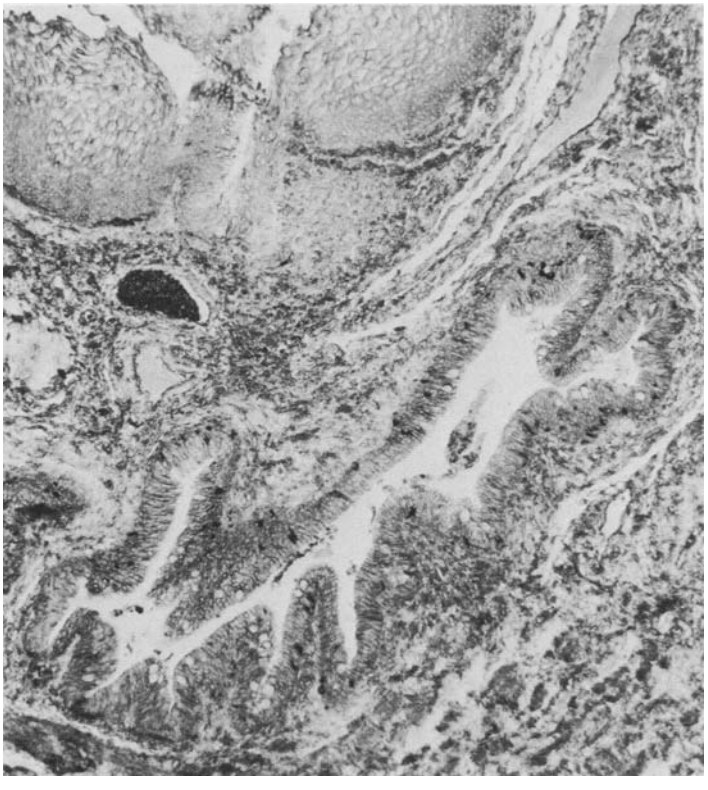

Fig. 7. Anal canal. This anal duct is particularly rich in calcitonin cells. Positive cells are lacking in the overlying squamous epithelium. (Anticalcitonin PAP ×80)

et al. 1985) have been documented in certain prostatic, urinary bladder and urethral neoplasms. Finally, increased concentrations of immunoreactive calcitonin have been reported in plasma samples from patients with prostatic and bladder carcinomas (Coombes et al. 1974).

Extrathyroidal C-cells have been described in bronchiolar and bronchial mucosa (Becker et al. 1980). Bioassayable calcitonin was present in thymus and parathyroids (Galante et al. 1968). Recently, calcitonin immunoreactivity has been detected in cells from thymic medulla (Müller-Hermelink 1985). Hillyard et al. (1978) identified relative deficiency of plasma-calcitonin in normal women. Coexistence of calcitonin and serotonin in the same thyroid C-cells was reported in horse, goat and sheep (Solcia et al. 1969).

Endocrine cells exhibiting α-hCG immunoreactivity have been thoroughly documented (Heitz et al. 1983; Solcia et al. 1985).

The great abundance of calcitonin cells in the prostate gland was very remarkable. In our previous work (Fetissof et al. 1983, 1984) we have not been successful in identifying calcitonin and somatostatin immunoreactivities. We have previously demonstrated that all argyrophil cells displayed serotonin immunoreactivity (Fetissof et al. 1983). However the present in-

vestigation showed that most argyrophil cells were calcitonin-producing. In view of these results, it follows that most serotonin cells are also calcitonin-containing. This implies co-expression of calcitonin and serotonin in the same endocrine cell. The small variability in calcitonin immunostaining suggests that throughout the whole cell life, there is transient reduction in calcitonin content. Therefore, calcitonin-positive and calcitonin-negative serotonin-storing cells might represent various successive stage in the cells functional cycle. In turn, hCG immunoreactive cells correspond to a subpopulation of serotonin cells. hCG immunoreactivity is probably related to α-subunit of gonadotropin.

Calcitonin and serotonin immunoreactivities were immunolocalized in most organs which originated from the urogenital sinus and more generally from the cloaca. This distinctive endocrine profile proves to be of value as a reliable marker of cloacal derived tissues. This is clearly demonstrated in the anal canal, demarcating ATZ from rectal type mucosa abruptly. Moreover, serotonin and calcitonin immunoreactivities in a stratified epithelium might indicate transitional type (urothelial) differentiation. Cloacal derived tissues constitute an additional site for extrathyroidal C-cells. In this sphere the role of these specialized cells remains to be established. It is beyond the scope of this paper to raise the problem of the histogenesis of the C-cells but we suggests that this extra-thyroid C-cell population might help to explain the occurrence of a relative deficiency of plasma-calcitonin in normal women. Finally, it is likely that calcitonin cells participate and proliferate in certain prostatic adenocarcinomas.

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