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Argyrophil cells in normal endometrial glands

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Summary. Normal endometrium from one hundred normal cases were examined histologically, using sections stained with the Grimelius method. Endocrine cells were demonstrated in 4 cases. Immunohistochemically, these cells were positive for anti-serotonin and anti-somatostatin antisera. Argyrophil granules were also observed in supranuclear or subnuclear regions of glandular cells in 17 cases, and argyrophilia was present in the apical region including the brush borders or microvilli of glandular cells in 6 cases. In these latter 23 cases argyrophilia seemed to be nonspecific, having no relation to endocrine type secretory granules, judging from the electronmicroscopic observations on the silver-impregnated sections. The presence of endocrine cells and the pattern of argyrophilia in glandular cells were similar to those found previously in endometrial glandular adenocarcinomas with argyrophil cells. This is the first report on the occurrence of endocrine cells in normal endometrial glands.

Key words: Argyrophil cell – Normal endometrial gland

Introduction

Various kinds of endocrine cells have been detected in the endodermally derived tissues such as the gastro-intestinal tract (Sidhu 1979; Solcia et al. 1975), lung (Capella et al. 1978; Tateishi 1973), urinary bladder and prostate gland (Fetissof 1983; di Sant Agnese 1984). However, there are only a few reports on the occurrence of endocrine cells in the mesodermally derived tissues such as the renal pelvis (Gordon 1963), ovary (Hidvegi 1982), and uterine cervix (Fox et al. 1964; Tateishi et al. 1975; Satake et al. 1982). The cells were found in areas of intestinal metaplasia of the renal pelvis but not in normal pelvic epithelium. In the uterus endocrine cells have been detected in the cervical gland in 2 out of 60 normal cervices examined, according to the report of Fox et al. (1964) and in 1 out of 18 cervices (1 out of 134 blocks), according to our previous study. However, there have been no reports on the presence of endocrine cells in the endometrium (Scully et al. 1984). The present examinations were performed to reveal the occurrence of endocrine cells in the normal endometrial gland by histochemical methods and electron microscopy.

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Materials and methods

One hundred normal endometria were examined; 29 were obtained from surgically removed myomatous uteri and 71 by curettage. In these endometria no tumour, hyperplastic or remarkable inflammatory changes were observed. The mean age of the patients was 37 years, ranging from 23 to 53 years. The phase of menstrual cycle was proliferative in 38, secretory in 39, postmenopausal in 2 and pregnant in 21. Endometria were fixed in formalin, and all sections were stained with haematoxylin and eosin and Grimelius method. Total number of blocks examined were 165.

Immunoperoxidase examinations were performed on the paraffin embedded tissues of 27 cases containing argyrophil cells, using the technique of Sternberger 1979. Sections were reacted with rabbit anti-serotonin (SERA-LAB, England), anti-gastrin, anti-somatostatin (DAKO, Denmark), anti-S100 protein (kind gift from H. Hidaka, Mie University School of Medicine, Japan), and anti-neuron specific enolase antisera (kind gift from K. Kato Institute for Developmental Research, Aichi Prefectural Colony). Swine antibody to rabbit immunoglobulins and peroxidase/rabbit antiperoxidase complex were purchased from DAKO (Denmark).

Tissue sections from the same block used for immunoperoxidase examinations were used. The sections were deparaffinized by xylene, dehydrated in graded ethanol, mounted in non-fluo-

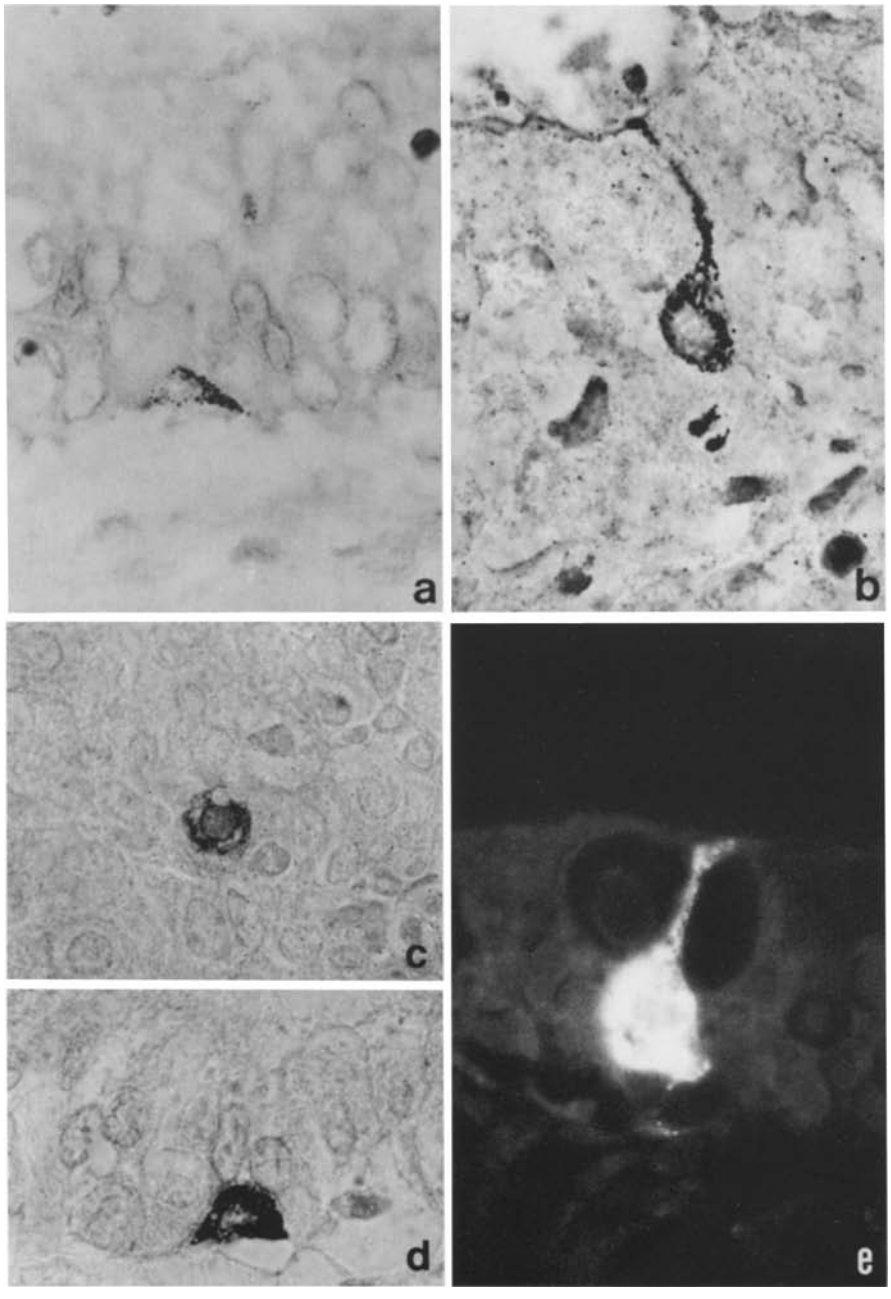

Fig. 1 a–e. Endometrial glands from cases with myoma uteri. **a** A triangular argyrophil cell is seen basal in the epithelium; **b** an elongated process of an argyrophil cell extends to the glandular luminal surface; (**a, b**) Grimelius stain, (**a**) ×990, (**b**) ×1,160; **c** Argyrophil cells show a reactivity to anti-serotonin and **d** anti-somatostatin antisera. Immunoperoxidase stain, (**c, d**) ×990, Autofluorescence cells (**d**). Autofluorescence method, ×1,160

rescein glycelin and examined with a fluorescent microscope with an UV filter (Enerbäck 1972).

Fourteen specimens of endometria were obtained from myomatous uteri removed by operation and fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.4. for 1–2 h. Post fixation was carried out in 1% osmium tetroxyde in 0.04 M phosphate buffer for 2 h, dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead (Sato 1979). Twelve specimens of endometrium were used for an electronmicroscopic study of silver-impregnated sections stained with the Grimelius method (Vassallo et al. 1971). Some paraffin sections were stained with Grimelius method, in which argyrophil cells were recognized, were re-embedded in Epon 812 by the inverted capsule method detaching them from slide glasses for an electronmicroscopic study.

Results

In 4 of 100 cases, a few, isolated argyrophil cells were observed. The cells were triangular or elongated in shape and contained many intracytoplasmic argyrophil granules in the basal region. They were compressed below by endometrial glandular cells (Fig. 1 a), extending their slender cytoplasm to the glandular luminal surface (Fig. 1 b). These

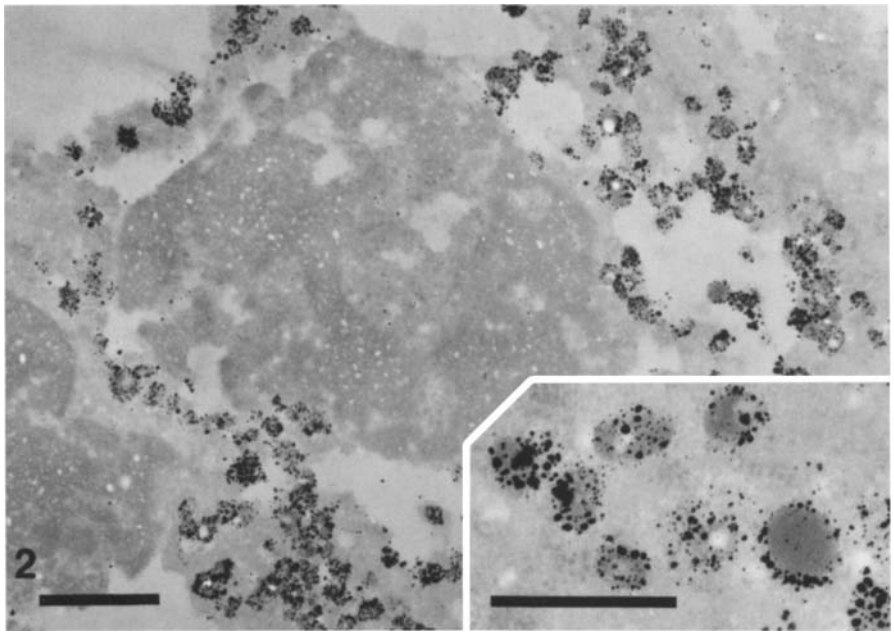

Fig. 2. Silver deposits are seen as grains on the round, dense granules. 10% formalin fixed, paraffin embedded, Grimelius silver, reembedded in Epon, ×16,000, Bar 1 μ, Inset: ×50,000, Bar 0.5 μ

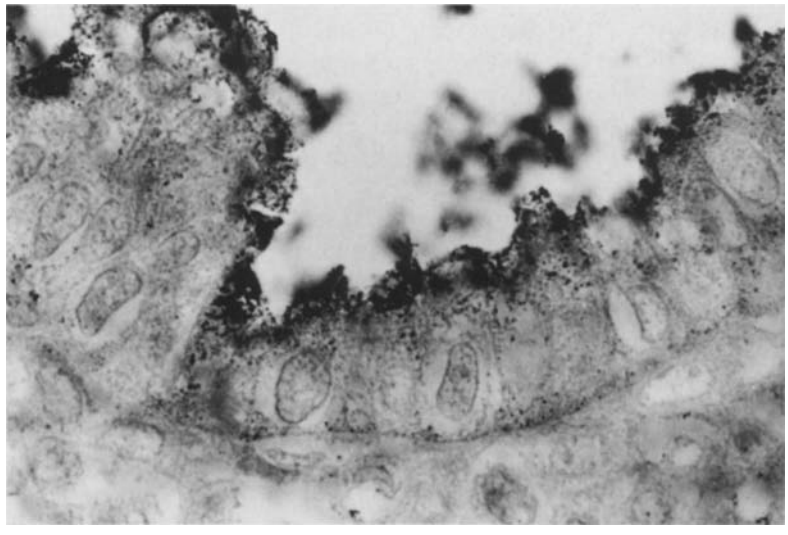

Fig. 3. Argyrophilia is present in the apical and subnuclear regions of glandular cells. Grimelius stain, ×990

cells corresponded to the endocrine cells that were present in other sites. Menstrual phase of 2 cases was proliferative and 2 secretory. These cases were 48, 47, 35 and 32 years in age, respectively. One of the cases contained positive cells for anti-serotonin and anti-somatostatin antisera and for autofluorescence (Fig. 1c–e), but no positive cell for anti-gastrin, anti-neuron specific enolase and anti-S100 protein antisera was found in the endometrial glandular epithelia. Electronmicroscopic observations on the cells, which were stained positively with Grimelius method, showed many round electron dense granules, having numerous silver grains (Fig. 2). The granules were measured from 160 nm to 210 nm in diameter. However, endocrine cells could not be detected in freshly fixed endometria for the electronmicroscopy.

Argyrophilia was also observed in endometrial glandular cells in 23 out of 100 cases. In 6 cases argyrophil granules were present in the apical region or at the microvilli of glandular cells (Fig. 3). In 17 cases they were seen in supranuclear or subnuclear regions of glandular cells. They showed no reactivity for immunoperoxidase examinations and autofluorescence. Submicroscopically, no endocrine type secretory granules were found in these cells, but silver grains were scattered diffusely or deposited together somewhat roundly in the cytoplasm. In some cases small round granules measuring about 170 nm in diameter were observed in

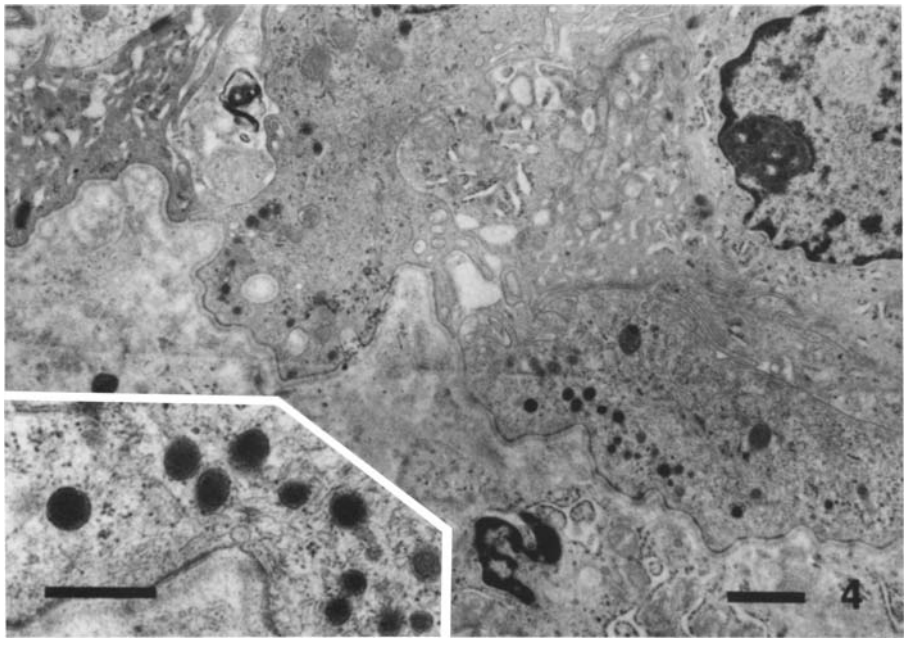

Fig. 4. Small electron dense granules are seen in the subnuclear region of glandular cell. Uranyl acetate and lead stain, ×10,000, Bar 1 μ, Inset: ×30,000, Bar 0.5 μ

the subnuclear region of glandular cells. These granules were few in number and present in a cluster or scattered (Fig. 4). By an electronmicroscopic observation of silver-impregnated endometria, many silver deposits were seen on the luminal surface of glandular cells (Fig. 5a), but not on the round granules in the subnuclear cytoplasm (Fig. 5b). A few silver grains seemed to be deposited together in some round secretory granules located in the supranuclear region (Fig. 5c). In one of the pregnant endometria, argyrophil granules were seen in the subnuclear region of glandular cells by light microscopy (Fig. 6a) and were confirmed submicroscopically to be located in subnuclear mucinous globules (Fig. 6b).

Discussion

In the present study two types of cells which showed argyrophilia were observed within the endometrial glandular epithelia. One is endocrine cell and the other is argyrophil glandular cell. Argyrophil cells which were observed in 4 of 100 endometria were endocrine cells judging from the light microscopic, histochemical and electronmicroscopic features. The cells contained serotonin and somatostatin. No correlation was noted between the presence of endocrine cells and the age and menstrual phase in these 4 cases. Fox et al. (1964) examined endometria of 60 cases and found no endocrine cells. Bannatyne et al. (1983) found argyrophilic granules in the apical cytoplasm of glandular cells in 2 endometria, examining endometria from 25 patients with non-neoplastic disease. Aguirre et al. (1984) reported that endocrine cells could not be identified in 7 normal endometrial curettings, but that argyrophil granules were observable in glandular cells. They diagnosed these granules as mucin and glycogen.

The great majority of tumours containing endocrine cells arise in tissues that normally contain endocrine cells. In the endometrium, hormone secreting small-cell carcinomas (Kuzuya et al. 1979; Olson et al. 1982; Matsuyama et al. 1984) and glandular adenocarcinomas with argyrophil cells have been reported. In the latter, two or three subtypes of argyrophil cells have been classified by the distribution of the granules and shapes of the cells. One of these types is the glandular cell itself, containing argyrophil granules in the apical region or throughout the cytoplasm, which were thought not to be endocrine granules but to be related to intracellular mucin and glycogen (Aguirre et al. 1984). The other corresponds to endocrine cells as observed in the gastro-intestinal tract and in other sites, and this has been confirmed by histochemical, submicroscopic, fluorescence and biochemical methods (Inoue et al. 1982; Bannatyne et al. 1983; Scully et al. 1984; Inoue et al. 1984). Argyrophil granules in the normal endometrial glandular cells were not so numerous as in glandular adenocarcinomas, and this made it difficult to determine the nature of the granules. Small round granules seen in the subnuclear region of glandular cells in the freshly fixed endometria for electronmicroscopy seemed to be similar to endocrine type

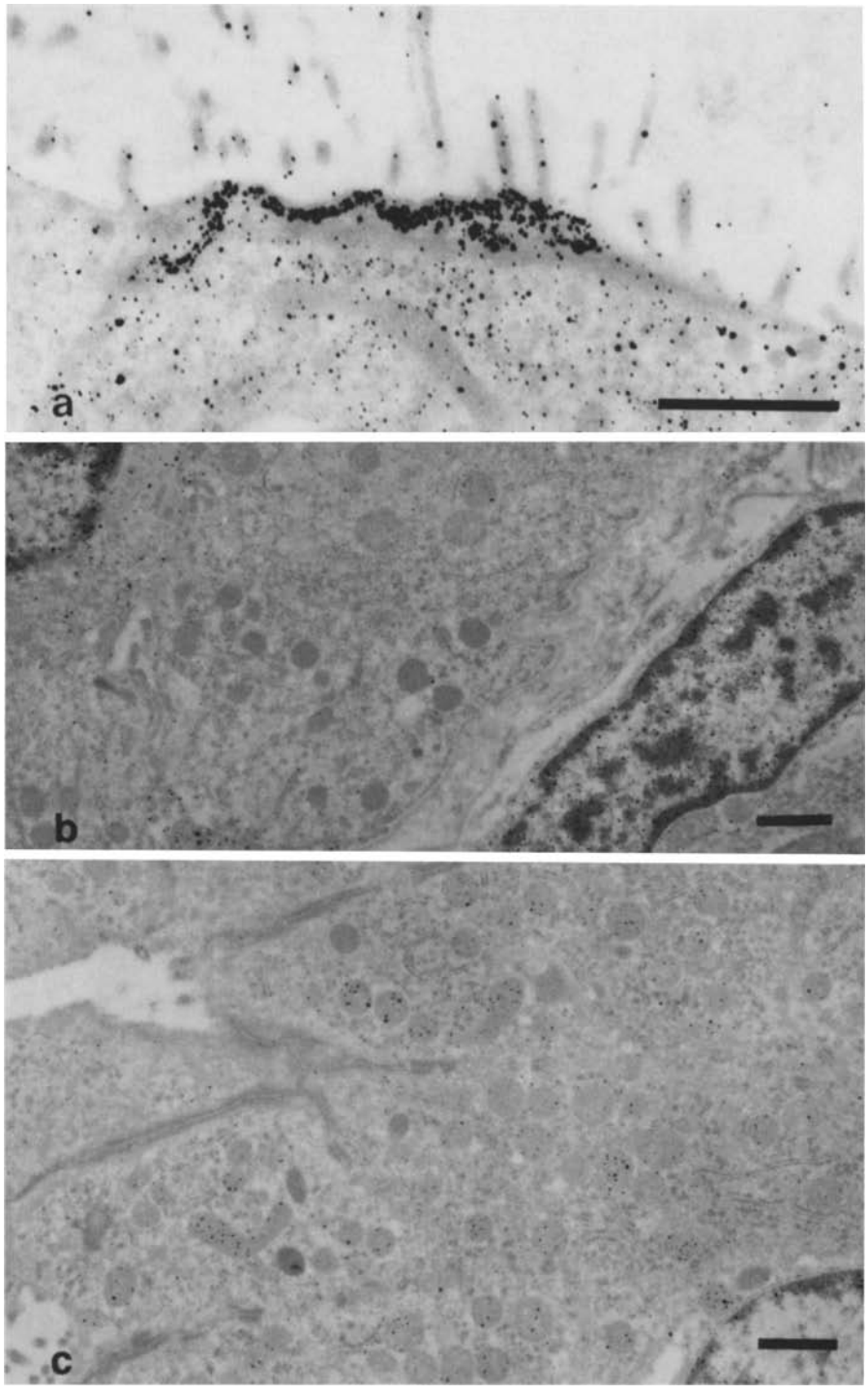

Fig. 5a, b, c. Silver deposits are seen on the luminal surface of the glandular cell (**a**) but not on round granules in the subnuclear region (**b**). A few silver grains deposit on the round granules in the supranuclear region. At left is the glandular lumen (**c**). (**a, b, c**) 2.5% glutaraldehyde fixed, Grimelius silver, uranyl acetate stain, (**a**) ×26,000, Bar 1 μ, (**b**) ×10,000, Bar 1 μ (**c**) ×10,000, Bar 1 μ

secretory granules, but this type of granule did not show argyrophilia in the silver-impregnated sections. Moreover, silver grains were deposited on the microvilli, brush borders and the mucinous globules of glandular cells. Therefore, the argyrophilia in glandular cells was thought to be nonspecific, having no relation to the endocrine type secretory granules. Many electron dense, round granules were seen in supranuclear or subnuclear regions, which showed mild argyrophilia, seemed to be exocrine secretory or lysosomal granules.

In conclusion, even in the normal endometrial glandular epithelia argyrophil cells are present and can be classified into two cell types, as in the endometrial glandular adenocarcinomas with argyrophil cells. Although the frequency and number of

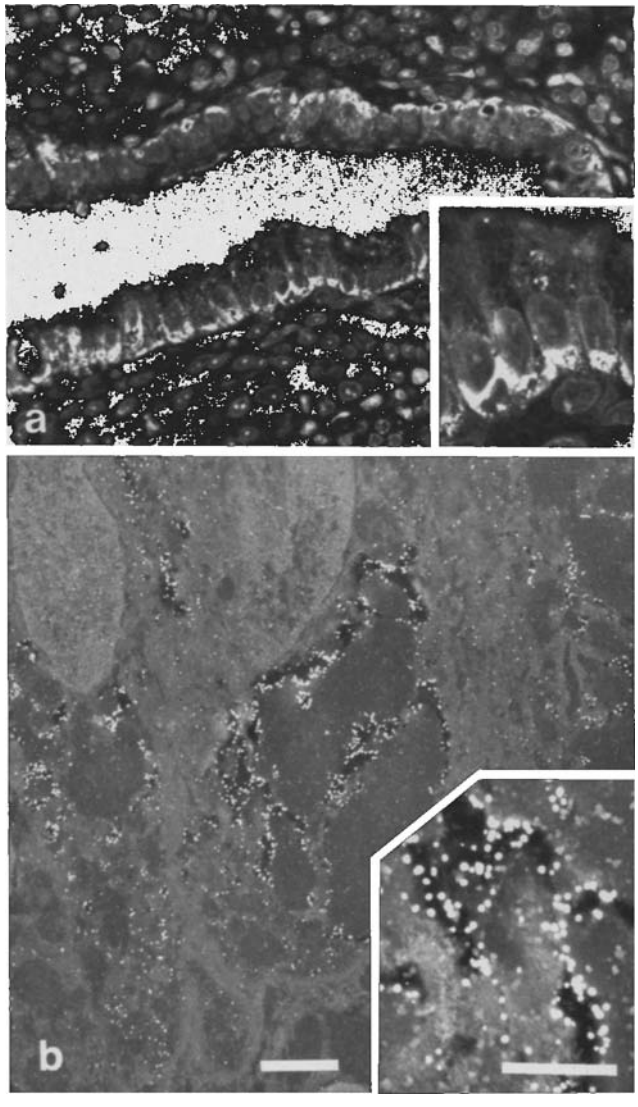

Fig. 6a, b. Light (**a**) and electron micrograph (**b**) of argyrophil granules seen in subnuclear regions, which were diagnosed as mucinous globules (**b**). (**a**) Grimelius stain, ×200, Inset: ×400 (**b**) 10% formalin fixed, Grimelius silver, reembedded in Epon, uranyl acetate stain, ×10,000, Bar 1 μ, Inset: ×30,000, Bar 0.5 μ

both endocrine cells and argyrophil glandular cells were much higher in endometrial glandular adenocarcinoma than in normal endometrium, they presumably increased during the course of neoplastic transformation.

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