

## Argyrophilic Cells in Carcinoma of the Female Breast

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**Summary.** Argyrophilic hormone storage granules were sought in the tissue specimens obtained from 52 benign breast lesions (13 normal breasts, 27 cases of fibrocystic disease, 9 fibroadenomas, 2 intraductal papillomas, and 3 cases of gynecomastia) and from 90 adenocarcinomas of the female breast. No argyrophilic cells were found in the normal breast tissue or in the benign lesions studied. In three of the carcinomas (3.3%) such granules were found in the tumor cells. Using electron microscopy, the argyrophilic granules were shown to be of moderate or high electron density with an average diameter of 165 to 170 nm. Ectopic hormone production was not observed clinically in any of these three patients. The absence of argyrophilic cells in normal and benign ductal and acinar epithelium, and their occasional presence in breast carcinomas favors the concept of the histogenesis of these cells through genomic derepression during the course of neoplastic transformation.

**Key words:** Breast carcinoma – Endocrine activity – Argyrophilic cells – Hormone granules.

### Introduction

Reports on the occurrence of argyrophilic granules in the cytoplasm of human breast cancer cells are few. Feyrter and Hartman (1963) found such granules in two colloid carcinomas showing carcinoid-like growth pattern, using the Bielschowsky-Gros method. Following this first report, argyrophilic granules have been described in 10 cases of breast carcinomas by Cubilla and Woodruff (1977). These authors classified their cases as carcinoid tumors on cytological and morphological grounds. Kaneko et al. (1978) described a male with bilateral

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breast carcinoids. Cohle et al. (1979) reported argyrophilic granules in a lobular carcinoma, and Azzopardi (1979) found such granules in the intraductal portion of an infiltrating breast carcinoma.

When breast carcinomas containing argyrophilic cells were studied using electron microscopy, electron dense hormone storage granules were found (Cubilla and Woodruff 1977; Kaneko et al. 1978; Cohle et al. 1979). In none of the 10 cases of carcinoid tumors were clinical signs of hormonal activity observed (Cubilla and Woodruff 1977). However, Kaneko et al. (1978) found increased noradrenalin secretion in their patient, and Cohle et al. (1979) reported an elevated ACTH secretion leading to Cushing's syndrome in their patient with immunohistochemically detectable ACTH present in her carcinoma cells. Feyrter and Hartman (1963) found minute amounts of 5-hydroxytryptamine (0.022 µg/g) in a breast carcinoma showing carcinoid-like growth in scattered areas. These authors also found in the literature one previous case of breast carcinoma with an increased urinary secretion of 5-hydroxyindoleacetic acid.

The origin of the argyrophilic cells in the neoplastic lesions of the human breast is unknown. It has been suggested that these cells are derived from the argyrophilic cells present in the normal ductal epithelium of the breast (Vogler 1947). This view was also held by Cubilla and Woodruff (1977), although they did not find argyrophilic cells in their 10 normal breast specimens or in four benign breast lesions. Feyrter and Hartman (1963) described argyrophilic granules in hyperplastic ductal lesions, and interpreted the positive cells as proliferating myoepithelial cells.

The present communication is the first systematic survey completed on the female breast carcinoma with special emphasis on the detection of the argyrophilic granules in the tumor cells. The aim was to obtain further insight into the hormonal activity of these tumors, and to make an attempt to clarify the biogenesis of the argyrophilic cells in breast carcinomas.

## Materials and Methods

The present series consists of 90 consecutive cases of female breast carcinomas submitted for study at the Department of Pathology, Jorvi Hospital, Espoo, during 1978 and 1979, and of 52 benign or normal breast specimens. The specimens were fixed in 10% neutral formalin, and processed for light microscopy according to routine procedures.

For the demonstration of the argyrophilic reaction, the technique of Grimelius (1968) was adopted. The presence of 5-hydroxytryptamine and catecholamines was assessed by utilizing the formaldehyde-induced fluorescence method (Björklund et al. 1972) in fresh tissue specimens frozen in liquid nitrogen followed by freeze-drying. The possible presence of a high tryptophan content in the hormone storage granules of the Grimelius-positive cases was studied with the combined formaldehyde and acetyl chloride vapour method (Partanen 1978). These and the formaldehyde-induced fluorescence specimens were studied with Leitz Orthomat fluorescence microscope fitted with the filter setting for catecholamine fluorescence. The coexistence of the reducing substances was demonstrated with Masson-Fontana ammoniacal silver (argentaffin) method.

For an electron microscopical study of the argyrophilic granules, representative pieces were cut from the paraffin blocks, deparaffinized in xylene and fixed in MacDowell's fixative overnight. Following a wash in veronal acetate-HCl buffer for 16 hours, the specimen was postfixed in 1% OsO<sub>4</sub> in collidine buffer for 1 h at +4°C. An en block-staining with 0.5% uranyl acetate in veronal acetate-HCl buffer was instituted, followed by embedding in Epon. The ultrathin sections were viewed and photographed with Jeol-JEM 100S electron microscope.

**Table 1.** Argyrophilic granules in the material studied

Type of the breast lesion	Number of cases	Number of Grimelius positive cases
<i>Female breast</i>		
Normal	5	0
Fibrocystic disease	27	0
Fibroadenoma	9	0
Aberrant breast	2	0
Intraductal papilloma	2	0
Lactating breast	4	0
Carcinoma		
ductal	83	3
lobular	4	0
medullary	1	0
apocrine	1	0
gelatinous	1	0
<i>Male breast</i>		
Gynecomastia	3	0

## Results

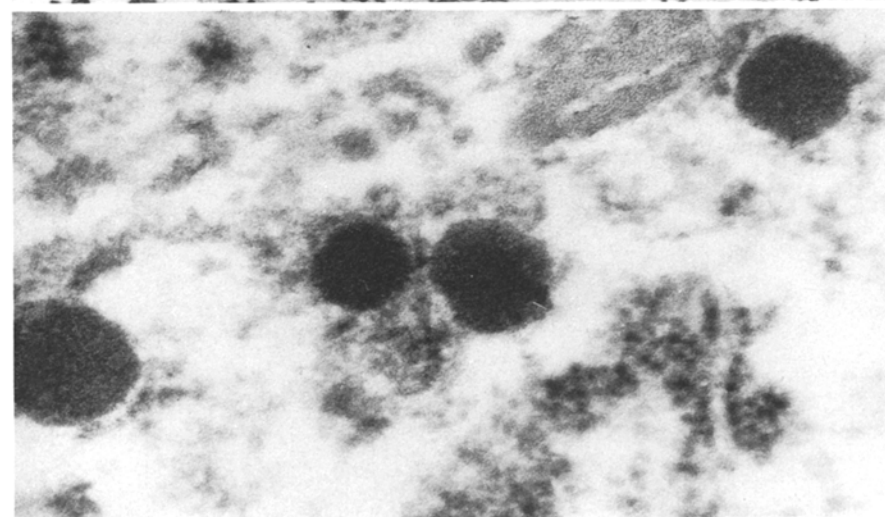
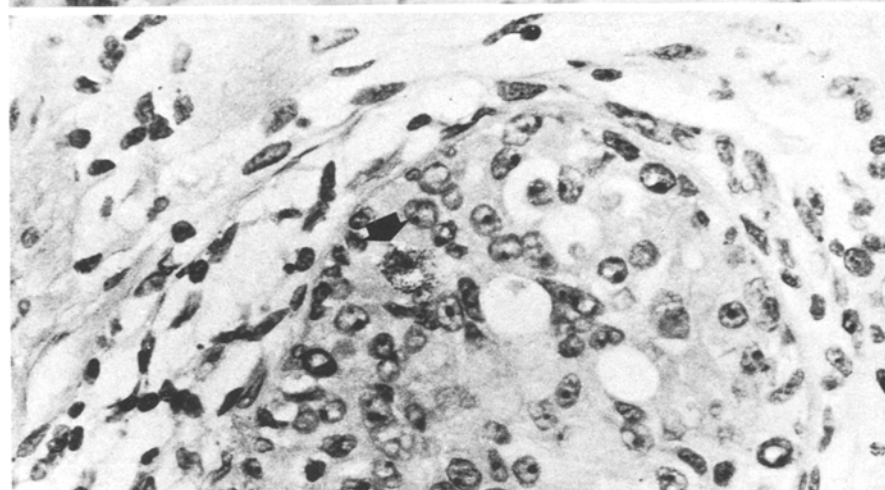
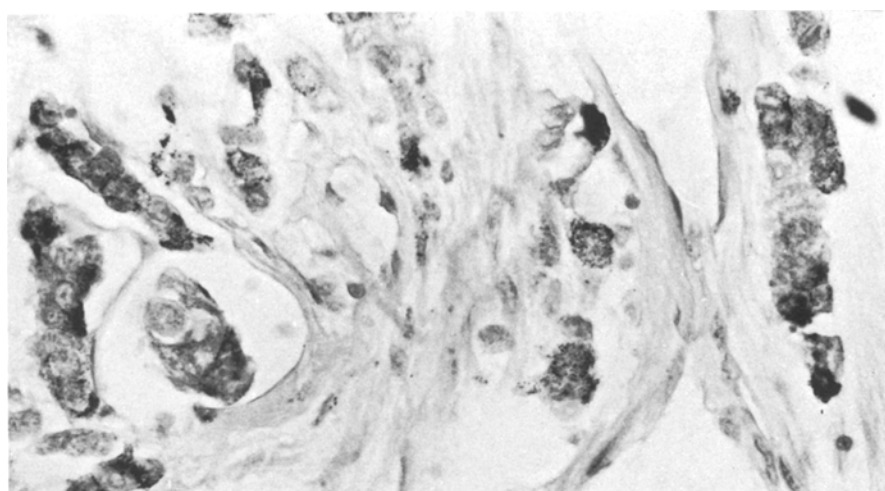
The results of Grimelius argyrophilic staining in the material studied are summarised in Table 1. All three carcinomas showing intracytoplasmic argyrophilic granules (Fig. 1) were histologically of the ductal type with irregular infiltrating borders and with their cells arranged in islands of varying size and shape between the dense collagenous stroma. In two cases (Cases 1 and 2), foci of intraductal growth were also found and in the latter case occasional argyrophilic cells were observed in that intraductal position (Fig. 2).

The results of the other histochemical reactions and the electron microscopical findings on these three carcinomas are depicted in Table 2. Dense intracytoplasmic hormone storage granules were detected in all three Grimelius positive cases (Fig. 3). Formaldehyde-induced fluorescence did not reveal any cells with

**Table 2.** Histochemical reactions and electron microscopical findings in the argyrophilic carcinomas

Case	Age	No of argyrophilic granules <sup>a</sup>	Argentaffin reaction positive	EM-granules present/average diameter (nm)	Combined fluorescence reaction	Axillary metastases/granules present in
1	32	++	—	+165	—	present +
2	51	+	—	+165	—	present —
3	56	+	—	+170	—	none

<sup>a</sup> + = occasional argyrophilic cells; ++ = argyrophilic cells regularly present, some clusters without granules only



specific fluorescence in the subareolar ductal epithelium of the normal breast, in the lesions of fibrocystic disease or in the three ductal carcinomas studied. In electron microscopy, tumor cells of all three carcinomas had an abundant rough endoplasmic reticulum and numerous ribosomes in the cytoplasm. Golgi complex was inconspicuous. In case 1, all the hormone storage granules were moderately electron dense, and contained no halo separating the membrane from the dense core. In case 2, moderately and highly electron dense granules (Fig. 3) were concomitantly present, as they were in case 3, where they were also devoid of the halo.

## Discussion

In the present study, no argyrophilic intracytoplasmic granules were observed in 7 specimens of the normal breast, in 4 lactating breasts and in 41 cases of benign breast lesions including three specimens of gynecomastia. This is consistent with the previous findings of Feyrter and Hartman (1963), and Cubilla and Woodruff (1977) in their 14 cases. Vogler (1947), Feyrter and Hartman (1963), and Azzopardi (1979) described argyrophilic granules in ductal cell proliferations, which according to the current opinions might be classified as lobular carcinomas or lobular in situ carcinomas (Haagensen et al. 1978).

Three of the 90 carcinomas of the female breast (3.3%) studied in the present work were shown to contain intracytoplasmic argyrophilic granules. One of the cases also disclosed such granules in the intraductal portion of an infiltrating carcinoma, which is an exceptional finding. Such a localization has been previously reported only twice; Cubilla and Woodruff (1977) described one case with concomitant intraductal carcinoma and carcinoïd, and Azzopardi (1979) found one case in bilateral infiltrating carcinoma.

Basing on the present and the previous findings, it seems probable that ductal linings of the normal breast do not contain argyrophilic cells. This is supported by the failure of the highly sensitive and specific formaldehyde-induced fluorescence technique to demonstrate any cells containing biogenic amines in the ductal linings of the breasts studied. This finding is in disagreement with that of Kaneko et al. (1978) who suggested the presence of such cells in the normal breast epithelium.

The presence of argyrophilic electron dense hormone storage granules in some lobular neoplasms, in infiltrating lobular and ductal carcinomas, and in all breast carcinoïds thus far studied suggests that in rare instances during

**Fig. 1.** Infiltrating ductal carcinoma cells clustered between the stromal dense collagen bundles in a 32-year-old woman. Intracytoplasmic argyrophilic granules are visible in many cancer cells. (Grimelius technique,  $\times 400$ )

**Fig. 2.** Infiltrating ductal carcinoma of a 51-year-old female demonstrating the differentiation of an argyrophilic cell in an intraductal position (*arrow*). (Grimelius technique,  $\times 400$ )

**Fig. 3.** An electron micrographic demonstration of the dense hormone storage granules in the cancer cells of the tumor shown in Fig. 1. (Original magnification  $\times 100,000$ )

the neoplastic transformation the breast epithelium acquires capacity to produce hormone storage granules, and most probably also physiologically active hormones. Due to the fact that the normal breast epithelium does not contain cells with endocrine granules, the biogenesis of these cells might occur through genomic derepression, thus differing from that of similar tumors (Apudomas) in most other organs (Pearse and Polak 1974). It has been suggested that argyrophilic lobular neoplasms are able to develop further into primary breast carcinoid tumors (Azzopardi 1979). As previously mentioned, Feyrter and Hartman (1963) have reported a case showing argyrophilic ductal proliferation and carcinoidlike colloid carcinoma concomitantly present in a lesion. Because the ductal carcinomas may contain argyrophilic granules, it is evident that in some rare instances difficulties are encountered in the classification of these tumors as ductal carcinomas or carcinoids. This difficulty is further accentuated by the well-known tendency to polymorphous growth patterns of breast carcinoma.

When breast carcinomas are considered in the framework of the derepression theory of the argyrophilic cells discussed above, some cases would also be expected to contain argentaffin cells if 5-hydroxytryptamine or catecholamines are produced. Indeed, one such case was reported by Dewitt (1978), although the reaction was not specified. Furthermore, in one case 5-HT was possibly found biochemically (Feyrter and Hartman 1963). For some obscure reason, the argentaffin reaction was negative in a case of bilateral carcinoid most probably secreting noradrenalin (Kaneko et al. 1978).

Although hormone storage granules have been demonstrated in breast carcinomas, clinically overt syndromes of ectopic hormone production have not been observed, except in one case, where an infiltrating lobular carcinoma produced ACTH (Feyrter and Hartman 1963; Cubilla and Woodruff 1977; Kaneko et al. 1978; Cohle et al. 1979). Electron microscopy does not give any clue to the hormone type. Grimelius reaction stains the limiting membrane of certain types of endocrine cells (Bussolati et al. 1971). Combined formaldehyde and acetyl chloride vapour demonstrates granules with a high tryptophan content, and granules in cells storing gastrin, glucagon and ACTH have been revealed by this method (Partanen 1978). Thus, in the present work, where this staining was negative, the hormones mentioned above can be excluded with high degree of certainty. Human breast carcinomas have been shown to synthesize and secrete human chorionic gonadotrophin and calcitonin for example, the latter being produced by cancer cells in tissue culture in over half of the cases (Coombes et al. 1975). Both hormones are normally stored in typical electron dense granules to which the argyrophilia of thyroid medullary carcinoma is to be attributed (Sundler et al. 1974). It is possible that the cytoplasmic granules in the different types of breast carcinomas store calcitonin.

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