Chromogranin-Reactive Endocrine Cells in Argyrophilic Carcinomas ("Carcinoids") and Normal Tissue of the Breast

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Breast carcinomas, either positive or negative with the Grimelius' silver procedure, benign fibroadenomas, duct papillomas, and areas of histologically normal breast tissue were tested immunocytochemically with the mouse monoclonal antibody LK2H10 directed against human chromogranin. This is regarded as a general stain for polypeptide-hormone–producing cells and tumors. In 3 of the 9 cases of argyrophilic carcinoma, but in none of 12 ductal infiltrating carcinomas, chromogranin-positive cells were found: the number of reactive cells was very low in 1 case, while in the other 2 carcinomas about 50% of the argyrophilic cells appeared stained. In areas of histologically normal breast tissue, rare argyrophilic chromogranin-positive cells were detected. This study is the first reported evidence concerning the presence of endocrinelike cells probably belonging to the diffuse neuroendocrine system in the normal mammary parenchyma. Our data are consistent with the endocrine nature of at least some of the breast argyrophilic carcinomas. (Am J Pathol 1985, 120:186–192)

FEYRTER AND HARTMANN[1] first suggested, on the basis of silver impregnation procedures, the endocrine nature of mucoid carcinomas ("carcinoids") of the human breast and of some basally located cells in the ductal system. They considered such cells part of the diffuse endocrine (Hellen Zellen) system. However, they were describing myoepithelial cells, which are not endocrine, as later shown by several histochemical and electron-microscopic studies.[2] Feyrter's observations[1] were dismissed; and, despite further investigations,[3,4] no further evidence of breast tissue endocrine cells appeared.

Recent reports[4–11] described a variety of argyrophilic breast tumors containing dense-core secretory granules and showing the typical features of carcinoids. The term "argyrophilic carcinomas" was suggested.[11] The endocrine nature of these tumors remains hypothetical.[12] Taxy et al[9] and Azzopardi et al[11] pointed out that no conclusive evidence has been presented on hormonal production. In addition, Clayton and co-workers[13] demonstrated that some of these breast argyrophilic carcinomas produce alpha-lactalbumin.

A monoclonal antibody has recently been raised against a human pheochromocytoma and found to react with a variety of endocrine tumors and cells in normal organs, while unreactive with nonendocrine tissues.[14,15] The antigen detected by this antibody has been associated with cytoplasmic dense-core "secretory" granules, and its target structure has been identified as human chromogranin.

Because the antibody is a general stain for polypeptide-hormone–producing cells and tumors,[14,15] we tested it against normal human breast epithelium and against breast carcinomas, mainly of the argyrophilic variety.

Materials and Methods

Human adult female breast specimens fixed in Bouin's fluid and routinely embedded in paraffin were employed. The following tissues were investigated: 1) histologically normal breast tissue, removed for aesthetic reasons (2 cases), or from mastectomies, taken

Supported by grants from the Italian C.N.R. (Rome) (84.00478.44), from the A.I.R.C. (Milan), and from M.P.I. (Rome).

Accepted for publication March 8, 1985.

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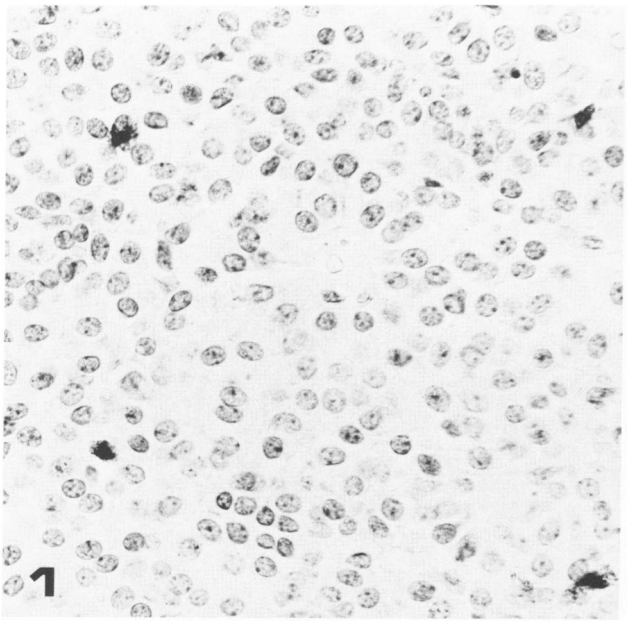


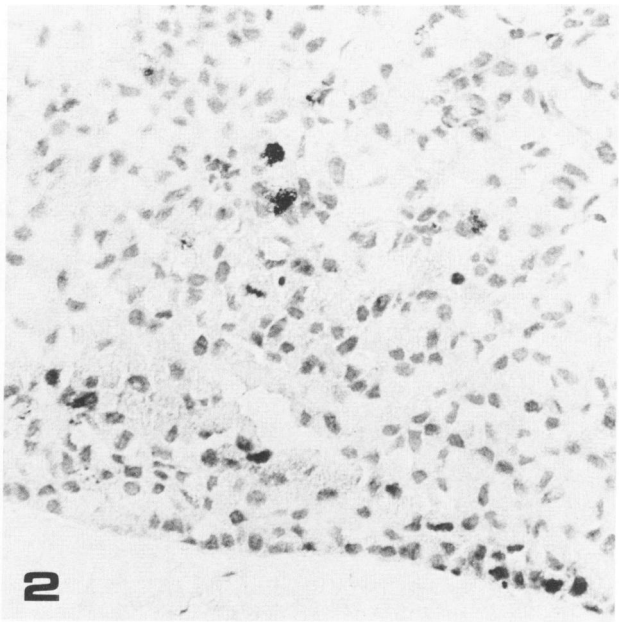


Figure 1—Case of breast argyrophilic carcinoma. Anti-chromogranin antibody immunoperoxidase staining reveals very few scattered cells showing a marked cytoplasmic reaction. (Nuclei counterstained with hemalum, ×250) **Figure 2**—Mucinous breast carcinoma. Most neoplastic cells contain mucin, as shown by selective staining procedures on parallel sections; scattered cells were argyrophilic (Grimelius procedure). Chromogranin staining reveals positive cells, distributed as are the argyrophilic cells. (×250)

far from the carcinomatous areas (25 cases); 2) areas of epitheliosis (papillomatosis) in cases of cystic disease (8 cases); 3) benign breast fibroadenomas (3 cases) and duct papillomas (6 cases); 4) ductal infiltrating carcinomas of the most common, not otherwise specified, variety (12 cases); 5) argyrophilic carcinomas (3 mucinous, 6 carcinoid-like), positive by the Grimelius[16] staining procedure. The histologic features of these tumors conform to those described in detail by other authors.[6,7,11] We adopted the Grimelius method,[16] and 30–70% of the neoplastic cells were positive.

Sections were hydrated, treated with the H_2O_2–periodic acid–sodium borohydride sequence[17] for elimination of endogenous peroxidase, and then processed by the immunoperoxidase procedure. The avidin–biotin–peroxidase complex (ABC) procedure[18] was employed with a minor modification so that we could avoid nonspecific staining.[19]

The mouse monoclonal antibody LK2H10 was employed as previously described[14] at a 1:50 dilution of the culture supernatant.

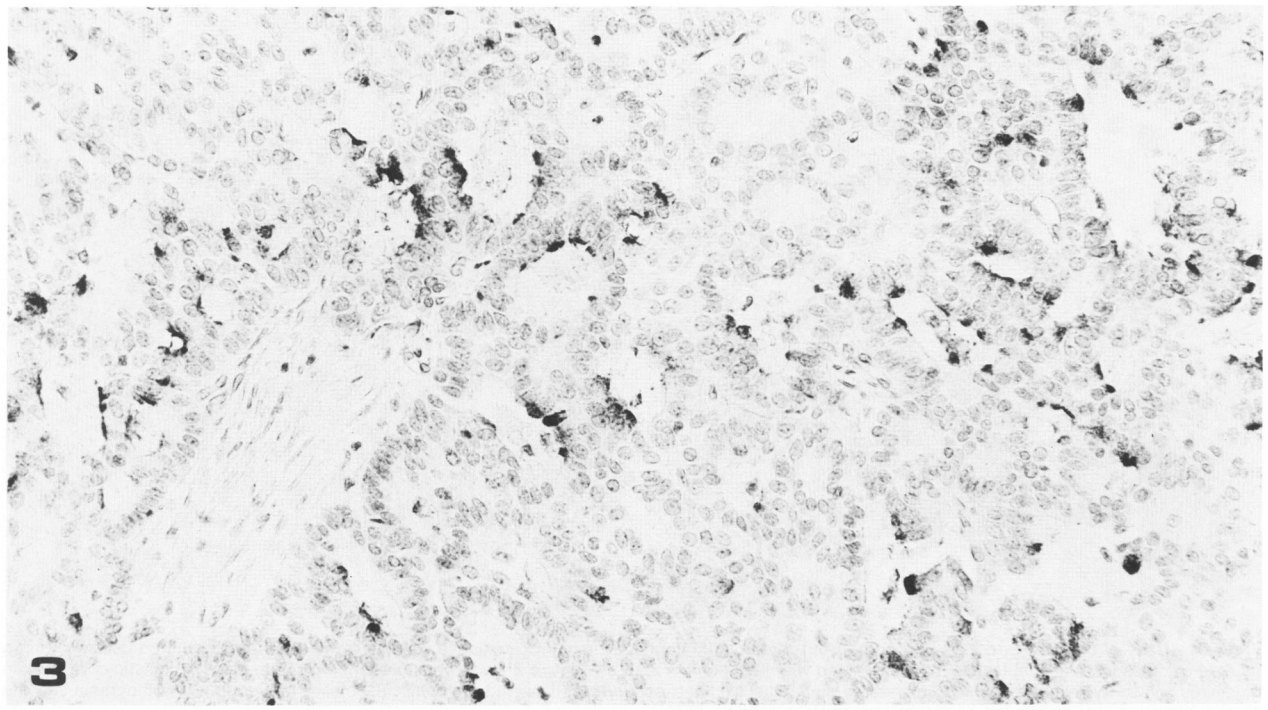
In those cases of histologically normal breast tissue which were positive to the monoclonal LK2H10, serial sections were cut. These were then stained with the Grimelius silver procedure[16] and with immunohistochemical procedures to test the presence of polypeptide hormones. The following antisera were employed: anti-calcitonin (Ortho Diagnostic Systems, Raritan, NJ), kit dilution; anti-somatostatin (Ortho), kit dilution; anti-bombesin (Milab, Malmö, Sweden), diluted 1:5000; anti-ACTH (Milab), diluted 1:900; anti-insulin (Ortho), kit dilution; anti-glucagon (Ortho), kit dilution; anti-gastrin (Milab), diluted 1:1280; anti-VIP (Milab), diluted 1:1250; anti-GIP (Milab), diluted 1:320; anti-prealbumin (Atab Atlantic Antibodies, Scarborough, Maine), diluted 1:1000; anti-cerulein (kindly supplied by Susan van Noorden, London), diluted 1:100.

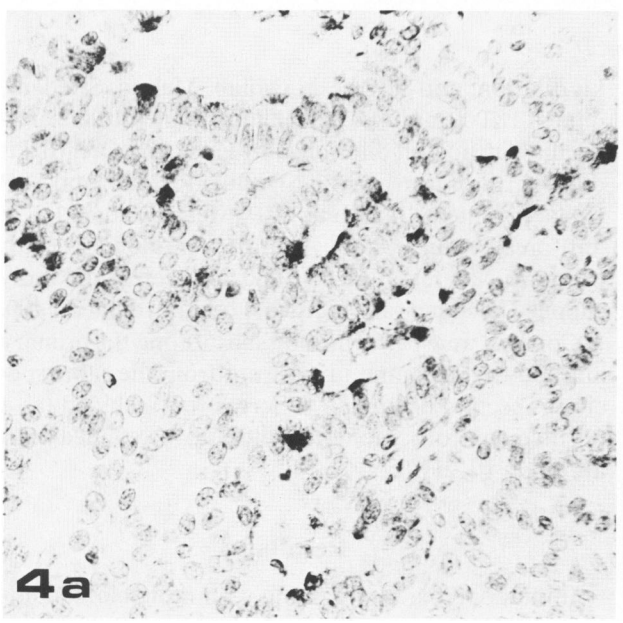
Controls were performed by substituting the primary antibody with nonimmune serum from the same species. A section of human pancreas was included (as a positive control) among the slides to be stained with antibody LK2H10.

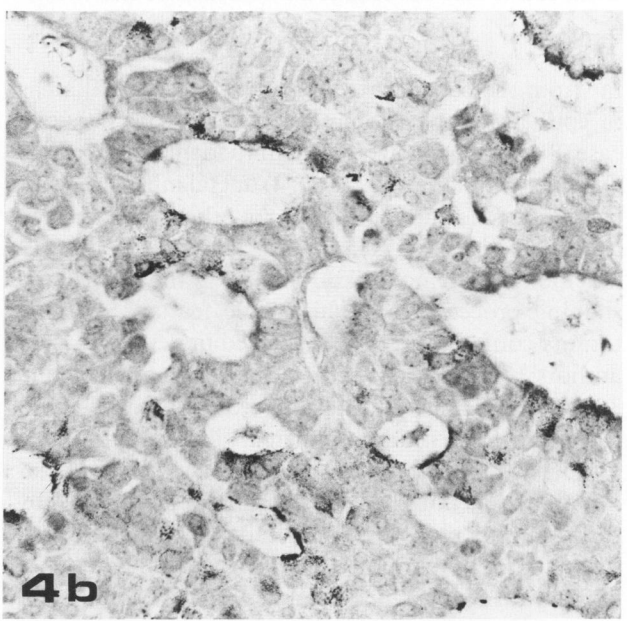
Results

Immunoperoxidase staining with monoclonal antibody LK2H10 was positive in 3 of 9 argyrophilic breast tumors. One was mucinous, and two were of the solid variety. In one tumor (Figure 1), only very few cells (<1%) reacted with the anti-chromogranin antibody, while in the other two about 50% of the argyrophilic cells appeared stained (Figures 2, 3, and 4a).

Staining with the antibody was finely granular and evenly distributed in the cytoplasm. In areas showing ribbonlike patterns and pseudoglandular structures, staining appeared mainly at one border, often the "luminal" one (Figures 3 and 4a). A similar pattern was noticed with silver staining on serial sections (Figure







Figures 3 and 4—Carcinoidlike argyrophilic carcinoma of the breast with solid and pseudoglandular patterns. The immunoperoxidase reaction for chromogranin (Figures 3 and 4a) is strongly positive in some neoplastic elements. The finely granular cytoplasmic reaction is mainly localized at one border. Nuclei counterstained with hemalum. The Grimelius method (Figure 4b) on a parallel section shows silver deposits with a distribution similar to that of chromogranin. (Nuclei counterstained with methyl green; **Figure 3**, ×100; **Figure 4a** and **b**, ×250)

4b), and the number of cells stained was higher than the number stained with the antibody.

All infiltrating ductal carcinomas, fibroadenomas, and papillomas were immunocytochemically negative. On serial sections they were also found unreactive by the Grimelius procedure.

Chromogranin-reactive (Ch) cells were found in histologically normal breast tissue from 2 mastectomy cases and from 1 case where the operation had been done for aesthetic reasons. Cases of cystic disease and benign papillomas were negative as well as tissue blocks from subareolar areas and nipple. Only 7 out of 66 nor-

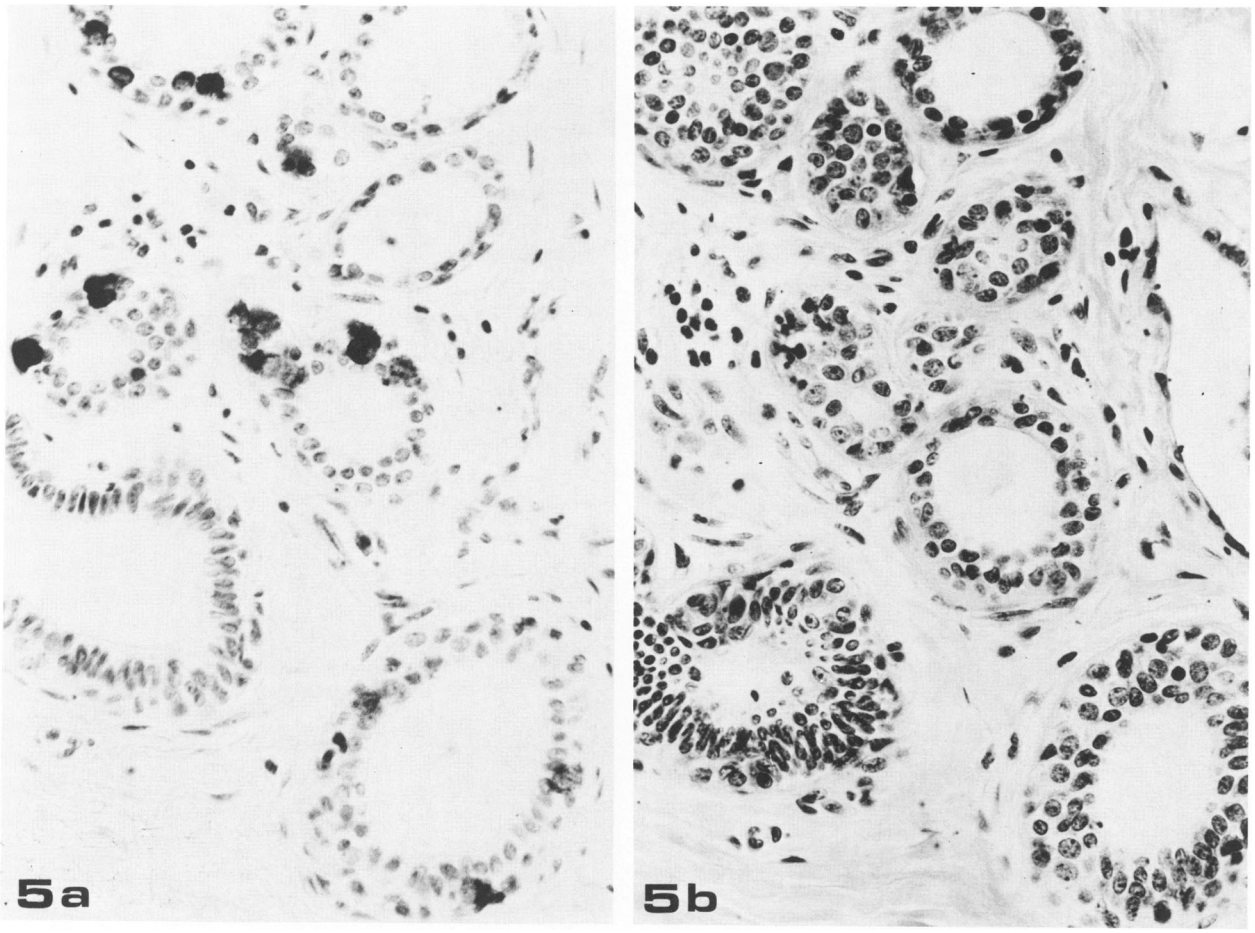


Figure 5—Histologically normal human breast tissue. **a**—Chromogranin-positive (Ch) cells (**a**) are present in lobular ductules, prevalent between the basal myoepithelial and luminal epithelial cells, which are both negative. **b**—A parallel section of the same lobule. (H&E, ×250)

mal breast tissue blocks stained positively for the antibody, and each of the 7 blocks had only scattered Ch cells, the total number being only about 300. Sometimes they were isolated or, more often, focally localized in some lobular ductules or intralobular and interlobular ducts. The cell bodies appeared between the basal myoepithelial and luminal epithelial cells, which were chromogranin-negative (Figure 5). The Ch cells were globoid or more often cylindrical or stellate, with slender cytoplasmic projections toward the basement membrane. The nucleus was round to oval. In some sections these cells reached the luminal border. The cytoplasm was finely granular, and the amount of granular staining varied from cell to cell. On parallel sections stained by the Grimelius procedure, cells showing a definite, although rather weak, argyrophilic staining were detected. These cells corresponded in number, distribution, and shape to the Ch cells, thus supporting the interpretation that the Ch cells are argyrophilic (Figures 6a and b).

All the other immunocytochemical reactions gave uniformly negative results.

Discussion

The existence of breast endocrine tumors has raised much interest and discussion. Whether argyrophilia and cytoplasmic dense-core bodies ("secretory granules") are sufficient evidence of the endocrine nature of a tumor is a debated point.

Taxy et al[9] found focal argyrophilia in a high proportion of the more common type of breast carcinomas and questioned the specificity of silver staining as a marker of endocrine differentiation and even the existence of breast carcinoids.

Azzopardi and co-workers,[11] in an extensive investigation, found only 5% of conventional infiltrating carcinomas to be argyrophilic by the Grimelius procedure, employed under strict technical conditions; they could, however, not produce any conclusive evidence of their endocrine nature.

Rare reports have appeared in literature on breast hormone-producing endocrine tumors: Kaneko et al[3] and Gould and Chejfec[20] demonstrated biochemically

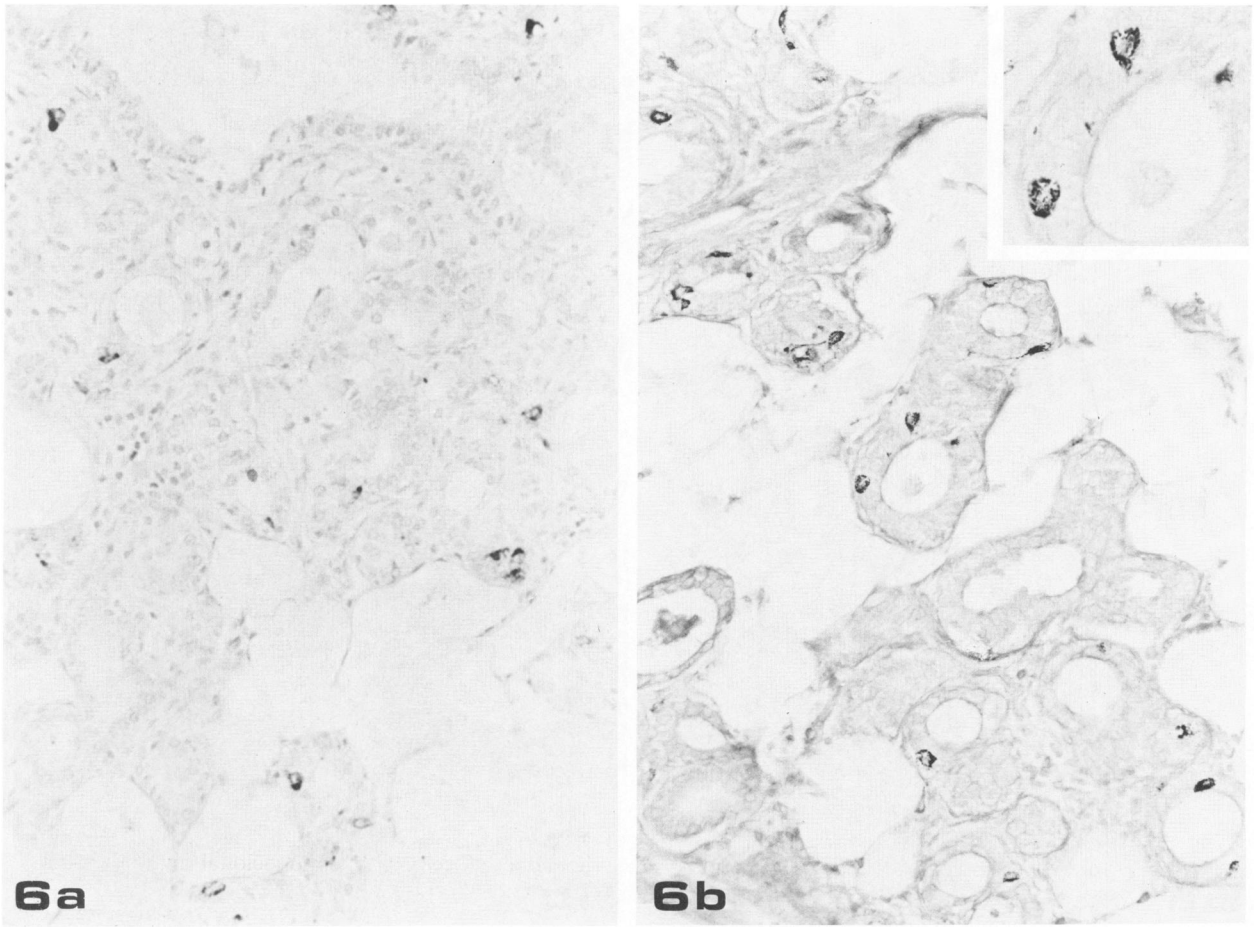


Figure 6—A lobule of histologically normal breast tissue stained, on parallel sections, for chromogranin (**a**) and with the Grimelius silver method (**b**). The Ch cells correspond in shape and distribution to the argyrophilic cells. The **inset** at higher magnification of one ductule shows the globoid or flasklike shape of these cells. Other cell types are negative. (×100; **inset**, ×250)

catecholamine production in breast tumors. Ectopic ACTH production was reported by Cohle et al[21] in a lobular carcinoma and in a ductal mammary carcinoma by Woodard et al.[22] Calcitonin production was demonstrated in a series of breast carcinomas[23]; interestingly, at least 1 case was mucoid. Unfortunately, these tumors were not studied by silver impregnation procedures, and it is therefore difficult to make comparisons with later studies on argyrophilic breast carcinomas.

Clayton et al[13] related the argyrophilia in breast tumors to the immunohistochemical staining for alpha-lactalbumin; this would exclude the endocrine nature of such tumors. Recently, however, Lee et al,[24] using the same anti-human alpha-lactalbumin serum, reported unexpected pancreatic endocrine cell staining. Additional studies are needed before the reaction with this particular anti-alpha-lactalbumin serum can be accepted as evidence of pure exocrine lactational activity.

Our data support the endocrine nature of some breast argyrophilic carcinomas. The monoclonal antibody LK2H10 reaction with chromogranin is a specific test for endocrine differentiation, as shown by extensive investigations in a variety of tumors and normal endocrine cells in different organs.[14] Wilson and Lloyd,[15] while studying the reactivity of a large series of nonendocrine tissues and tumors with monoclonal antibody LK2H10, also studied 3 cases of breast carcinoma and 2 cases of normal mammary tissue and found they were all negative. Their results are not in contradiction with the data presented in this study, given the rarity of the Ch cells in normal breast tissue.

In breast carcinomas, on parallel sections, we noticed a discrepancy between results obtained with the reaction for chromogranin and with argyrophilic Grimelius staining. Only a part of the argyrophilic tumors (3 of 9) were immunocytochemically positive. This finding seems to offer two alternative interpretations: argyrophilia in a part of breast tumors might be related to other factors of nonendocrine nature; this interpretation would agree with the suggestion of Azzopardi

et al[11] that breast argyrophilic carcinomas form a nonhomogeneous spectrum of tumors. Alternatively, these breast tumors might be multihormonal, a property known to be common to most neuroendocrine neoplasms,[25] only a part of the endocrine cells being chromogranin-reactive. Staining with anti-chromogranin antibody is negative[15] in poorly granulated endocrine tumors; this seems, however, hardly the explanation for our chromogranin-negative argyrophilic breast carcinomas, because the Grimelius silver staining also is related[7] to cytoplasmic secretory granules.

The histogenesis of breast argyrophilic carcinomas has also been under discussion[9,12,26] because in normal breast parenchyma endocrine cells have never been demonstrated previously. We believe that besides secretory epithelial and myoepithelial cells, an endocrine-like argyrophilic chromogranin cell type is normally present. Although its hormonal product has still to be defined, in the light of the presented evidence it should be included in the series of endocrine cells of the diffuse neuroendocrine system (DNES).[27] Whether it contains chromogranin only or, as in the case of most of DNES cells, it produces some other specific polypeptide, cannot be said at present. All the tests we have performed with numerous anti-polypeptide hormone antibodies and with anti-prealbumin sera, known to cross-react with families of prohormones,[28] were negative.

The chromogranin-reactive argyrophilic cells demonstrated in this study in the normal breast ducts and ductules were few in number, focally located in sparce "endocrine ductule–lobular units." This might explain the failure of other workers[3,4] to detect them despite careful search. It should be mentioned that in some adult human tissues such as the bronchi[29,30] endocrine cells are very scanty. Bronchial endocrine cells are, however, frequent in human fetuses.[31] An extensive study in rudiments of mammary gland from fetuses and infants of different ages to test the presence of chromogranin-reactive endocrine cells would be worthwhile.

The importance of the present findings in mammary pathology remains to be fully exploited, and further studies are needed to ascertain the incidence of Ch cells, especially in tumor pathology. Small-cell neuroendocrine (oat-cell) breast carcinomas have recently been described[32,33] as a variant of mammary carcinoids.

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Acknowledgments

The authors greatly appreciated the secretarial help of Miss Monica Musso and the assistance of Mr. Nino Ferraro in obtaining photomicrographs. Dr. Michael Moster, of the University of Turin, kindly reviewed the manuscript.