Neuroendocrine Cells in Dysplastic Bronchi

Ultrastructural Observations and Quantitative Analysis of Secretory Granules and the Golgi Complex

Victor E. Gould, MD, Aristomenis D. Yannopoulos, BS, Sheldon C. Sommers, MD, and John A. Terzakis, MD

Ultrastructural and biochemical studies have suggested that bronchopulmonary carcinoids and oat cell carcinomas may be derivatives of neuroendocrine cells; their amine and/or peptide secretory capabilities may at times be reflected in clinical hormonal syndromes. This investigation was prompted by the hypothesis that dysplastic neuroendocrine bronchial cells may also exhibit structural and functional aberrations of their secretory apparatus. Surgical specimen samples from 5 human dysplastic bronchi were studied ultrastructurally; 7 normal bronchi served as controls. Golgi complexes of dysplastic cells were distinctly less prominent than those of the controls. Moreover, the Golgi vesicles of dysplastic cells appeared significantly smaller than their counterparts in normal cells ($P < 0.01$). Also, dysplastic neuroendocrine cells displayed significantly fewer secretory granules per cell than the controls ($P < 0.05$). These findings indicate structural abnormalities in the secretory apparatus of neuroendocrine cells in dysplastic bronchi and correlate with experimental observations of aberrant hormonal production associated with bronchial dysplasia. Thus, the possibility arises that bronchial epithelial dysplasias may be detected and monitored through laboratory determinations of their secretory products. (Am J Pathol 90:49–56, 1978)

THE PRESENCE OF NEUROENDOCRINE CELLS in the tracheobronchial tree is well established as is the basic structural and functional relationship between those cells and the enterochromaffin and APUD systems.[1-3] The actual functional significance of the tracheobronchial neuroendocrine cells in adult mammals is as yet unclear.[1-4] However, it has become clear that neoplasms derived from those cells, ie, carcinoids and oat cell carcinomas may produce detectable amine and/or peptide materials which at times may be responsible for important clinical hormonal manifestations.[5] The intriguing observation that "dysplastic bronchial epithelium" was associated with demonstrable levels of a prohormone (Big-ACTH)[6] suggested that nonneoplastic and possibly preneoplastic bronchial neuroendocrine cells may be the locus of demonstrable structural alterations of their secretory apparatus. This provocative

From the Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, and the Departments of Pathology, Lenox Hill Hospital and Columbia University College of Physicians and Surgeons, New York, New York.

Supported in part by the Council for Tobacco Research USA and the Otho S.A. Sprague Memorial Institute.

Accepted for publication August 19, 1977.

Address reprint requests to Dr. Victor E. Gould, Department of Pathology, Rush Medical College, 1753 West Congress Parkway, Chicago, IL 60612.

notion prompted us to undertake this ultrastructural investigation on the qualitative and quantitative features of the secretory granules and the Golgi complex of neuroendocrine cells in dysplastic bronchial epithelium and to compare the findings with those in their normal counterparts. A preliminary report on this investigation has been recently published.[7]

Materials and Methods

A series of electron microscopic studies had previously been made for investigation of the alterations with aging.[4] The material comprised all suitably preserved, surgically resected lung specimens obtained during a 3-year period. It comprised material from 5 patients with areas of dysplastic bronchial epithelium and from 7 patients with histologically normal bronchial epithelium, which served as controls; the groups were matched for age and sex. The basic pathologic diagnosis in the patients with bronchial dysplasia included 1 case of moderately and 1 of poorly differentiated adenocarcinoma, 1 of undifferentiated small cell carcinoma, and 2 of extensive bronchial epithelial hyperplasia with multiple tumorlets. The 7 control patients included 3 with bronchoalveolar carcinomas, 1 with papillary exophytic squamous cell carcinoma, 1 with caseating pulmonary tuberculosis, 1 with chronic bronchitis and bronchiectasis, and 1 with lipid pneumonia. Multiple sections of bronchi from areas uninvolved by the basic process were obtained and, although bronchial epithelial dysplasia was present in the experimental group, the normal bronchial epithelial architecture was retained by the controls. Bronchial dysplasia was defined as epithelial hyperplasia of basal cell type, moderate loss of polarity, nuclear pleomorphism and hyperchromasia, without the presence of differentiated ciliated or nonciliated cells. Mitoses were restricted to the basal levels of the epithelium. The degree of bronchial dysplasia varied within the experimental group and within individual cases; however, the severity of the changes was invariably lower than the description of bronchial carcinoma *in situ* as recently outlined.[8]

A minimum of 30 neuroendocrine cells per case were examined ultrastructurally. The diameters of all available Golgi vesicles and cisternae were measured on photographic prints at magnifications that averaged 28,400 for the normal and 28,100 for the dysplastic cases. An average of 62.2 Golgi vesicles and 21.9 cisternae were measured in the specimens from normal individuals; an average of 25.8 vesicles and 17.5 cisternae were measured in the specimens from patients with dysplasia. The numbers and size distributions of the neurosecretory granules were also determined. Comparisons of the normal and dysplastic groups were made using the statistical Mann-Whitney test.[9]

Results

The Golgi complexes appeared consistently more conspicuous in the control neuroendocrine cells than in their dysplastic counterparts. Comparision of Golgi vesicle diameters in the neurosecretory cells of dysplastic and normal bronchial epithelium showed the vesicles to be significantly smaller in the dysplastic cases ($P < 0.01$ by the Mann-Whitney test). The mean diameters of the dysplastic vesicles were 145.5 nm and 399.1 nm for the normal group (Figures 1 and 2). The distribution of Golgi vesicle diameters is shown in Table 1. The cisternal mean diameters were 368.3 nm for the dysplastic cases and 485.1 nm for the normal cases. No signif-

Table 1—Distribution of Golgi Vesicle Diameters (Mean Percentages)

Diameter (nm)	Dysplasia (%)	Controls (%)
<100	14.2	0.4
100–200	32.0	18.6
200–300	26.2	41.1
>300	37.6	39.9

icant statistical difference in Golgi cisternae sizes was found between the two groups.

The average sizes and distribution of sizes of neurosecretory granules did not differ significantly in the dysplastic and normal epithelial cells. The mean sizes were 123.1 nm ± 29.3 nm for the dysplastic group and 105.2 nm ± 31.6 nm for the normal group. The size distributions of the granules are listed in Table 2.

However, dysplastic cases had significantly fewer neurosecretory granules per cell ($P < 0.05$ by the Mann-Whitney test) (Figures 3 and 4). The means and standard deviations were 33.9 ± 24.7 granules in the dysplastic cases and 71.3 ± 31.1 in the normal cases. The numbers of secretory granules per (averaged) cell per case are listed in Table 3. The granules' core density was distinctly lower and more variable in the dysplastic cells.

Discussion

Neuroendocrine cells from 5 patients with bronchial dysplasia and 7 normal controls were studied ultrastructurally. The diameters of the Golgi cisternae and the sizes of the secretory granules were not appreciably different from those of the normal controls. However, dysplastic neuroendocrine cells had less conspicuous Golgi complexes and significantly smaller Golgi vesicles than those of normal cells ($P < 0.01$). Significantly fewer secretory granules were present in dysplastic cells than in the controls ($P < 0.05$) and their electron density varied considerably.

The tracheobronchial epithelium includes cells with granules capable

Table 2—Size Distribution of the Neurosecretory Granules

	Dysplasia (%)	Controls (%)
<90 nm	34.8	48.1
90–120 nm	55.0	38.8
120–170 nm	8.7	10.3
>170 nm	1.5	2.8

Table 3—Numbers of Secretory Granule per (Averaged) Cell per Case

	Dysplasia	Controls
10–20	1	0
20–49	3	2
50–89	1	3
90–120	0	2

of different degrees of reactivity with silver salts. Given the known derivation of the tracheobronchial tree from the foregut, the relationship of these cells with the enterochromaffin system was inferred. It was also suspected that both groups of cells were part of a complex and highly dispersed endocrine system.[10] However, only during the past decade has it become apparent that both cell groups, as well as many others, were part of a neuroectodermally derived endocrine system with common biosynthetic pathways capable of producing amine and peptide hormones. Based on their cytochemical and ultrastructural characteristics, all of those cells are encompassed within the APUD system.[1,11,12]

Tracheobronchial neoplasms thought to be derivatives of APUD cells include carcinoids, most if not all small (oat) cell carcinomas, and other less well-defined neuroendocrinomas.[2,3,5,13,14] Although some of these neoplasms may produce "ectopic" hormones that result in clinical syndromes, many more may contain neurosecretory granules in the absence of clinically apparent hormonal activity.[3,5] This apparent discrepancy may be explained as follows: a) secretory materials may be produced in insufficient amounts to stimulate the target organs, b) the secreted material may not be extruded from the cells due to failure of the microtubular-filamentous system, and c) neoplastic cells may secrete aberrant materials incapable of provoking target organ responses.[5]

The secretory process of normal and neoplastic neuroendocrine cells includes several sequential steps: a) the proper message from the nucleus, b) secretion initiation within the cisternae of the rough endoplasmic reticulum, c) concentration, sugar addition, if any, and acquisition of the membrane within the Golgi complex, d) maturation and intracytoplasmic transport via the microtubular-filamentous system, and e) granule ejection by emiocytosis.[15,16] In neoplastic cells such a complex mechanism may be arrested or deviated at any point, resulting in the production of unusual materials[3] and not seldom multiple hormone-like substances which may be synchronously or asynchronously produced.[17] Biochemically, most, if not all, hormones are initially produced as part of a larger, inactive complex (prohormone) that is subsequently trypsinized, liberating the active hor-

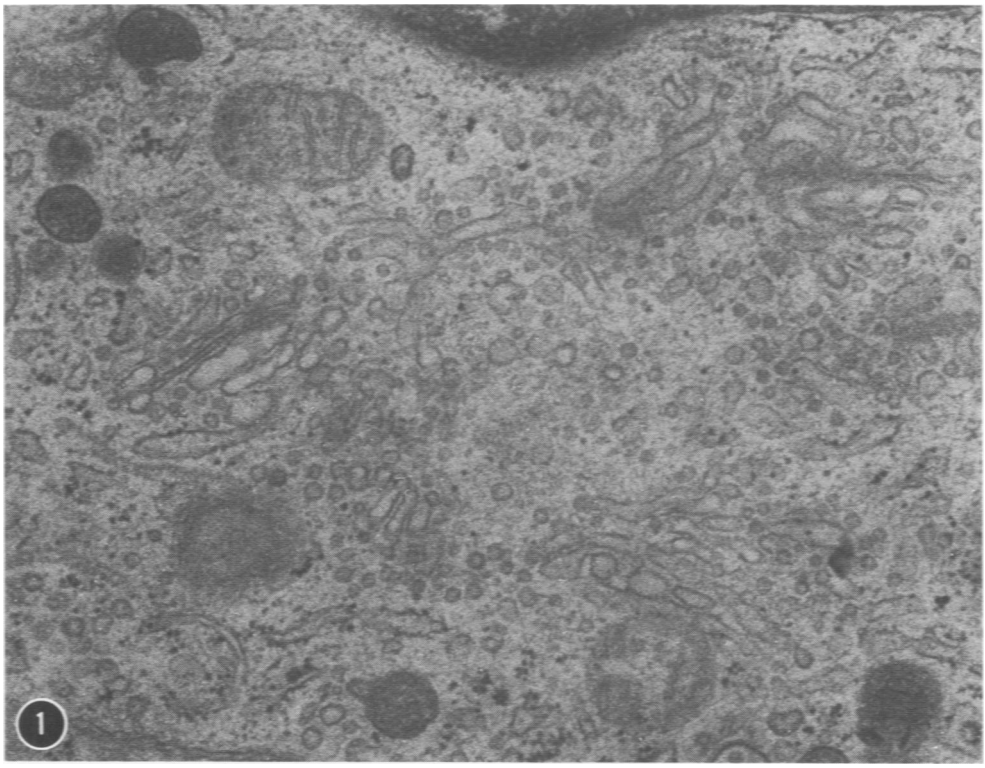
mone; these studies have led to the concepts of proinsulin, progastrin, and pro (big) ACTH.[18-22] Several studies have also suggested that neoplastic neuroendocrine elements may produce predominantly prohormones but, due to lack of the crucial converting enzyme system, may be clinically hormonally "silent." [3] These biochemical data fit earlier qualitative ultrastructural observations of "immature" granules in some neuroendocrine neoplasms.[22-24]

Our observation of quantitative anomalies and certain qualitative changes in the secretory apparatus of dysplastic bronchial neuroendocrine cells suggests that these may also be the site of arrested or deviated secretory activity. This confirms in human material the initial observation of high pro-ACTH levels associated with bronchial dysplasia in dogs.[7] Moreover, since bronchial dysplasia often accompanies neoplasms other than oat cell type, the amine and/or peptide materials detected in a variety of pulmonary carcinomas [5] may be the result in part of abnormal secretory activity by the dysplastic bronchial epithelium rather than the neoplasms themselves. Similarly, it is tempting to speculate that some paraneoplastic-type syndromes associated with various nonneoplastic chronic pulmonary diseases [25] may be a reflection of aberrant secretory activity by dysplastic neuroendocrine bronchial cells.

References

1. Pearse AGE: The APUD cell concept and its implications in pathology. Pathol Annu 9:27–41, 1974
2. McDowell EM, Barret LA, Trump BF: Observations on small granule cells in adult human bronchial epithelium and in carcinoid and oat cell tumors. Lab Invest 34:202–206, 1976
3. Gould VE: Neuroendocrinomas and neuroendocrine carcinomas. APUD-cell system neoplasms and their aberrant secretory activities. Pathol Annu 12 (Vol. II): 1977 (In press)
4. Terzakis JA, Sommers SC, Andersson B: Neurosecretory appearing cells of human segmental bronchi. Lab Invest 26:127–132, 1972
5. Sherwood L, Gould VE: Ectopic hormone syndromes and multiple endocrine neoplasia. Endocrinology. Edited by L DeGroot. New York, Academic Press, Inc., 1977
6. Gewirtz G, Yalow RS: Ectopic ACTH production in carcinoma of the lung. J Clin Invest 53:1022–1032, 1974
7. Gould VE, Sommers SC, Terzakis JA: Ultrastructure of neuroendocrine cells in normal and dysplastic bronchi. Am J Pathol 86:23A–24A, 1977 (Abstr)
8. Melamed MR, Zaman MB, Flehinger BJ, Martin N: Radiologically occult in situ and incipient invasive epidermoid lung cancer. Am J Surg Pathol 1:5–15, 1977
9. Snedecor GW, Cochran EG: Statistical Methods. Ames, Iowa, Iowa State University Press, 1971
10. Feyrter F: Die peripheren endokrinen (parakrinen) Drüsen. Lehrbuch der speziellen pathologischen Anatomie, Vol 11-12. Edited by Kaufmann-Staemler, De Guyter, Berlin, 1969
11. Pearse AGE: The cytochemistry and ultrastructure of polypeptide hormone-pro-

ducing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 17:303–313, 1969
12. Pearse AGE: The APUD Cell Concept and its implications in pathology. Endocrine Pathology Decennial, 1966–75. Edited by SC Sommers. New York, Appleton-Century-Croft, 1975, pp 162–163
13. Bensch KG, Corrin B, Pariente R, Spencer H: Oat cell carcinoma of the lung: Its origin and relationship to bronchial carcinoid. Cancer 22:1163–1172, 1968
14. Tsuji K, Hayata Y, Sato M, Shimosato Y, Fukushima Y: Neuronal differentiation of oat cell carcinoma *in vitro* by dibutyril cyclic adenosine 3′, 5′-monophosphate. Cancer Lett 1:311–318, 1976
15. Lacy PE: Endocrine secretory mechanisms. Am J Pathol 79:170–188, 1975
16. DeRobertis EDP, Saez FA, DeRobertis EMF Jr: Cell biology, sixth edition. Philadelphia, W. B. Saunders Co., 1975, pp 575–593
17. Hammar S, Sale G: Multiple hormone producing islet cell carcinomas of the pancreas: A morphological and biochemical investigation. Hum Pathol 6:349–362, 1975
18. Steiner DF, Cunningham D, Spigelman L, Aten B: Insulin Biosynthesis: Evidence for a precursor. Science 157:697–700, 1967
19. Steiner DF, Oyer PE: The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc Natl Acad Sci USA 57:473–480, 1967
20. Yalow RS, Berson SA: Size heterogeneity of immunoreactive human ACTH in plasma and in extracts of pituitary glands and ACTH-producing thymoma. Biochem Biophys Res Commun 44:439–445, 1971
21. Gregory RA, Tracy HJ: Isolation of two "big gastrins" from Zollinger-Ellison tumour tissue. Lancet 2:797–799, 1972
22. Gewirtz G, Schneider B, Krieger DT, Yalow RS: Big ACTH: Conversion to biologically active ACTH by trypsin. J Clin Endocrinol Metab 38:227–230, 1974
23. Gould VE, Benditt EP: Ultrastructural and functional relationships of some human endocrine tumors. Pathol Annu 8:205–230, 1973
24. Gould VE, Benditt EP: Ultrastructural and functional relationships of some human endocrine tumors.[12] pp 190–193
25. Walker CO: Chronic duodenal ulcer. Gastrointestinal Diseases. Edited by MH Slessinger, JS Fordtran. Philadelphia, W. B. Saunders Co., 1973, pp 665–691



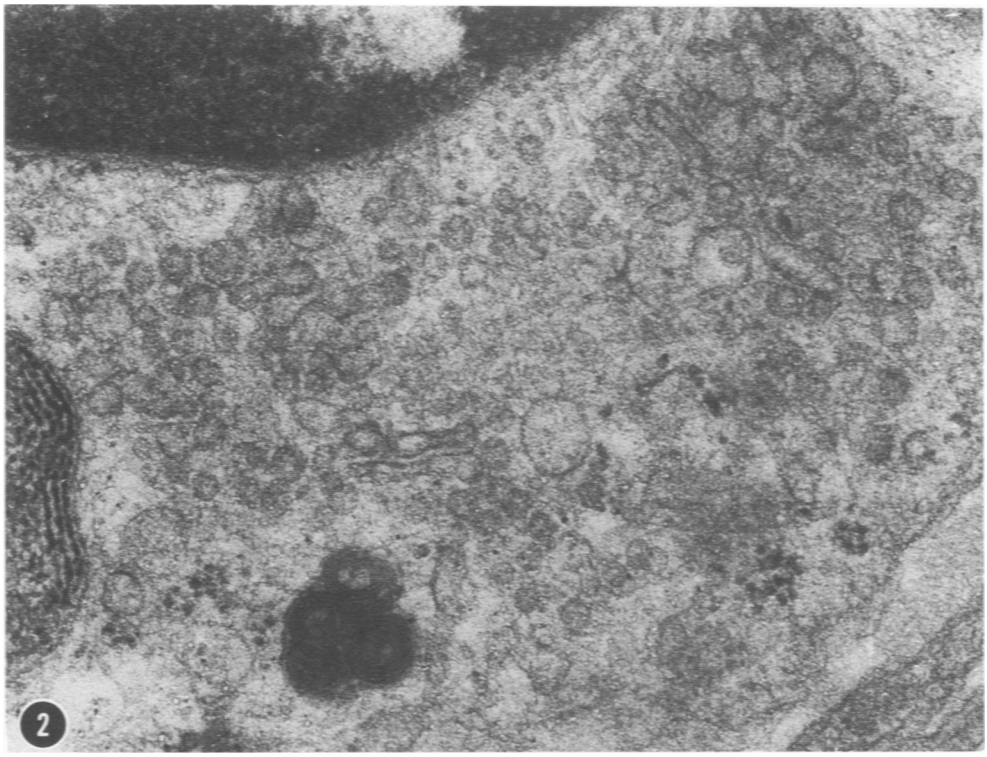
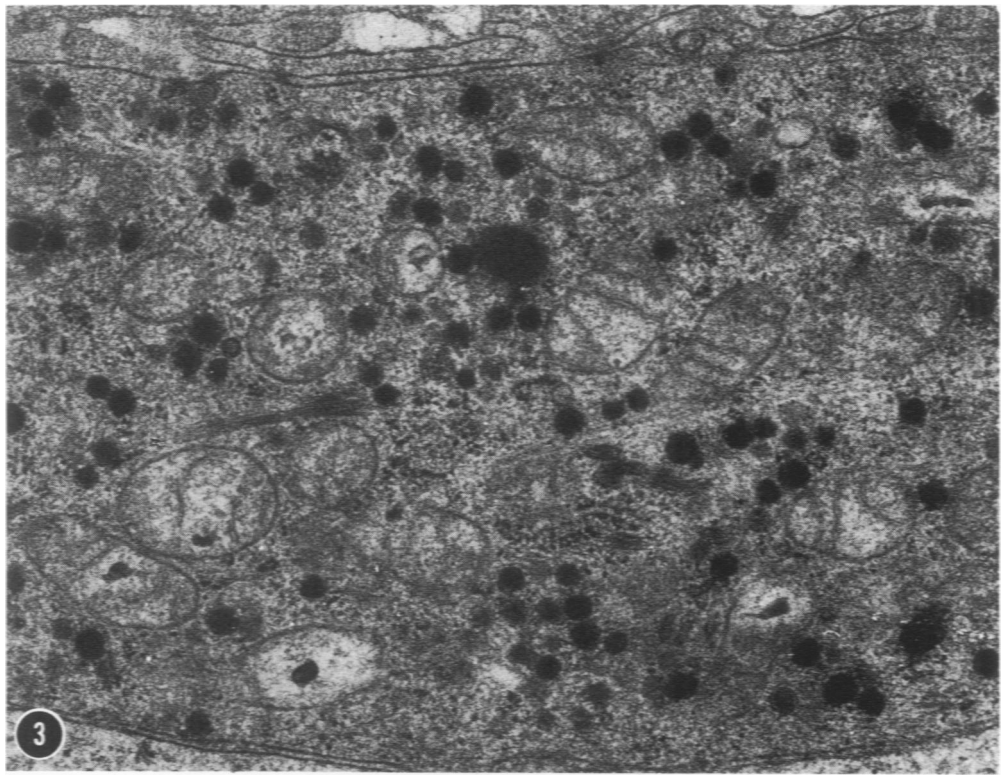


Figure 1—Neuroendocrine cell in normal control bronchus. Notice prominent Golgi complex with rather uniform parallel arrays of cisternae and abundant vesicles. (× 41,000) **Figure 2**—Neuroendocrine cell in dysplastic bronchial epithelium. Given the higher magnification, the Golgi complex is evidently less conspicuous than the control. Note scarcity of cisternae and irregular size of vesicles. (× 128,000)



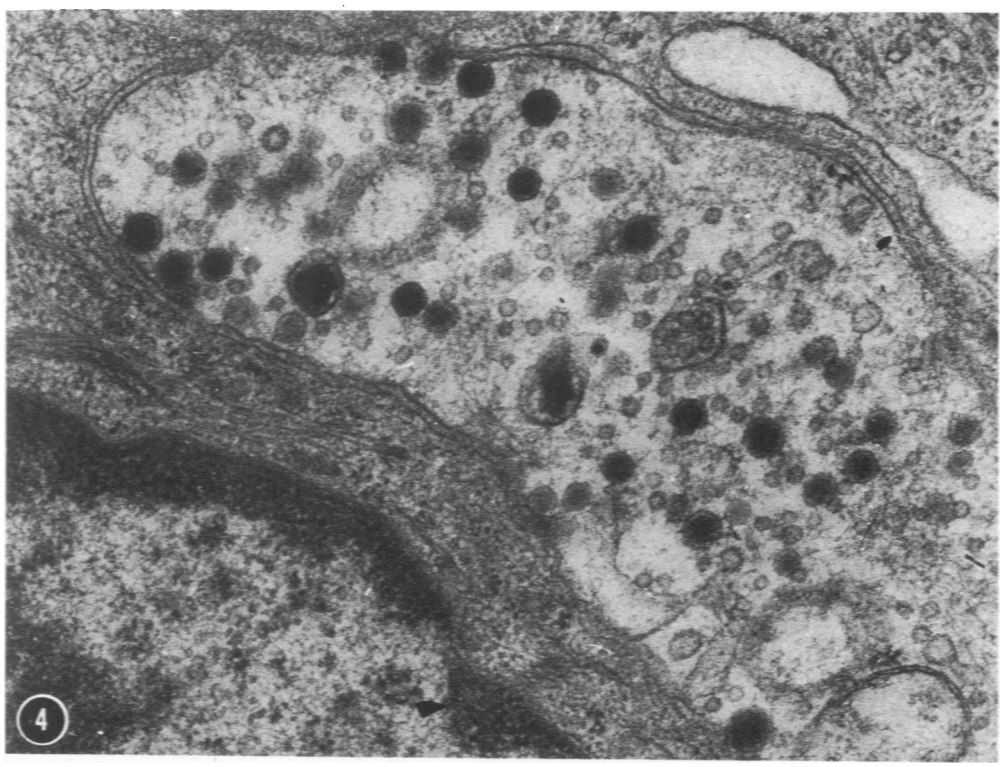


Figure 3—Neuroendocrine cell in normal control bronchus. Note abundant neurosecretory granules of rather uniform size and electron density. (× 41,000) **Figure 4**—Neuroendocrine cell in dysplastic bronchial epithelium. Relatively few granules are present. Note their irregular size and electron density and the abundant "empty" vesicles. (× 42,000)