Ultrastructure of Nitrosoheptamethyleneimine-Induced Lung Tumors in European Hamsters

Hildegard Reznik-Schüller, Priv. Doz, Dr., DVM

The ultrastructure of lung tumors induced in European hamsters through chronic subcutaneous treatment with nitrosoheptamethyleneimine (NHMI) is described. The neoplasms demonstrated either adenomatous (adenomas and adenocarcinomas) or mixed (tumors with areas of adenoid and squamous differentiation) pattern. Adenomatous areas consisted of Clara cells and of cells with lamellated inclusion bodies in their cytoplasm. Cells in areas with squamous differentiation contained cytoplasmic filaments and filament bundles as well as dense-cored granules. (Am J Pathol 93:45–52, 1978)

BIOCHEMICAL INVESTIGATIONS of transformed cells have mostly involved the whole tissue, since no method exists for separating the altered from the normal cells. The primary problem has been that often the target cell is not known at the time the tumor is microscopically visible. This study forms part of a series aimed at characterizing experimentally induced tumors by electron microscopy techniques. By this means, it will be possible to see which of the many different cells of the tissue were transformed and thus produced the gross tumor. This will provide a basis for comparing biochemical examinations of carcinogen–tissue interactions in different species. The cyclic nitrosamine nitrosoheptamethyleneimine (NHMI) is known to induce pulmonary and upper digestive tract tumors in rats.[1-3] In the European hamster, the target organs are the respiratory tract (nasal cavities and lungs), with up to 100% pulmonary tumors developing after relatively short periods of subcutaneous treatment.[4] In the rat, all of the lung tumors were squamous (squamous carcinomas and papillomas) after intragastric administration, whereas in the European hamster, adenoid (adenomas and adenocarcinomas) and squamous (papillomas and squamous and mixed carcinomas) patterns were reported.[4] In this paper, the ultrastructure of NHMI-induced lung tumors in European hamsters is described and compared with tumors induced in the same species and in Syrian golden hamsters by other nitrosamines.[5-9]

From the Frederick Cancer Research Center, Frederick, Maryland.

Supported in part by Public Health Service Contract NO1-CP12148 from the Division of Cancer Cause and Prevention, National Cancer Institute.

Accepted for publication May 22, 1978.

Address reprint requests to Hildegard Reznik-Schüller, Priv. Doz, DVM, Veterinary Pathologist, Chemical Carcinogenesis, Frederick Cancer Research Center, P. O. Box B, Frederick, MD 21701.

0002-9440/78/1010-0045$01.00

Materials and Methods

Ten male European hamsters (strain Mhh-EPH) were housed one per plastic cage. They received a pellet diet (RMH-TMB, Hope Farms, Woerden, The Netherlands) and water *ad libitum*. The animals were given a subcutaneous injection once a week with 1/5 LD_{50} (66 mg/kg body weight) NHMI dissolved in olive oil until they showed clinical signs of respiratory tract tumors (breathlessness and loss of body weight). They were then fixed *in situ* by vascular perfusion with 2% cacodylate-buffered glutaraldehyde under hexobarbital–Na anesthesia (Evipan-Na, Bayer, Leverkusen, FRG). Samples of macroscopically visible lung tumors were excised, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812 (Ladd Research Industries, Burlington, Vt.). Sections were cut on an LKB Ultrotome III (LKB, Bromma, Sweden). Semithin sections (1 μ thick) were stained with toluidine blue. Ultrathin sections were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips 201 electron microscope at an accelerating voltage of 40 kV.

Results

All the animals had nasal cavity tumors and multiple pulmonary neoplasms. Under light microscopic examination, the lung tumors were frequently seen to be situated near segmental bronchi and bronchioles and they showed an adenoid or mixed (areas with adenoid and poorly keratinized squamous differentiation) pattern (Figures 1 and 2). Using electron microscopy, it could be seen that adenoid neoplasms (adenomas and adenocarcinomas) and the areas with adenoid differentiation in mixed tumors consisted predominantly of Clara cells and of cells morphologically resembling Type II alveolar epithelial cells. The latter typically exhibited lamellated inclusion bodies and various amounts of smooth endoplasmic reticulum in their cytoplasm (Figure 3) whereas the former demonstrated abundant smooth endoplasmic reticulum only. Ciliated cells were also occasionally found. In areas which appeared histologically to be of squamous differentiation, electron microscopy revealed numerous cells which possessed varying amounts of dense-cored granules and cytoplasmic filaments (Figures 4 and 5). It was observed that cells with many dense-cored granules had few filaments and those with many filaments had few granules.

Discussion

The present findings demonstrate that NHMI-induced lung tumors in the European hamster have an ultrastructure similar to that of pulmonary neoplasms in this species after treatment with *N*-dibutylnitrosamine [5] and in Syrian golden hamsters after treatment with diethylnitrosamine,[6–8] dibutylnitrosamine,[6–8] and nitrosomorpholine.[9] In the cited investigations, as well as in the present studies, lung tumors with adenoid composition consisted mainly of proliferated Clara cells and of cells with lamellated inclusion bodies. The latter organelles are normally found in Type II

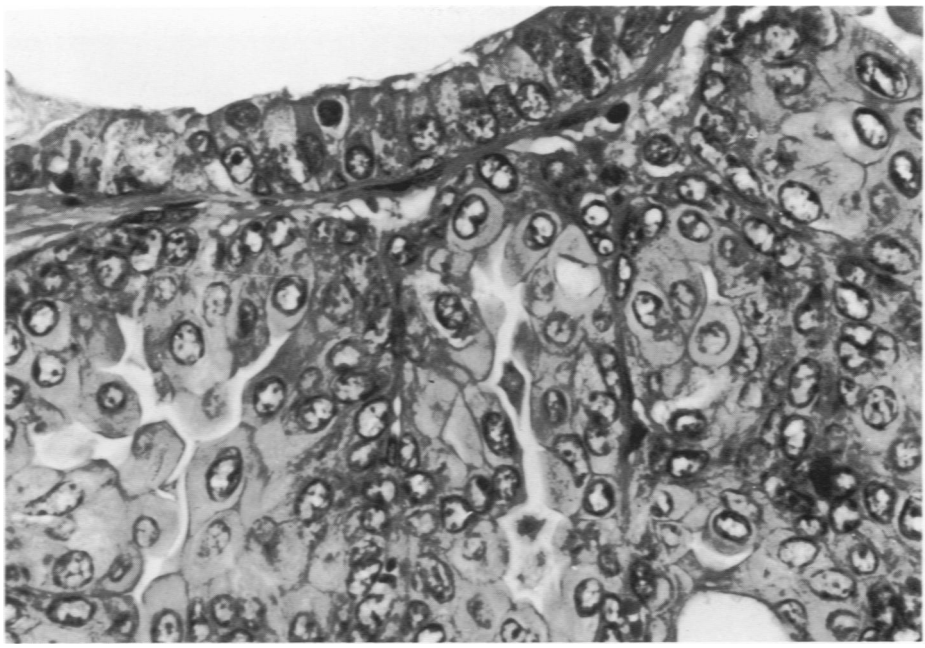
alveolar epithelial cells exclusively [10-13] and are believed to be the source of surfactant,[10-13] a surface active material consisting of phospholipid components.

Human alveolar cell carcinomas are composed of cells with such lamellated inclusion bodies and have therefore been interpreted as deriving from alveolar epithelial cells.[14] However, serial sacrifice studies in Syrian golden hamsters have demonstrated that such tumor cells develop from the bronchial Clara cells.[6,9] It is therefore most likely that the tumor cells described have originated from the bronchial Clara cells. Cells containing dense-cored granules, which in the present case were found in tumor areas with squamous differentiation, were also reported to proliferate in the bronchial epithelia of Syrian hamsters during early nitrosamine-induced carcinogenesis.[7-9] These cells are regarded to be of the APUD type (Amine Precursor Uptake and Decarboxylase active), although admittedly only one of the criteria (presence of dense-cored granules) for cells belonging to the APUD series was investigated. APUD-type cells are numerous in the fetal airways of humans [15,16] and various mammals,[16,17] but they are only occasionally found in adults. They are believed to be the source of bronchial carcinoids and oat cell carcinomas in humans.[18-20] It is of interest that such cells in the present studies were found in tumorous areas of squamous differentiation and that their content of dense-cored granules was small when large numbers of cytoplasmic filaments were present. These findings agree with the results of serial sacrifice studies in Syrian golden hamsters, in which gradual loss of dense-cored granules coincided with increasing occurrence of cytoplasmic filaments and filament bundles.[8] It was concluded that this process represents the morphologic expression of a transformation of APUD cells into squamous cells (squamous metaplasia), a concept which gains further support from the present paper.

References

1. Lijinsky W, Tomatis L, Wenyon CEM: Lung tumors in rats treated with N-nitrosoheptamethyleneimine and N-nitrosooctamethyleneimine. Proc Soc Exp Biol Med 130:945–949, 1969
2. Garcia H, Lijinsky W: Tumorigenicity of five cyclic nitrosamines in MRC rats. Z Krebsforsch 77:257–261, 1972
3. Taylor HW, Lijinsky W: Tumor induction in rats by feeding heptamethyleneimine and nitrite in water. Cancer Res 35:812–815, 1975
4. Reznik G, Mohr U, Lijinsky W: Carcinogenicity of subcutaneously injected N-nitrosoheptamethyleneimine in European hamsters. J Natl Cancer Inst 61:239–243, 1978
5. Reznik-Schüller H, Mohr U: The ultrastructure of N-dibutylnitrosamine induced pulmonary tumours (adenocarcinomata) in European hamsters. Br J Cancer 32:230–238, 1975
6. Reznik-Schüller H: Ultrastructural alterations of nonciliated cells after nitrosamine

treatment and their significance for pulmonary carcinogenesis. Am J Pathol 85:549–554, 1976
7. Reznik-Schüller H: Proliferation of endocrine (APUD-type) cells during early N-diethylnitrosamine-induced lung carcinogenesis in hamsters. Cancer Lett 1:255–258, 1976
8. Reznik-Schüller H: Ultrastructural alterations of APUD-cells during nitrosamine induced lung carcinogenesis. J Pathol 121:79–82, 1977
9. Reznik-Schüller H: Sequential morphologic alterations in the bronchial epithelium of Syrian golden hamsters during *N*-nitrosomorpholine induced pulmonary tumorigenesis. Am J Pathol 89:59–66, 1977
10. Buckingham S, Avery ME: Time of appearance of lung surfactant in the foetal mouse. Nature 193:688–689, 1962
11. Klaus M, Reiss OK, Tooley WH, Piel C, Clements JA: Alveolar epithelial cell mitochondria as source of the surface-active lung lining. Science 137:750–751, 1962
12. Creasey JM, Pattle RE, Shock CJ: Ultrastructure of inclusion bodies in type II cells of lung, human and subsimian. J Physiol 237:35–37, 1974
13. Pattle RE, Gandy G, Shock C, Creasey JM: Lung inclusion bodies: Different ultrastructure in simian and non-simian mammals. Experientia 30:797–798, 1974
14. Coalson JJ, Mohr JA, Pirtle JK, Dee AL, Rhoades ER: Electron microscopy of neoplasms in the lung with special emphasis on the alveolar cell carcinoma. Am Rev Respir Dis 101:181–197, 1970
15. Bensch KG, Gordon GB, Miller LR: Studies on the bronchial counterpart of the Kultschitzky (argentaffine) cell and innervation of bronchial glands. J Ultrastruct Res 12:668–686, 1965
16. Cutz E, Chan W, Wong V, Conen PE: Ultrastructure and fluorescence histochemistry of endocrine (APUD-type) cells in tracheal mucosa of human and various animal species. Cell Tissue Res 158:425–437, 1975
17. Hage E: Histochemistry and fine structure of endocrine cells in foetal lungs of the rabbit, mouse and guinea-pig. Cell Tissue Res 149:513–524, 1974
18. 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
19. Bensch KG, Gordon GB, Miller LR: Electron microscopic and biochemical studies on the bronchial carcinoid tumor. Cancer 18:592–602, 1965
20. Hattori S, Matsuda M, Tateishi R, Nishihara H, Horai T: Oat-cell carcinoma of the lung: Clinical and morphological studies in relation to its histogenesis. Cancer 30:1014–1024, 1972



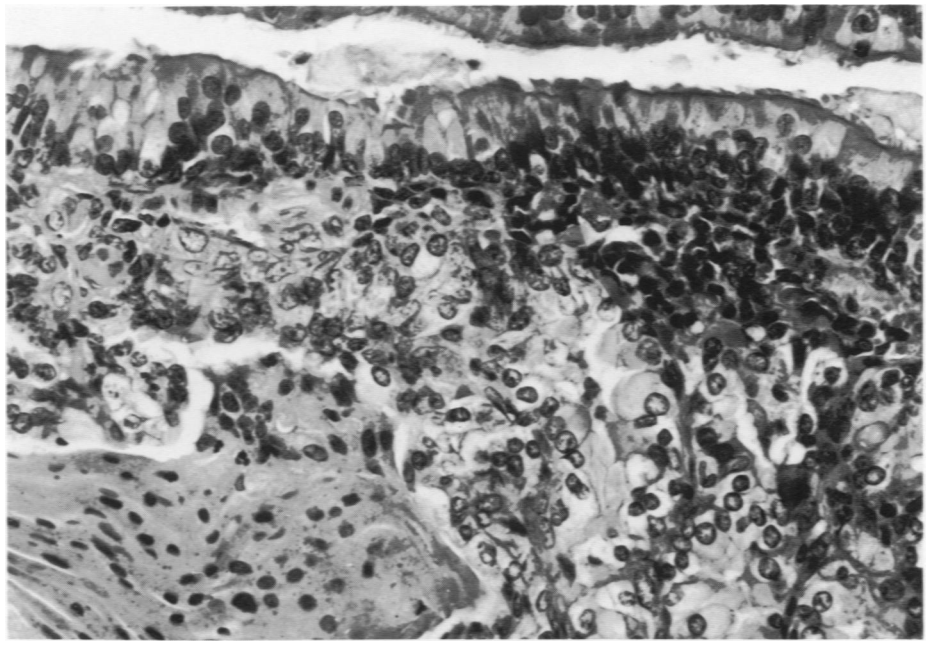


Figure 1—Lung tumor with adenoid pattern in an animal treated for 40 weeks with 1/5 LD_{50} NHMI. The tumor is typically located adjacent to a bronchiole. Semithin section. (Toluidine blue, × 100) **Figure 2**—Lung tumor with mixed pattern in an animal treated for 42 weeks with 1/5 LD_{50} NHMI. *Top.* The neoplasm is adjacent to a segmental bronchus. *Lower left.* The tumor shows squamous differentiation, whereas on the *right* it is adenoid. Semithin section. (Toluidine blue, × 100)

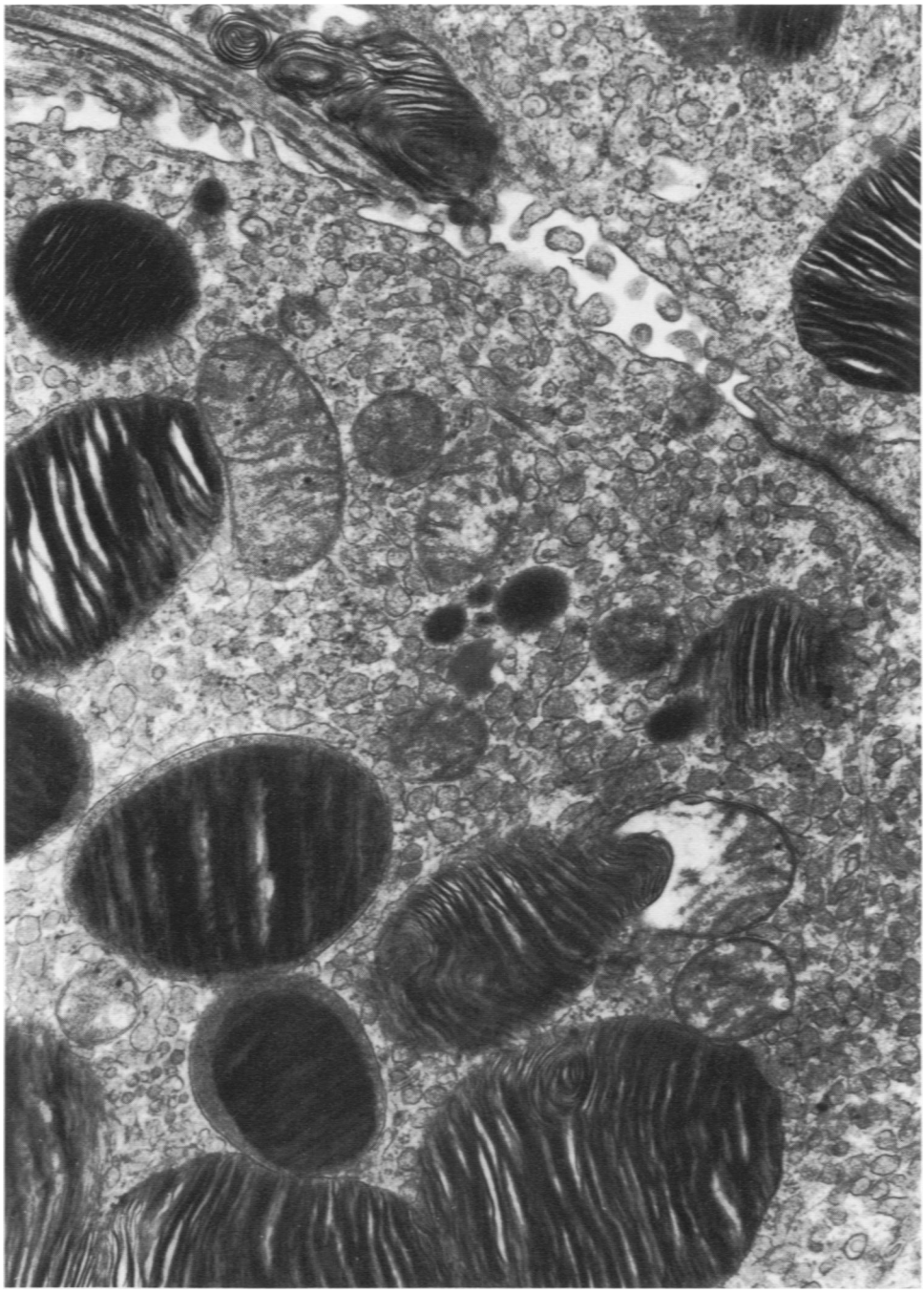


Figure 3—Tumor cells of adenoid neoplasm. The cytoplasm demonstrates abundant smooth endoplasmic reticulum (which is normally a typical feature of Clara cells) and lamellated inclusion bodies (which are normally found in alveolar Type 2 cells exclusively). Electron micrograph. (× 30,000)

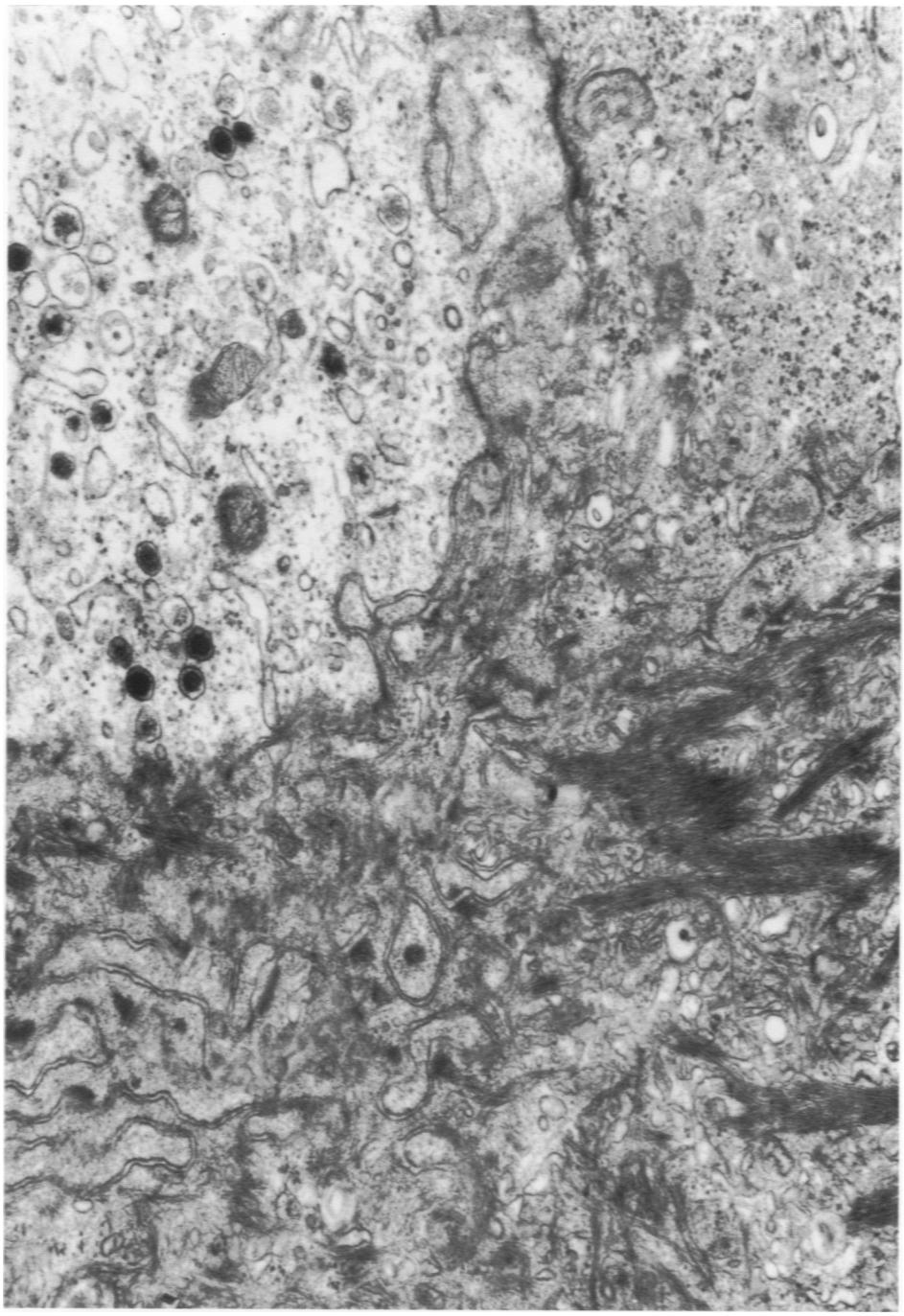


Figure 4—Tumor cells of neoplasm with squamous differentiation. *Upper right.* The cell demonstrates neuroendocrine dense-cored granules. *Lower right.* The cell possesses abundant cytoplasmic filaments. Electron micrograph. (× 30,000)

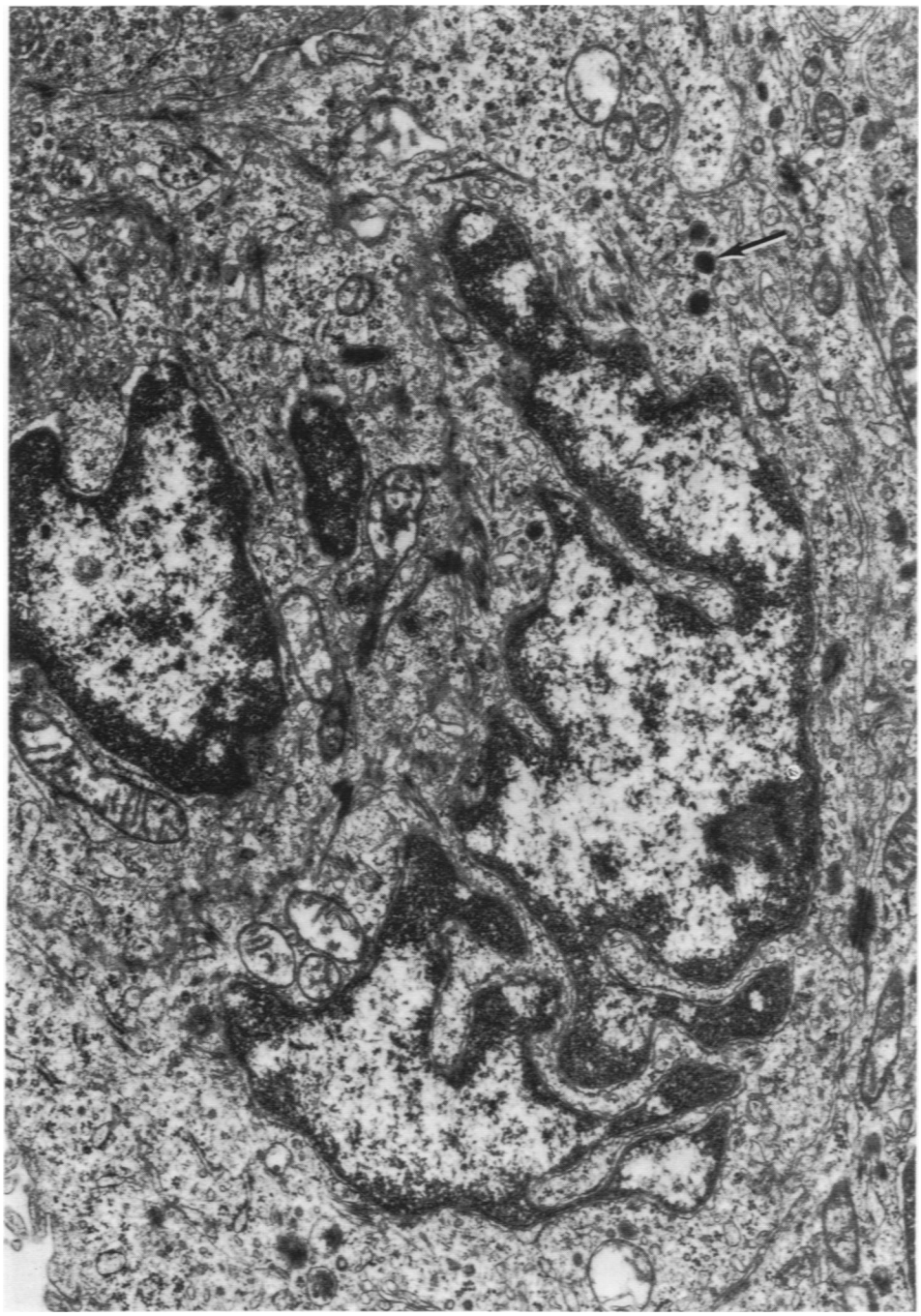


Figure 5—Tumor cells of area with squamous differentiation. Due to their very scanty dense-cored granules (*arrow*) these squamous cells are difficult to recognize as deriving from APUD-type cells. Electron micrograph. (× 20,000)