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Human Anaplastic Thyroid Carcinoma in Tissue Culture.* (32466)

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Herein is reported the isolation and maintenance in culture of a human anaplastic thyroid carcinoma. Although many stable lines of human cancer cells have been established, we believe that this is the first human thyroid cancer cell line.

Origin and characteristics of the culture. The culture was established from a 1 × 2 cm metastasis of giant and spindle cell thyroid cancer in the kidney of a 73-year-old man (Memorial Hospital No. 26-62-75) on December 30, 1965. The specimen was obtained aseptically approximately 2½ hours after death. The tumor nodule was dissected from the cortex of the kidney and minced into fragments of roughly 3 mm diameter. Ten to 12 of these fragments were placed into milk dilution bottles and fed with 10 cc of Eagle's minimum essential medium(1) containing 20% a–gamma calf serum, with kanamycin sulfate and streptomycin at a concentration of 100 gamma per ml for each. The bottles were laid on their side and incubated at 37°C. No plasma clot or other matrix was used. Medium was changed at approximately one week intervals depending upon pH of the medium and appearance of the cells.

There was a sparse outgrowth of mixed cell types from several of the tissue fragments. It was nearly 4 months before there were complete monolayers in the original bottles. Sub-culturing was first attempted by selectively scraping, with a rubber policeman, those areas where epithelioid cells predominated. Growth of these subcultures was more rapid and consisted predominantly of large and small epithelioid cells with granular cytoplasm (Fig. 1). Subsequent passages were accomplished by trypsinization, utilizing 0.25% trypsin for 5 minutes at 37°C. As

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of May 1967 the cells have been in culture for 17 months and have gone through 24 consecutive passages. Aliquots of the cells in the tenth passage were frozen at —70°C in tissue culture medium containing 10% glycerin and have been successfully recultivated after 6 months in the frozen state.

Heterotransplantation of the cell line. A

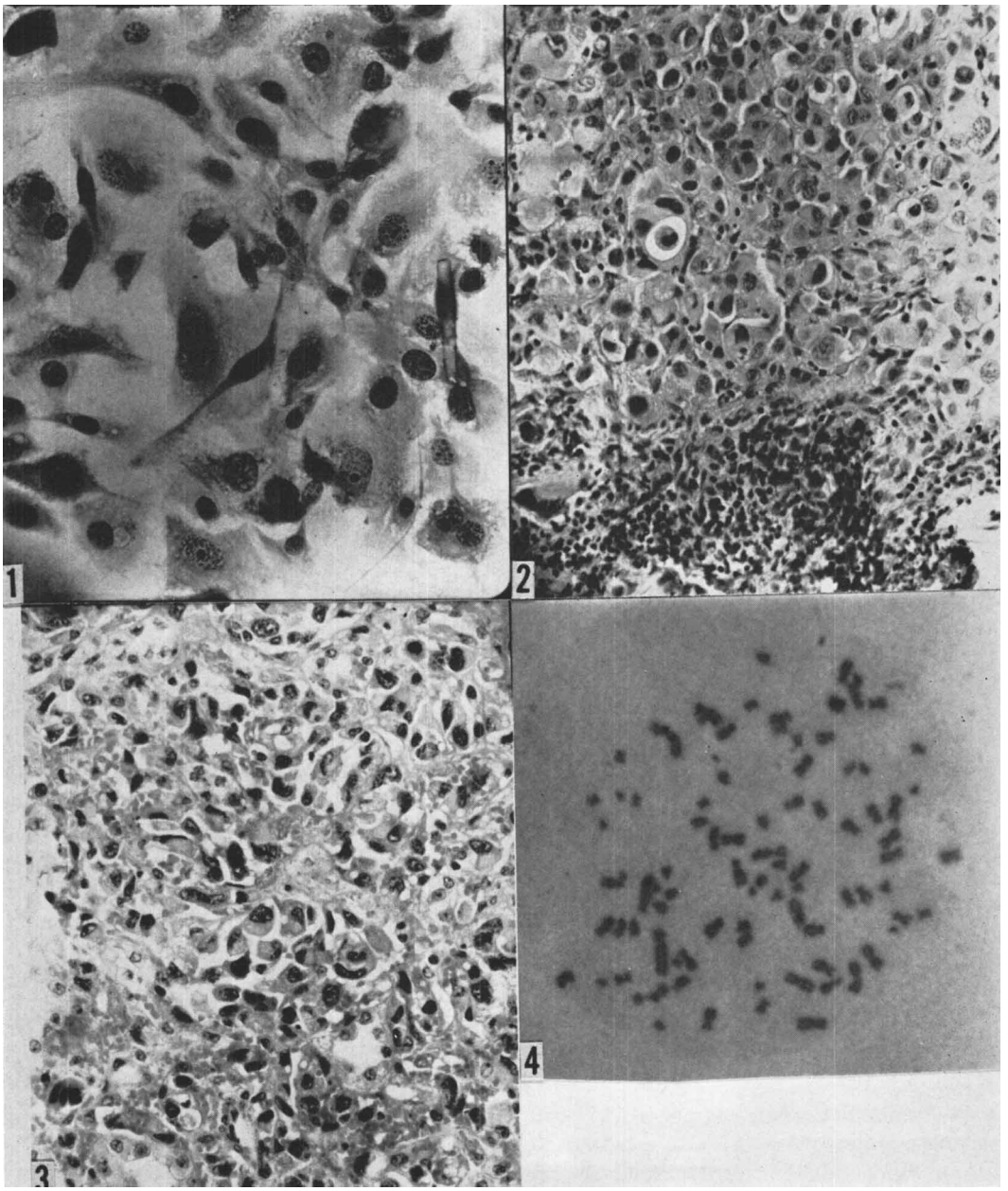

FIG. 1. Large and small epithelial-like cells with few spindle shaped fibroblastic cells in 4 month old culture. × 250.

FIG. 2. Hyperchromatic nuclei in pleomorphic epithelial cells with mitotic figures in heterotransplanted tumor nodule. Note rejection reaction. × 250.

FIG. 3. Anaplastic thyroid carcinoma in kidney parenchyma from which explants were obtained. × 100.

FIG. 4. Hypotetraploid cell with abnormally long chromosomes. × 400.

suspension containing half a million cells in tissue culture salt solution (without serum) was injected subcutaneously into each of 8 seven-day-old rats which had been made tolerant to human cell antigens(2) by intravenous injections of J-111 cells on the day of birth. Five of these eight rats developed subcutaneous nodules up to 4 mm in diameter. These were removed 21 days later for histologic study. The nodules were anaplastic carcinoma resembling the tumor from which the culture originated (Fig. 2 and 3). An attempt was also made to transplant the tumor by intravenous inoculation of 1,000,000 cells into rats less than 24 hours of age, but all the recipients became cyanotic and died of asphyxia within two hours of injection, perhaps because of the large size of the cells.

Chromosomal analysis of the cultured cells. Cells of passage numbers 7, 9 and 15 were prepared for chromosomal observation by conventional techniques of colcemid and hypotonic pretreatments, acetic alcohol fixation, preparation of flame-dried slides, and acetic orecin staining. Twenty cells representing all 3 passages were counted. Numbers of chromosomes per cell were: 59, 77, 77, 78, 78, 79, 81, 81, 82, 84, 85, 86, 86, 87, 87, 92, 92, 92, 154, and 171 (Fig. 4). Thus there was no clear cut mode and the chromosome numbers were mainly in the hypotetraploid range. One hundred consecutive metaphase cells were surveyed and estimates were made of their ploidy classes. Forty-six were in the range of 75 to 95 (tetraploid range); 40 in the range of 150 to 200; and 14 in the range of 300 to 400. In all cells, including those with a count of 92 chromosomes, there was evidence of a considerable degree of structural rearrangement, resulting in new chromosomal types. Dicentrics were prevalent, ring chromosomes were found occasionally, and cells with one or more chromatid breaks or gaps were frequent. Many metaphases had very small paired acentric fragments of unknown significance.

Summary. A cell line has been established from a nodule of human giant and spindle cell carcinoma of the thyroid metastatic to a kidney. The culture has been maintained through more than 24 passages over a period of 1½ years. Its identity as a cancer cell line was established by its growth on heterotransplantation into immunologically tolerant rats with reproduction of the histologic appearance of the original tumor. Chromosomal studies showed a high degree of aneuploidy and polyploidy with numerous abnormalities of chromosomal form.

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Effect of 1-Methyl-2-Mercaptoimidazole (Methimazole-Tapazole On DNA Content of the Chicken Thyroid Gland.*† (32467)

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In the determination of the thyroid hormone secretion rate (TSR) in the fowl, the goitrogen methimazole or tapazole has been used to block the recycling of I^{131} from the metabolism of L, thyroxine-I^{131}(1). As the term goitrogen indicates, it depresses the synthesis of the thyroid hormones and as a result stimulates an increased secretion of TSH which acts upon the thyroid glands to increase its weight and size to form the goiter. However, the goitrogen does not influ-

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