Induction of Neoplasia *in vitro* in Hamster Thyroid Tissue by SV40 And Adenovirus 7-SV40 "Hybrid" (Strain LLE46). (31528)

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Newborn hamster kidney tissue transformed *in vitro* by simian virus 40 (SV40) and injected into newborn or adult hosts, produced tumors which histologically contained areas of carcinoma with tubular differentiation(1-3). Cell cultures derived from hamster liver, lung, and salivary gland have also been transformed *in vitro* by SV40; however, when these cells were injected into hamsters they produced fibrosarcomas with no epithelial elements (4,5).

The LLE46 strain of adenovirus 7 (adenovirus 7-SV40 "hybrid" virus)(6) also transformed hamster kidney tissue *in vitro*(7). Rabson *et al* transformed explant cultures of newborn hamster kidney with LLE46 and produced tumors by transplanting the cells into irradiated adult hosts(8). Histologically, these neoplasms were predominantly undifferentiated tumors similar to adenovirus 12 induced tumors, although they did contain small areas of SV40 type fibrosarcoma. Black and Igel, however, observed that neoplasms produced by LLE46 transformed weanling hamster kidney cells were fibrosarcomas containing areas of carcinoma and were similar to neoplasms produced by SV40 transformed kidney tissue(9).

LLE46 transformed salivary gland cells produced tumors with histologic components characteristic of both SV40 and adenovirus type neoplasms. The predominant element was an SV40 type fibrosarcoma intermixed with small areas of adenovirus-type tumor cells(5).

In the present experiments we have studied the effects of four oncogenic DNA viruses (SV40, LLE46, polyoma and adenovirus 12) on explant cultures of newborn hamster thyroid tissue to determine whether differentiated epithelial thyroid neoplasms could be produced.

Materials and methods. Viruses. The strain of polyoma virus used was originally isolated from a cell-free filtrate of an extract of a parotid gland tumor of a C_3H/Bi mouse. The details of the derivation of this strain of virus have been given by Dawe and his associates(10). The polyoma virus preparation in the present studies was grown in P388 D_1 cells maintained in medium #199 with 20% fetal bovine serum (FBS) and contained $10^{6.0}$ tissue culture infectious doses ($TCID_{50}$) per ml when titrated by limiting dilution in P388 D_1 cells.

SV40 strain VAC777, obtained from Dr. Paul Gerber, Division of Biologics Standards, National Institutes of Health was grown and titrated in African green monkey kidney (GMK) cell cultures maintained in medium #199 with 2% FBS. The pool we used contained $10^{7.94}$ $TCID_{50}$ per ml.

Adenovirus 12 was obtained from the American Type Culture Collection and passed in cultures of HEp-2 cells maintained in medium #199 with 2% FBS. The pool that we used titered $10^{8.5}$ $TCID_{50}$ per ml in primary human embryo kidney (HEK) cells.

LLE46, the adenovirus 7-SV40 "hybrid," obtained from Dr. Wallace P. Rowe, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, was passed in primary GMK cell cultures maintained in medium #199 with 2% FBS. The pool used in the present experiments contained $10^{5.2}$ $TCID_{50}$ per ml when titrated in GMK cells.

Newborn hamster tissues. Newborn Syrian hamsters obtained from the Animal Production Section of the National Institutes of Health were killed on the day of birth; the thyroid glands were removed with aseptic technique and minced into 1 mm explants for culture.

Culture methods. Medium #199 with 20% FBS was used as growth medium in all experiments and all cultures were incubated at 37°C. The explants were grown in two-ounce prescription bottles (Brockway Saniglas) as

previously described(1).

Cultures were trypsinized with 0.25% trypsin in Dulbecco's tris buffer free of calcium and magnesium for 30 minutes at 37°. The cells were then resuspended in growth medium and passed to other bottles.

Injection of cell suspensions into animals. Cell suspensions were prepared by scraping confluent layers from glass bottles with a rubber policeman. Three-week-old male hamsters which had received 400 r total body X-radiation 24 hours earlier were injected subcutaneously with 1.0 ml of cell suspension containing approximately 1×10^8 cells.

Animals that developed tumors after injection of cells were killed with ether and autopsied. Tissues were fixed in Zenker-formol solution and sections stained with hematoxylin and eosin.

Immunofluorescent methods. Antisera against "T" antigens were obtained from hamsters bearing large transplanted tumors induced by SV40 and the Gomen strain of adenovirus 7. Fluorescent antibody techniques and tests for reactivity and specificity of the sera have been previously described (11).

Attempts to isolate virus from the transformed cells. Tube cultures of GMK and HEK cells were obtained from Microbiological Associates and Flow Laboratories, respectively. LLE46 and SV40 transformed cells were trypsinized, suspended in 1 ml of maintenance media and placed in the GMK and HEK culture tubes. The tube cultures were incubated on a roller drum and observed for 21 days.

Experiments and results. Five two-ounce prescription bottles were prepared, each with 25 one-mm explants of thyroid tissue. Elongated and cuboidal cells generally spread from most clumps within the first 24 hours. On the second day of growth, four of the culture bottles were separately infected with 1 ml of undiluted preparations of the following viruses: polyoma, adenovirus 12, SV40 and LLE46. One bottle was kept as a virus-free control.

Within 48 hours after infection, cytopathic effects (CPE) were severe in cultures infected with polyoma virus, but were absent in SV40 infected cultures. A mild adenovirus type of CPE developed in adenovirus 12 and LLE46 infected cultures.

Within 2 weeks, tissues infected with LLE46 and SV40 developed evidence of transformation characterized by an accelerated growth phase, rapid development of acid pH in freshly fed media and growth of cells in multiple layers and clumps.

Adenovirus 12 and polyoma infected tissues did not transform and grew no better than uninfected controls.

Twenty-eight days after infection all culture bottles were trypsinized and equal cell suspensions were transferred to 2 new bottles. The LLE46 transformed cells were cuboidal, somewhat granular, and grew more rapidly than did the SV40 transformed cells which were polygonal and elongated and tended to grow in clumps. Interspersed among the SV40 transformed polygonal and elongated cells were frequent 1-2 millimeter circular areas containing nests of cuboidal epithelial cells. Both the LLE46 and SV40 transformed thyroid cells have grown well for 6-7 months and have been repeatedly subcultured by trypsinization. Adenovirus 12 infected cells, polyoma infected cells and uninfected control tissue grew poorly and contained only small numbers of elongated cells when the cultures were discontinued on the 50th day.

Immunofluorescent studies demonstrated SV40 "T" antigens in more than 95% of the cells transformed by SV40 while both SV40 and adenovirus 7 "T" antigens were present in 95% of the LLE46 transformed cells.

Attempts to isolate SV40 and adenovirus from the transformed cells by growth of the cells in contact with HEK and GMK monolayers were unsuccessful.

Forty to fifty days after infection, SV40 and LLE46 transformed cells were scraped from 32-ounce culture bottles. The cells from each bottle (approximately 1×10^8) were suspended in 1 ml of mixture #199 and injected subcutaneously into an irradiated 3-week-old-hamster. All injected animals developed progressively growing tumors within 20-40 days.

Hamsters injected with LLE46 transformed

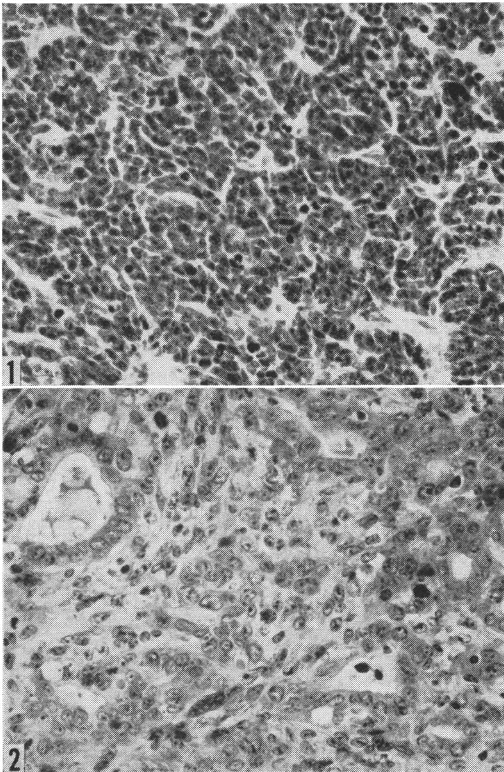

FIG. 1. Section of tumor produced in hamster by injection of LLE46-transformed hamster thyroid cells. It is composed of sheets of small cells with hyperchromatic nuclei similar to those seen in tumors induced by oncogenic adenoviruses. H. and E. × 400.

FIG. 2. Section of tumor produced in hamster by injection of SV40-transformed hamster thyroid cells. Well-formed follicular elements are dispersed in a fibrosarcomatous stroma. H. and E. × 400.

thyroid cells developed neoplasms which grossly were soft and white with areas of hemorrhagic necrosis. Histologically, these neoplasms were undifferentiated malignant tumors consisting of small, closely packed cells with darkly staining nuclei similar to tumors induced by oncogenic adenoviruses (Fig. 1).

Tumors produced by the injection of SV40 transformed cells were grossly firm and white. Microscopically, the neoplasms were predominantly fibrosarcomas, however, they contained areas of adenocarcinoma with well defined follicular structures (Fig. 2).

Discussion. In subcutaneous tumors produced in hamsters by the LLE46 virus, Huebner and his associates observed that some neoplasms histologically resembled SV40 fibrosarcomas, others resembled adenovirus-induced neoplasms and still others were bimorphic with mixtures of the SV40 and adenovirus tumor cell types(6).

Hamster kidney tissue transformed by the LLE46 virus produced tumors which were either of the SV40 type or predominantly of the adenovirus type with a small SV40 component(8,9). More evident histological mixing was observed in tumors produced by LLE46 transformed hamster salivary gland (5), while LLE46 transformed hamster thyroid tissue produced tumors which thus far have been composed of cells exclusively of the adenovirus type.

Injected SV40 transformed thyroid tissue produced neoplasms which were predominantly fibrosarcomas, but they also contained areas of adenocarcinoma with well defined follicular structures. Thus it seems that while SV40 does not induce epithelial transformation in hamster salivary gland, liver, and lung, it does do so in both kidney and thyroid tissue.

At present, studies are in progress to determine the ability of the tumors produced by SV40 transformed and LLE46 transformed thyroid cells to trap iodide. Tumor homogenates are also being bioassayed for thyrocalcitonin content.

Summary. Explant cultures of newborn hamster thyroid tissue were readily transformed by SV40 and the LLE46 strain of adenovirus 7. The SV40 and adenovirus 7 "T" antigens were present in the majority of LLE46 transformed cells, while the SV40 "T" antigen was present in SV40 transformed cells. When LLE46 transformed cells were injected into hamsters, they produced tumors with histological characteristics of adenovirus-type neoplasms. Tumors produced by SV40 transformed cells were predominantly fibrosarcomas, but also contained areas of adenocarcinoma with well defined follicular structures.

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Monoclonal γ-Globulins in Ferrets with Lymphoproliferative Lesions.* (31529)

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In mustelids, Aleutian disease (AD) of mink has been the only disease described in which gammopathies resembling those observed in man had occurred(1-3). However, recently ferrets have been found to have a condition analogous to, if not identical with, AD in mink in which a systemic proliferation of lymphoid elements occurs with concomitant hypergammaglobulinemia and vasculitis(4).

Since previous attempts to transmit AD to ferrets resulted in infectivity as measured by transmission of AD back into mink, with no change in serum proteins(5), ferrets from different breeders were obtained for AD transmission to determine the influence of genotype and previous exposure to AD. During the course of these studies, ferrets from a particular ranch were observed to have hypergammaglobulinemia(4). As the result of this observation, the survey reported herein was made to establish the incidence of hypergammaglobulinemia and the occurrence of myeloma-like immunoglobulins in a representative ferret population.

Materials and methods. Ferrets of both light and dark color phases ranging in age from 1 to 4 years and about equally distributed in males(49) and females(43) were used in this survey. The sera harvested from 92 blood samples obtained from ferrets on 3 ranches, as indicated in Fig. 1, were analyzed electrophoretically to quantitate the level of *gamma* globulins and to determine if monoclonal hypergammaglobulinemia existed.

Quantitative estimates of the *gamma* globulins were made with paper electrophoresis at pH 8.6, 0.075 ionic strength veronal buffer in a Spinco model R electrophoretic cell with a potential difference of 75 volts, (2.5 mamps) for 16 hours. The electrophoretically resolved proteins were stained with bromphenol blue and the paper strips scanned with a Beckman RB integrating densitometer. Additional evidence of myeloma-like globulins was sought by zone electrophoresis on cellulose acetate and by immunoelectrophoresis. The Beckman model R101 Microzone cell was employed for electrophoresis with cellulose acetate using veronal buffer at pH 8.6, 0.075 ionic strength at 250 volts (4-6 mamps) for

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