by direct injection of the thymus gland include splenomegaly and lymphadenopathy, increase of bone marrow lymphocytes, increase of gamma globulin, positive Coombs' antiglobulin test, development of follicles within the thymus gland, plasma cell development in the thymus, spleen and lymph nodes and anemia, possibly of the autoimmune type.

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In vitro Transformation of Hamster Kidney Cells by Human Adenovirus Type 12.* (29060)

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The discovery of the oncogenic potential for Syrian hamsters of adenovirus type 12 provided the first direct evidence that a virus of human origin can induce cancer(1). This observation has been confirmed and extended to include adenovirus type 18(2). More recently it has been shown that cell cultures can be established *in vitro* from the virus-induced tumors(3,4). Unfortunately, a detailed analysis of an oncogenic virus-host cell relationship is exceedingly difficult if dependence must be placed on tumorigenesis *in vivo* as an experimental model, with or without subsequent *in vitro* cultivation of the tumor cells.

This report describes the transformation of hamster cells *in vitro* by adenovirus type

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12. It is anticipated that many new parameters of the tumorigenic potential of this virus can be explored with this model.

Materials and methods. Virus. Adenovirus type 12 (Huie strain) was obtained from Dr. R. J. Huebner as the prototype stock(5). This preparation was twice passaged in KB cells maintained in Eagle's medium supplemented with 10% fetal bovine serum. The second passage stock used in the experiments reported herein had a titer of 4×10^7 pfu/ml in fetal human kidney cell cultures.

Cell cultures. Kidneys were removed from 12 to 72 hour-old Syrian hamsters and minced. A portion of the minced tissue was placed in culture in Rose chambers(6), some tissue in plasma clots and some under full cellophane sheets. Cultures were prepared in duplicate, one set being incubated at 32°, the other at 37°C. The remaining tissue was disrupted with trypsin and the resultant cell suspension planted into tubes and cultured at 37°C.

Cultures of human fetal kidney cells were prepared by trypsinization. After outgrowth of the primary culture, secondary and tertiary monolayer cultures were prepared in a like manner.

All cultures were grown and maintained in Eagle's medium supplemented with 10% fetal bovine serum. The medium was changed one or two times a week as indicated by acid production.

Virus assays. Assays were done by the plaque method on secondary or tertiary fetal human kidney cultures. The overlay medium consisted of 5% fetal bovine serum in Eagle's medium containing 0.9% agar.

Virus inoculation. Eight of the 20 tube cultures of trypsinized hamster kidney cells were inoculated at time of planting with 0.1 ml (4×10^6 pfu) of stock virus. Twenty-four coverslips of kidney fragments were covered with one drop (*ca* 0.05 ml) undiluted virus prior to embedding of fragments in a plasma clot (12 cultures) or covering with a cellophane membrane (12 cultures). A like number of cultures was initiated in an identical manner without exposure to virus.

Results. A rapid outgrowth of epithelial and fibroblast cells occurred in the tube and plasma clot cultures of non-infected cells at 37°C. Similar but slower growth appeared in the plasma clot cultures held at 32°C. Inoculated cultures exhibited an initial retardation of growth under the same conditions; however, they apparently recovered full growth potential by the second week of cultivation. Outgrowth was scanty for fragment cultures (infected and uninfected) under cellophane.

After a period of several weeks all cultures appeared to be in poor condition, *e.g.*, cells were very granular, many piled up into cord-like arrangements, and in some areas the cell monolayer retracted into clusters. Only fibroblast cells remained in the tubes. Both fibroblasts and epithelial cells remained in the Rose chambers.

In time some cultures were lost due to breakage of coverslips in Rose chambers and inadvertent rotation of tubes in the stationary racks.

After 5 weeks of cultivation, an island of epithelial outgrowth appeared in one virus-inoculated tube. Subsequent examination did not reveal any marked progress of this outgrowth. However, by the eighth week a number of areas of epithelial growth were seen in all 4 of the inoculated tube cultures. These appeared first as discrete islands of polygonal cells which remained tenaciously adherent to each other and sharply circumscribed from the background cells. In time the central portion of many such islands consisted of an area piled many cells deep. Indeed, they often had the appearance of a hat crown surrounded by a brim of epithelial cells. These morphological transformations were observed likewise in 2 of the Rose chambers (Fig. 1 and 2 and Table I). None were observed in any of the uninoculated control cultures.

After observing and recording these alterations for 2 weeks (10 weeks after initiation of the cultures), the tube cultures of transformed cells were trypsinized and passed to additional tubes and bottles. In each case only the tightly packed islands of epithelial cells grew. They have since been successfully subcultured at intervals of 3 to 7 days. It may be noted that these cells retain the same

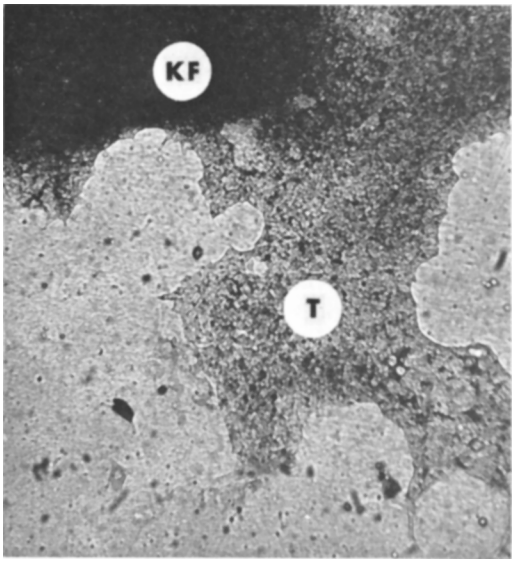

FIG. 1. 9-week-old culture of newborn hamster kidney tissue maintained under a cellophane membrane in a Rose chamber. T—transformed cellular outgrowth from kidney fragment KF.

character of tenacious adherence to one another. In addition, they appear to be highly susceptible to damage by extended trypsin treatment, and to adverse conditions of cultivation, *e.g.*, alkaline or acid medium, infrequent medium changes, etc. Attempts to subculture 6 tubes of the uninoculated control cells at this time (10 weeks after planting) in a like manner were unsuccessful.

Preliminary attempts were made to demonstrate the presence of infectious virus in the transformed cells. For this purpose 10^2, 10^3, 10^4, and 10^5 untreated cells, 2 batches of 10^5 cells treated by ultraviolet irradiation (one for 1 second and one for 10 seconds), and 0.5 ml of medium harvested from transformed cell cultures each were inoculated in duplicate into plates containing a solid monolayer of virus sensitive fetal human kidney

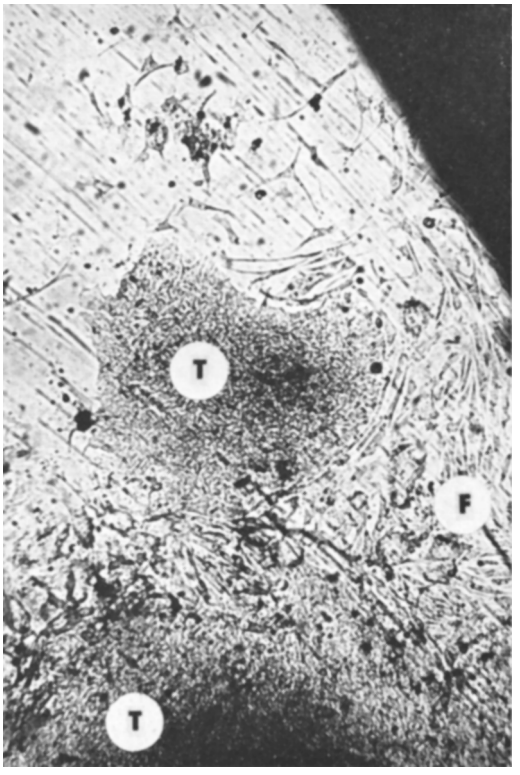

FIG. 2. 10-week-old culture of newborn hamster kidney cells grown in a plasma clot in a Rose chamber. T = transformed foci in a background of old fibroblasts—F.

TABLE I. Appearance of Morphological Transformation in Cultures of Newborn Hamster Kidney Inoculated with Type 12 Adenovirus.

Type of culture		Temp of incubation (°C)	Results	
Vessel	Tissue		Inoc with virus	Uninoculated controls
Tubes	Trypsinized cells in monolayer	37	4/4*	0/10
Rose chambers	Tissue fragments in plasma clot	32	0/4	0/4
		37	1/5	0/6
	Tissue fragment under cellophane membrane	32	0/2	0/0
		37	1/3	0/4

* Numerator = No. of cultures showing transformation. Denominator = No. of cultures in sample after 10 weeks of cultivation.

See text for conditions of inoculation and original number of cultures.

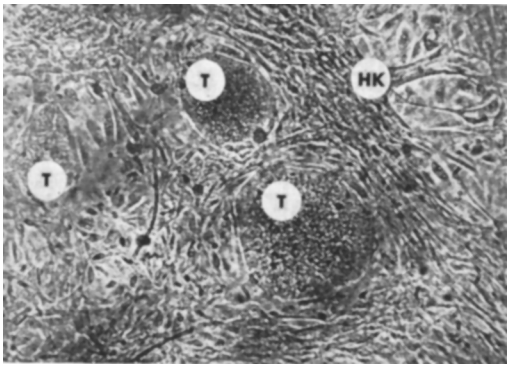

FIG. 3. Foci of transformed kidney cells—T—after 1 week's growth under agar on a monolayer of fetal human kidney cells—HK.

cells. After 2 hours adsorption these cultures were overlaid with agar medium. Careful examination of the cultures over a period of 4 weeks failed to reveal a single area of cytopathogenic activity in the indicator cells. Control cultures inoculated with virus stock exhibited maximum cytopathic effects within 10 days. It is interesting to note that the transformed cells were able to continue to divide into expanding foci even under the agar medium (Fig. 3).

An important discovery in the developing technology of viral oncology was the demonstration of the viral complement-fixing antigen in adenovirus induced tumors(7). Cell suspensions of the 4th and 5th tissue culture passage of the transformed cells were tested for adenovirus type 12 complement fixing antigen (C-F) by Dr. Wallace P. Rowe at the Nat. Inst. of Allergy & Infectious Diseases. These tests revealed that these cells had acquired adenovirus type 12 C-F antigens at a level of 1:8 at the 4th passage and 1:4 at the 5th passage. (The low level of activity may be partially due to the low concentration of cells in the suspension submitted for testing.)

In addition to the evidence cited above that these cells contain adenovirus type 12 C-F antigens, it was deemed wise to test them also for ability to react with antiserum to SV-40 virus. For this purpose, 5th passage transformed cells were grown on coverslips in test tubes. The cells were then washed and fixed with acetone, and reacted with fluorescein labeled gamma globulin prepared from the serum of rabbits hyperimmunized with purified SV-40 virus. Subsequent examination by conventional ultraviolet microscopy failed to reveal fluorescence characteristic of SV-40 infection.† (This same serum previously had been shown to be both highly reactive and specific with cells infected with SV-40 virus.)

Discussion. Evidence that the observations recorded here on *in vitro* transformation of hamster cells by adenovirus type 12 are valid includes the following: a) The virus inoculum is specifically neutralized by anti-adenovirus type 12 immune serum. b) The inoculum was only 2 passage levels from the prototype stock which has been demonstrated to be oncogenic *in vivo* in hamsters. c) Conditions of virus passage in this laboratory precluded contamination by other oncogenic viruses, *i.e.,* no other viral agents have been utilized in the rooms employed for these experiments. d) The transformation has been observed in 3 types of cultures, one of which employed a cellophane membrane which completely separated the cells from the medium added to the culture. This type of culture chamber virtually precludes the possibility of a secondary virus being inoculated onto the cells or of other cell types being superimposed upon the original culture. e) The morphological appearance of the transformed cells is unlike both that observed with SV-40 virus infection of similar cells by Dr. John L. Riggs, working in different quarters in this Laboratory, and that in the published micrographs of cells transformed by polyoma virus. f) Type-specific C-F antigen appears in tumors induced *in vivo* by type 12 adenovirus(8), and such antigen was demonstrable also in cells transformed *in vitro* by this virus in the experiments reported herein. g) These transformed cells failed to react with SV-40 specific fluorescein labeled antibodies. Therefore, the results presented clearly demonstrate successful *in vitro* transformation of

† We are indebted to Dr. John L. Riggs of this laboratory for the examinations by fluorescent microscopy.

Syrian hamster cells by human adenovirus type 12.

A significant question yet to be resolved is why *in vitro* transformation by adenovirus type 12 has not been observed frequently before this time. Certainly many laboratories have been striving to obtain this result. Kitamura *et al*(3) mention in passing an apparent *in vitro* transformation, but such results have yet to be published. It would appear that the procedures chosen for this investigation circumvented barriers imposed by the techniques employed by others, which prevented the necessary virus-cell association required for transformation. Investigations are now in progress to determine which variables in the test system are significant for consistently obtaining *in vitro* transformation by this virus.

Summary. *In vitro* transformation by human adenovirus type 12 of cells derived from newborn hamster kidneys is described. Such transformation was obtained in 3 different types of kidney cell cultures: trypsinized cells, tissue fragments in plasma clots, and tissue fragments separated from the culture medium by a continuous cellophane membrane. This transformation included morphological alteration of the cells, recovery of the ability to divide, and development of an adenovirus type 12 specific complement-fixing antigen without the presence of detectable infectious virus. The transformed cells did not react with SV-40 specific fluorescein tagged antibodies. The malignant potential of these cells *in vivo* is under test. It is anticipated that the procedures employed in these experiments will offer new prospects for the study of human viral oncogenesis.

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Histamine Metabolism in the Brain of Conscious Cats.* (29061)

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Histamine is present in the brain(1,2), and the metabolism of histamine in the brain of anaesthetized cats has previously been studied by perfusion of the cerebral ventricles with radioactive histidine and histamine(3, 4). In the present experiments these radioactive substances were injected into the cerebral ventricles of conscious cats, thereby avoiding possible effects of anaesthesia and of the dissection of the brain for ventricular perfusion.

Methods and materials. A Collison cannula was implanted into the lateral ventricle of 5 cats, as described by Feldberg and Sherwood(5). The injections through the cannula of radioactive substances were made 9 days, or longer, after the implantation. The substances were dissolved in 0.2 ml Tyrode solu-

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