were added for determination of radioactivity in a Packard scintillation counter. The samples were counted for 20 to 50 minutes to obtain statistical significance, and correction was made for quenching by channel ratio. The organic extracts used for chromatography were dried completely, then 50 to 100 μl of ethanol was added for application to Eastman silica gel sheets. This heptane extraction was followed sequentially by identical procedures with two other solvent mixtures: heptane and 1.5 percent isoamyl alcohol, and heptane and 3 percent isoamyl alcohol. The heptane extracted 90 percent of the Δ^9THC, 10 percent of the 11-hydroxy THC, and none of the 8,11-dihydroxy THC, whereas the heptane containing 1.5 percent isoamyl alcohol extracted the remaining Δ^9THC, 60 percent of the total 11-hydroxy THC, and 20 percent of the 8,11-dihydroxy THC. The final extraction with heptane and 3 percent isoamyl alcohol recovered the remaining 11-hydroxy THC, and 60 percent of the total 8,11-dihydroxy THC. The amounts of each of the three compounds were determined by simultaneous equations. The precision and specificity of the method was confirmed three times on three thin-layer chromatography systems (hexane : acetone, 3 : 1; chloroform : ethanol, 19 : 1; chloroform : acetone, 9 : 1). The partitions in all four tissues were the same. The standard error of the mean for these procedures was 1.1 percent ($N = 16$).

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12. Two 0.5-ml fractions were taken from each homogenate and from the final residue after the three organic extractions. To each fraction 1.5 ml of NCS solubilizer (Nuclear-Chicago) was added, and the tissue was digested for 1 to 2 days (until a clear solution was obtained). Two drops of a 1 percent solution of $SnCl_2$ were added (to reduce chemifluorescence) plus 1 ml of ethanol and 10 ml of phosphor. There was, however, considerable variation between duplicates.
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Isolation of RNA Virus from Papillary Tumors of the Human Renal Pelvis

Abstract. *Virus particles were detected by electron microscopy in three papillary cancers of the human renal pelvis. Similar particles were seen in cells from all three tumors in primary culture. An RNA virus was isolated from these tumors. The serum of tumor patients contains neutralizing antibodies against virus isolated from two of the tumors. Initial studies suggest that this agent is not a known human RNA virus.*

There is evidence that viruses may play some role in the causation of certain human tumors, such as cancer of the cervix and breast, Burkitt's lymphoma, and sarcomas (*1*). We report here studies of a virus found in papillary transitional cell cancers of the human renal pelvis.

Papillary transitional cell carcinomas of the renal pelvis were removed from three adult males. The tumors (T_1, T_2, and T_3) were minced finely, and were then placed in flasks (area, 25 cm^2) containing RPMI-1640 medium with 20 percent fetal calf serum, penicillin (400 unit/ml), and streptomycin (100 μg/ml). In addition, minced tumors were suspended in RPMI-1640 medium and then passed through a 0.22-μm filter. Throughout the procedures all specimens were handled separately. The filtrates were then placed on cell cultures of human fibroblasts, testicle, and kidney, as well as on cultures of specific pathogen-free (SPAFAS) chicken embryo fibroblasts (CEF). Normal kidney from each tumor patient was subjected to trypsin digestion and was then propagated in cell culture with the same outgrowth medium. Culture medium from these cells was negative for bacteria and mycoplasma. All work was carried out in a laboratory where there had been no previous studies on RNA viruses of animals.

The original tumor specimens, contiguous and grossly normal kidney, and other control (skin and muscle) and tumor tissue culture cells were prepared for electron microscopy by being fixed in 3 percent glutaraldehyde, osmium tetroxide, and uranyl acetate, and then being embedded in Epon. Fine-structure studies of the original tumors showed two types of virus particles. One type was present mainly in extracellular membrane-bound vesicles; the particles had a diameter of 85 to 120 nm, and resembled C-type virus (Fig. 1). The other particles had a diameter of 60 to 90 nm, and resembled the A-type virus described in simian mammary tumors (*2*), human fibrosarcomas, and breast cancer (*3*). We observed virus particles in the cytoplasm, but not in the nucleus. The tumor cells in culture often showed several large membrane-bound vesicles containing virus particles of varying size and morphology (Fig. 2). The tubular epithelium of normal kidney from two patients had occasional A-type virus particles. Budding of the virus particles from the cell membrane was not observed; thus, it is not known how this virus matures. Others who have observed similar virus-like particles in human tumors either failed to demonstrate budding (*3*), or have seen it only after special treatment of the cells in culture (*4*).

The primary tumor cells in culture grew slowly. The cells were small and appeared to be epithelial; cells in several flasks showed foci of spontaneous degeneration characterized by round, refractile cells that detached from the surface of the flasks. Replicate cultures were chronically infected with infectious virus found in supernatant fluids. Normal kidney cells in culture grew well, and spontaneous cytopathology did not occur. Within 7 days after being planted, virus was isolated from the medium of tumor cells in culture by inoculating filtered culture medium from tumor cells onto CEF cultures. Human tumor fluids, when assayed on CEF cultures, had titers of 100 to 10,000 $TCID_{50}$ per milliliter (*5*). The culture fluids from normal kidney cells had no demonstrable virus when assayed on CEF cultures. Primary cells from the three tumors have been maintained in culture for over 6 months and continue to produce infective virus.

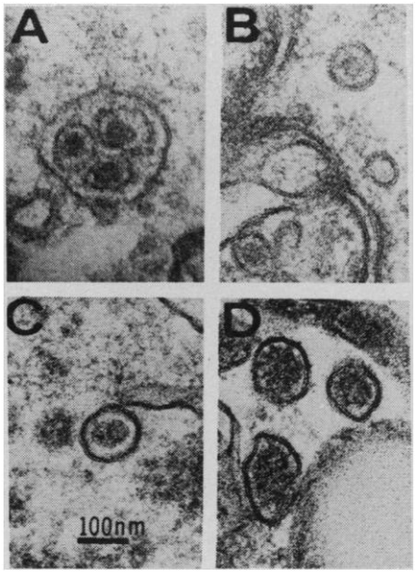

Fig. 1. Virus particles in the original tumor specimens. (A) Extracellular particles in T_1 (× 70,000). (B) Intracellular particles in T_1 (× 70,000). (C and D) Intracellular particles in cells of T_2 (× 70,000).

Ultrafiltrates from tumor homogenates, and from the tissue culture medium of the tumor cells, produced a cytopathic effect in cultures of human fibroblasts, human testicle, and CEF. No cytopathic effect was seen in inoculated human kidney cells. The changes in human fibroblast and testicle cultures appeared gradually, and foci of degeneration were discrete and limited, while a lytic infection occurred in CEF cultures. From 5 to 7 days after inoculation, CEF cells began to round up and detach from the surface of the flask. This cytopathic effect continued until day 10, at which time no healthy cells could be observed. Virus titers of 100 to 1000 $TCID_{50}$ per milliliter could be detected in fluids from CEF cultures from 5 to 10 days after inoculation. The cells manifesting a cytopathic effect contained virus similar in morphology to that seen in the original tumors (Fig. 1); no such effect was observed in uninoculated CEF cultures.

Virus from T_1 and T_2 passaged once in CEF cultures was characterized by techniques described in (*6*). As we isolated the viruses, we designated them EFMU-1, EFMU-2, and EFMU-3. The CEF cultures harvested on the fifth day after infection with virus were frozen and thawed (three times), and the cellular debris was removed by centrifugation at 3,000*g* for 15 minutes. Supernatant fluids containing virus were then banded, by centrifugation (100,000*g*, 16 hours), at the interface of a 15 to 60 percent (weight to volume) discontinuous gradient of sucrose-tris(hydroxymethyl)aminomethane (tris) buffer (*p*H 7.4) in a Spinco SW 27 rotor. The banded material at the interface was removed and again centrifuged in a 15 to 60 percent (weight to volume) linear gradient of sucrose-tris buffer (*p*H 7.4) (200,000*g*, 3 hours, SW 40 rotor). The gradients were fractionated, and each fraction was studied for absorption (254 nm) and density (by micropycnometry). Virus infectivity was located in the fraction with a density of 1.14 to 1.17 g/ml, as assayed on CEF cultures. Similar studies on uninoculated CEF cultures failed to detect an infectious agent.

To determine whether a labeled precursor of RNA was incorporated into the virion, a 1.5-ml inoculum of virus from tumor cells was placed onto each of the three CEF culture flasks containing 3×10^7 cells. Culture medium (15 ml) containing [^{14}C]orotic acid (10 μc/20 ml; specific activity, 60.8 mc/mmole; Radiochemical Centre, Amersham) was then added to each flask. Fluids were harvested 5 days after inoculation. Maximal radioactivity (600 count/min) was located in the fraction with a buoyant density between 1.14 to 1.17 g/ml after centrifugation in a linear sucrose gradient, as previously described.

Synthetic templates (*7*, *8*) and the endogenous reaction (*9*) were used to assay the virus that was present in bands in sucrose gradients for RNA-directed DNA polymerase. Murine leukemia virus preparations (*10*) were controls. Viruses from T_1 and T_2 passaged once in CEF with poly(rA) · $oligo(dT)_{10}$ and poly(rC) · $oligo(dG)_{12-18}$ templates (*7*) incorporated increasing amounts of radioactive label into the material insoluble in acid with time. In a typical endogenous reaction (*7*), murine leukemia virus incorporated 2400 count/min, and EFMU-2 incorporated 2600 count/min after 90 minutes. In this reaction (*7*), prior treatment of virus from T_2 with 50 μg/ml of pancreatic ribonuclease A (Sigma) for 25 minutes at 25°C reduced by two-thirds the number of radioactive counts incorporated into the product, as compared to that obtained without prior treatment of virus. The enzyme activity of murine leukemia virus was reduced by one-half after similar ribonuclease treatment. The absence of dATP in the reaction mixture completely abolished the enzymatic reaction of both viruses. These data indicate that the viruses tested have an RNA-directed DNA polymerase. The activity and similarity of the enzyme from the viruses isolated from T_1 and T_2 to the enzyme of other oncornaviruses (RNA oncogenic viruses) has not yet been determined.

Studies of T_1 virus from human cell cultures, with the orcinol (*11*) and Lowry (*12*) techniques, showed that it contained RNA and protein. Virus infectivity is destroyed by ether. The virus does not agglutinate or adsorb

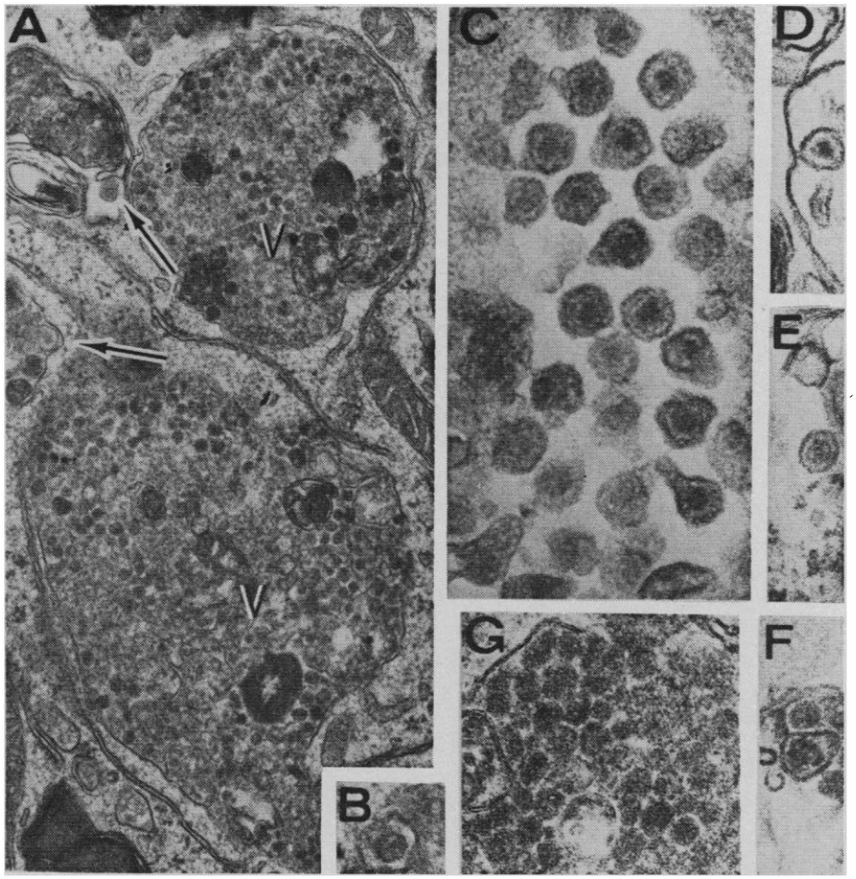

Fig. 2. Virus particles in tissue culture cells. (A) Culture (63 days) from T_1 containing A-type particles in cytoplasmic vesicles (*v*) and extravesicular C-type particles (arrows) (× 30,000). (B) Virus resembling C-type particles in 63-day culture of cells from T_1 (× 70,000). (C) Extracellular particles in CEF, 10 days after inoculation with filtered fluids from T_1 (× 70,000). (D, E, and F) Virus resembling C-type particles in human testicular cells, 37 days after inoculation with filtered fluids from T_1 (× 70,000). (G) Cytoplasmic vesicle in a human testicular cell containing particles, 37 days after inoculation with filtered fluids from T_1 (× 70,000).

human erythrocytes. Also, infectious virus was not produced when susceptible CEF cultures were treated with 50 μg/ml of 5-bromo-2-deoxyuridine 4 hours prior to inoculation with virus from tumor fluids.

The virus neutralization studies indicate that the agents isolated from all three tumors are the same virus. These data show that the serum of three tumor patients neutralized the virus taken directly from two of the three tumors, as well as the virus passaged once in CEF cultures. These studies were carried out by inoculating susceptible CEF cultures with mixtures of serum and virus and then taking the cytopathic effect as the end point of neutralization. In all neutralization studies, we maintained a constant virus concentration, and varied the serum concentrations. Serum from a fourth patient, with an identical tumor that has not yet been studied, also neutralized all three viruses. Also, rabbit and hamster antiserums prepared against EFMU-1 virus from primary tumor cultures specifically neutralized the virus derived from tumor or CEF cultures. These data suggest that the virus in the tumor and that passaged once in CEF are similar. Control studies were done with serums from normal, age-matched patients, from patients with renal adenocarcinoma of the kidney, and from animals before they were injected with the virus. Virus neutralizing antibody was not detected in any of the control serums. Also, specific antiserums against the seven known serotypes of simian foamy virus (*13*), diluted 1 : 10, did not specifically neutralize the virus.

During the past 18 months we have attempted to isolate virus directly from renal adenocarcinomas (hypernephromas), from renal tumors in cell culture, and from normal adult kidney. Thus far, we have failed to demonstrate an agent associated with these tumors by virological and morphological techniques, and we have not detected virus in normal kidney removed from patients of different ages with benign and neoplastic diseases of the urinary tract.

We believe that this is the first report of the isolation of a virus, with similar characteristics, from three malignant human tumors as well as from tumor cells in primary culture. Our initial studies suggest that this virus is not a known human RNA virus, and is therefore either a new human virus or an animal virus commonly associated with these tumors. Although we have established that EFMU has certain characteristics in common with known oncornaviruses, it is unlike known RNA tumor viruses in that it is a cytopathic agent, and it does not appear to mature by budding from the cell membrane.

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Somatosensory Cortex: Structural Alterations following Early Injury to Sense Organs

Abstract. *In mouse somatosensory cortex there are discrete cytoarchitectonic units, called "barrels." Each barrel is related to one sensory vibrissa on the muzzle. Individual vibrissae were carefully injured at birth; 12 to 43 days later, the corresponding barrels proved to be absent. Evidently the sensory periphery has an important influence on the structure of the somatosensory cortex.*

It is of great interest to know whether long-term or permanent alterations in the somatosensory periphery may produce lasting changes in the brain. In the mouse, the vibrissal pad and its cortical representation in the somatosensory field (SI) provide a particularly good situation in which to analyze the structural dependence of the cerebral cortex on the sensory periphery (*1*).

The vibrissal pad contains about 25 large mystacial hairs each of which, along with its associated set of sensory receptors, constitutes a discrete tactile sense organ. Each vibrissa is identifiable at birth, and each is known to project to a distinct cytoarchitectonic unit in the somatosensory cortex of the opposite side. These cytoarchitectonic units, or "barrels," are confined to layer IV of the SI, a layer largely populated by small neurons. The number and arrangement of the vibrissae are constant from animal to animal, and the same is true for the barrels in the cortex. There is also a striking topological similarity in the arrangement of the vibrissae and that of cortical barrels (compare d and e in Fig. 2 with a in Figs. 1 and 2) (*2*). This topologic con-