metabolites formed from BaP and BA by *Beijerinckia* B-836 suggests that these intermediates are not formed from arene oxide precursors since *trans* configuration would then pertain (*3*). It is possible that eukaryotic microorganisms utilize a monooxygenase enzyme system for the oxidation of aromatic hydrocarbons. Thus, *Cunninghamella bainieri* oxidizes naphthalene through *trans*-1,2-dihydroxy-1,2-dihydronaphthalene (*15*), and various fungi produce phenolic metabolites similar to those formed by hepatic microsomes (*16*). In contrast, prokaryotic organisms utilize a dioxygenase enzyme system, and *cis*-dihydrodiols have been identified as intermediates in the bacterial oxidation of several different nonphenolic aromatic hydrocarbons (*17*). The generality of this contrast awaits the isolation of bacteria from different genera that are capable of oxidizing such substrates. Bacterial metabolism to dihydrodiols is of particular interest since several recent reports have established that dihydrodiols of BaP and BA can be further activated by mammalian monooxygenases and can subsequently bind to DNA (*18*).

DAVID T. GIBSON
VENKATANAYARANA MAHADEVAN
Department of Microbiology, University of Texas at Austin, Austin 78712
DONALD M. JERINA
HARUHIKO YAGI
HERMAN J. C. YEH
National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014

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Isolation of Type C Virions from a Normal Human Fibroblast Strain

Abstract. *Type C virions were spontaneously released from cultures of a diploid human cell strain. The virions have properties of known type C RNA tumor viruses and share antigenic determinants with the major interspecies-specific antigen (p30) of simian sarcoma virus. Antiserum to reverse transcriptase of gibbon ape leukemia virus inhibits the reverse transcriptase of the putative human virions and that of simian sarcoma virus, but has no effect on the corresponding enzymes of avian or murine RNA tumor viruses.*

It has been postulated that the genetic information for type C tumor viruses resides in the DNA of normal cells (*1*). Type C viruses can be activated from selected mammalian cell cultures by prolonged propagation in vitro or by exposure to x-irradiation, halogenated pyrimidine derivatives, or inhibitors of protein synthesis (*2*). We now report on the characterization of so-called HEL-12 virions which are released spontaneously from a human fibroblast cell strain.

HEL-12 cells were derived in August 1974 from the lungs of a spontaneously aborted 8-week embryo. The cells grew as fibroblast like monolayers in medium containing fetal calf serum and antibiotics (*3, 4*). Weekly tests for mycoplasma contamination were negative (*5*). Type C virions were not detected by electron microscopy or assays of culture fluids for RNA-instructed DNA polymerase (reverse transcriptase) after the cells were subcultured for a few generations (*6, 7*). However, type C virions were released spontaneously after HEL-12 cells were serially cultured for 6 months. Spontaneous virion production was observed on four different occasions after virus-free, frozen cells were reinitiated in culture (*8*). Despite their conversion to virion releasers, HEL-12 cells maintained a modal distribution of 46 normal chromosomes throughout their life span in culture.

Thin-section electron microscopy of HEL-12 cells revealed typical type C virions, both free in the extracellular space and budding from plasma membranes (Fig. 1) (*6*). The HEL-12 virions incorporated [^{3}H]uridine; in sucrose equilibrium

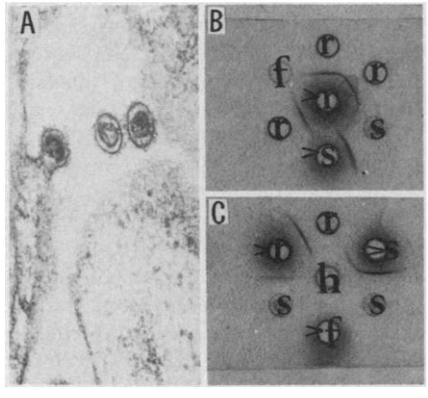

Fig. 1. Morphological and immunological properties of HEL-12 virions. HEL-12 cells were fixed with 2 percent glutaraldehyde in sodium cacodylate buffer, *p*H 7.3, and with 1 percent osmium tetroxide in collidine buffer, *p*H 7.3. Fixed cells were dehydrated in ethanol, embedded in Epon, and thin-sectioned. Sections were stained with uranyl acetate and lead citrate and examined in an RCA-EMU 4 electron microscope (*6*). (A) Electron micrograph of free and budding HEL-12 virions (× 62,000). (B and C) Double immunodiffusion analysis. The HEL-12 virions were purified by equilibrium centrifugation in sucrose gradients and examined for relatedness to R-MuLV (*r*), SiSV (*s*), and R-FeLV (*f*) with the use of monospecific antiserums to purified R-MuLV p30 (>*r*), SiSV p30 (>*s*), and R-FeLV p27 (>*f*). Slides contained 1 percent noble agar and 2 percent polyethylene glycol. Wells were filled with a total of 30 μl of undiluted antiserum or 15 μg of gradient purified virus. After overnight at room temperature, slides were washed for 3 days with six changes of normal saline, dried, and stained with amido black (*9*).

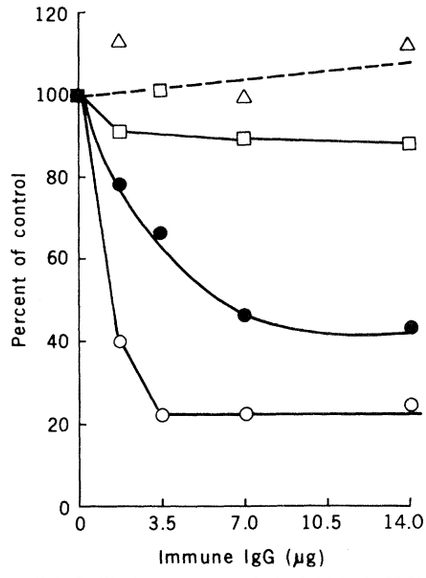

Fig. 2. Relatedness of the reverse transcriptases of HEL-12 virions and SiSV. Gradient-purified SiSV (grown in marmoset lung fibroblasts) and RSV (grown on chick primary embryo fibroblasts) were purchased frozen. G-MuLV was propagated in ER Thy/V cells (*14*). G-MuLV and HEL-12 virions were concentrated from spent tissue culture fluids and purified by equilibrium sucrose gradient centrifugation (*4*). All viruses were stored at −90°C in 50-μl portions in TMG buffer (10 m*M* tris-HCl, *p*H 7.8, 7 m*M* 2-mercaptoethanol, and 10 percent glycerol). Virus dilutions in TMG containing equivalent reverse transcriptase activity were disrupted with 0.1 percent NP-40 detergent for 20 minutes at 0°C. Each tube contained [^{3}H]deoxythymidine triphosphate (TTP) (approximately 25,000 count/min) incorporated into acid-precipitable material in 40 minutes with 1 μg of poly(A)·oligo(dT)$_{(12\text{-}18)}$ as template). Dilutions (5 μl) of disrupted virus were incubated with 5 μl of a solution of bovine serum albumin (50 mg/ml) in 0.6*M* KCl and 10 μl of dilutions of an immunoglobulin G fraction (IgG) of rat antiserum to the reverse transcriptase of GALV or serum from an unimmunized rat. All IgG dilutions were made in 0.1*M* tris-HCl, *p*H 8.0. After incubation for 2 hours at 0°C, the samples were added to a 180-μl reaction mixture containing 0.025 percent NP-40, 25 m*M* tris-HCl, *p*H 8.1, 7 m*M* 2-mercaptoethanol, 0.2 m*M* $MnCl_2$, 1 μg of poly(A)·oligo(dT)$_{(12\text{-}18)}$, 60 m*M* KCl, 0.0083 m*M* TTP (Sigma) and 5 μc of [^{3}H]-TTP (Schwarz/Mann, specific activity, 57 c/mmole). Reactions were carried out for 40 minutes at 37°C and were terminated by the addition of 25 volumes of ice-cold 5 percent trichloroacetic acid containing 2 percent sodium pyrophosphate; radioactivity in the acid precipitable fraction was then determined. Results are expressed as percentages of control reverse transcriptase activity in the absence of immune serum. The sources of viral reverse transcriptase were: △, RSV; □, G-MuLV; ●, SiSV; and ○, HEL-12 virions.

gradients they band at a density of 1.17 to 1.18 g/ml; and they contained reverse transcriptase which utilizes both poly(A)·oligo(dT)$_{(12\text{-}18)}$ (polyadenylate·oligodeoxythymidylate) and virion RNA as templates (*4*, *7*). To characterize HEL-12 virions immunologically, we examined purified culture fluids in double diffusion tests against monospecific antiserums to the interspecies-specific antigen of Rickard feline leukemia virus (R-FeLV), simian sarcoma virus (SiSV), and Rauscher mouse leukemia virus (R-MuLV) (*9*–*11*). The following precautions were taken to exclude contamination of HEL-12 cultures with these viruses. The three virus stocks were purchased (Electro-Nucleonics, Bethesda, Md.) after HEL-12 cells had become virion producers, and the virus lots were disrupted with Nonidet P-40 immediately after we received them (*4*). Moreover, samples of R-FeLV, SiSV, and R-MuLV were not handled in rooms where HEL-12 cells were processed. A component of HEL-12 virions was precipitated by antiserum to the interspecies-specific antigen (p30) of SiSV (SiSV p30) (Fig. 1). This precipitin line was continuous with the line between wells containing SiSV and antiserum to SiSV p30. The result suggests that HEL-12 virions are antigenically related to SiSV. Comparable amounts of antiserum to the R-MuLV p30 and the interspecies-specific antigen (p27) of R-FeLV did not produce precipitin lines with HEL-12 virions, although SiSV p30 cross-reacted with R-MuLV p30 (Fig. 1). Cells from a human myelogenous leukemia expressing a type C virus also reacted strongly with antiserum to SiSV p30, but less strongly with antiserum to R-MuLV p30, and not with an antiserum to R-FeLV p27 (*12*).

The primate origin of unknown type C virus isolates has been strengthened by the use of antibodies against purified reverse transcriptase of gibbon ape leukemia virus (GALV) and SiSV (*13*). We compared the effects of GALV antibody on reverse transcriptase activity in HEL-12 virions, Gross murine leukemia virus (G-MuLV), Schmidt-Ruppin strain of Rous sarcoma virus (RSV), and SiSV (*6*, *10*, *14*). Reverse transcriptase of RSV was stimulated slightly at most antiserum dilutions tested and G-MuLV was inhibited only 12 to 15 percent at the highest antiserum concentration used (Fig. 2). In contrast, reverse transcriptase of SiSV was inhibited by 58 percent and the enzyme of HEL-12 virions by 75 percent. The greater inhibition of reverse transcriptase of HEL-12 virions which was observed in three experiments suggests that the enzyme is more closely related to that of GALV than to SiSV.

During the past year, we have examined ten human cell strains for the induction of endogenous type C viruses by iododeoxyuridine (*2*, *8*). Particles with the morphological, antigenic, and enzymatic properties of type C viruses were repeatedly, but transiently induced from four of ten cell strains (*8*). Two inducible human cell strains, including HEL-12, also released type C virions spontaneously after serial propagation. The possibility that HEL-12 cells became infected with exogenous viruses from the growth medium or fetal calf serum has been excluded by repeated negative assays of medium and serum for reverse transcriptase activity and by electron microscopy. We have scrupulously observed the conditions necessary to avoid infection of the cultures with known RNA viruses. The type C viruses grown in our laboratory are all of murine origin as are the cells that we propagate (*4*). Simian RNA tumor viruses were not grown in the laboratory and those we used were purchased frozen and disrupted immediately. Our antigenic analyses indicate that contamination was unlikely.

In conclusion, this appears to be the first documented isolation of type C virions from a diploid human cell strain. However, further work is necessary to establish unequivocally the origin of the HEL-12 virions. Similar virions have recently been isolated and characterized from human leukemic cell cultures (*13*, *15*), although their relation to HEL-12 virions is unknown.

SANDRA PANEM
EDWARD V. PROCHOWNIK
FRANK R. REALE, WERNER H. KIRSTEN
Departments of Pathology and
Pediatrics, University of Chicago,
Chicago, Illinois, 60637

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Supraependymal Cells of Hypothalamic Third Ventricle: Identification as Resident Phagocytes of the Brain

Abstract. *Cells lying on the ventricular surface of the hypothalamic ependyma of the tegu lizard exhibit the pseudopodial and flaplike processes characteristic of macrophages found elsewhere. Since they ingest latex beads, they may be considered a resident phagocytic system of the brain. The importance of ependyma and ventricular phagocytes as a first line of defense against viral invasion of the brain, as well as their role in the pathogenesis of certain virus-related diseases, is suggested by a number of experimental and clinical observations.*

Recent studies of the ventricular surface of the hypothalamus, with the scanning electron microscope, have revealed cells lying free upon the ependyma (*1*). The cells described in these studies were found in both the supraoptic and infundibular recesses of the third ventricle in several species—monkey, cat, mink, rat, and rabbit—but their function was left an open question. The ultrastructural characteristics of a cluster of large cells on the wall of the infundibular recess of the rat suggested to some workers that the cells were neurons (*2*).

One of us (*3*) has found supraependymal cells throughout the major ciliated portion of the third ventricle of the mouse as well as in the nonciliated recess region. These cells exhibit a variety of forms, have one or more pseudopodial processes extending over the adjacent ependyma, have a usual size of 8 to 10 μm (overall range, 5 to 20 μm) and vary widely in number (from few to 20 to 30) and distribution from one brain to another. The association of clusters of these cells with mounds of debris in the mouse suggested that the cells may have a phagocytic function.

This report describes the phagocytic activity of supraependymal cells in the hypothalamic third ventricle of the tegu lizard, *Tupinambis nigropunctatus*, in response to the presence of latex beads injected into the third ventricle and also included in a culture medium. The tegu lizard was used in this study, since we have found that large numbers of supraependymal cells (50 to 150) are predictably located on a nonciliated vertical depression of the third ventricular wall (Fig. 1a). This depression corresponds to the ventricular ependymal organ or paraventricular organ previously described with light microscopy (*4*).

For the study of normal morphology with the scanning electron microscope, the brains of one female and one male tegu lizard were rapidly removed under sodium pentobarbital anesthesia and immersed in a 1.5 percent phosphate-buffered glutaraldehyde fixative (*p*H 7.4). The ventricles were opened immediately to permit rapid fixation, and diencephalic blocks were dissected while submerged in fixative. The blocks were fixed over 24 hours and dehydrated in ethyl alcohol (through a graded series up to 100 percent), which was then replaced with 100 percent amyl acetate. The blocks were critical point dried with liquid CO_2 and coated with gold-palladium in a vacuum evaporator (*5*). Cells were examined and photographed with the use of a JEOL U-3 scanning electron microscope at an accelerating voltage of 20 kv and a usual tilt angle of 20° to 30°.

To investigate phagocytosis, 25.0 μl of Bacto-latex beads, 0.81 μm in diameter (Difco Laboratories, Detroit, Michigan), were injected into the third ventricle of the anesthetized tegu lizard during direct visualization of the roof of the third ventricle. After 15 minutes the brain was removed. One side of the third ventricle was placed in fixative and the other side was placed in a dish of tissue culture medium NCTC-109 (Microbiological Associates, Bethesda, Maryland) to which several drops of the latex bead solution were added. After 15 minutes at room temperature, the incubated side was also placed in fixative. Subsequent fixation, dehydration, and drying followed the procedures described above for the normal controls.

In all specimens examined, supraependymal cells lay scattered singly and in clusters over the ventricular ependymal organ and were seen rarely on the larger ciliated surface of the third ventricle. In brains not exposed to latex beads all the cells were somewhat flattened, ranged in size from 8 to 10 μm, and had a relatively smooth cell body surface, that is, without folds or microvilli (Fig. 1b). All cells had several cytoplasmic extensions over the surrounding ependymal surface. Some extensions were long, narrow, tapering, and branched, while others were broad flaps with occasional branching extensions. The appear-

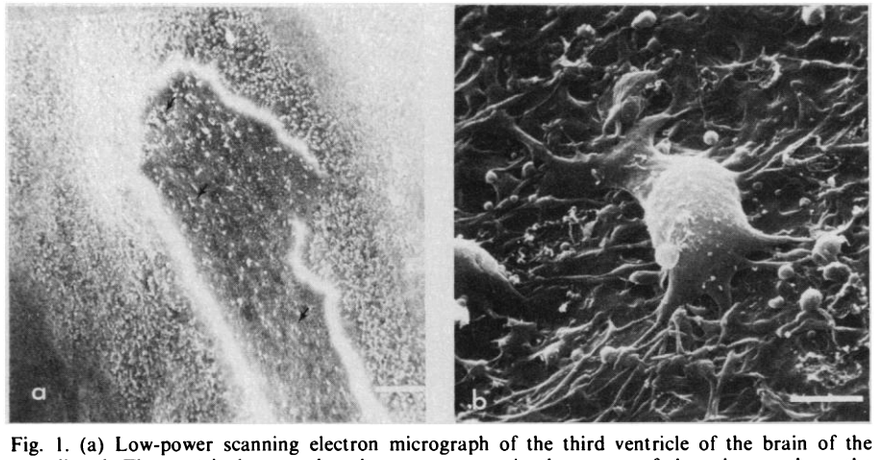

Fig. 1. (a) Low-power scanning electron micrograph of the third ventricle of the brain of the tegu lizard. The ventricular ependymal organ appears in the center of the micrograph as the vertical nonciliated region with a brightly reflecting border. Many supraependymal cells can be seen lying on this organ singly and in clusters (arrows). Cilia are visible on the rest of the ependymal surface. Bar represents 100 μm. (b) Supraependymal cell lying on the ventricular ependymal organ, showing many tapering branching pseudopodial extensions and one broad cytoplastic flap which also branches. The spherical structure lying on the cell body may be a platelet. Bar represents 5 μm.