An Epithelial Cell Line with Chronic Polyoma Infection Established from a Spontaneous Mouse Pancreatic Adenocarcinoma

Edward H. Leiter, Frank J. Malinoski, and John J. Eppig

The Jackson Laboratory, Bar Harbor, Maine 04609

ABSTRACT

The establishment of an epithelial cell line from a mouse pancreatic adenocarcinoma is described. The cell line, designated LTPA, was aneuploid and exhibited many transformed growth properties (rapid growth rate, failure to show density-dependent inhibition of growth, ability to grow in defined medium). A type C oncornavirus was isolated from the culture medium, and electron microscopy also revealed the presence of intracisternal type A particles. LTPA cells carried a persistent polyoma infection which produced only low levels of cytopathic effects. A mycoplasma contamination was also carried. When injected s.c. into Swiss nu/nu mice, LTPA cells formed ductular structures which were destroyed by inflammatory reactions within 3 weeks.

INTRODUCTION

The establishment in continuous culture of human pancreatic carcinoma cells and of pancreatic cells from hyperplastic nodules in azaserin-treated rats has recently been reported (12, 14, 23). The spontaneous occurrence of pancreatic carcinoma in inbred mice is quite rare (20), and this proved to be of particular interest because it carries a transformed growth properties (rapid growth rate, failure to show density-dependent inhibition of growth, ability to grow in defined medium). A type C oncornavirus was isolated from the culture medium, and electron microscopy also revealed the presence of intracisternal type A particles. LTPA cells carried a persistent polyoma infection which produced only low levels of cytopathic effects. A mycoplasma contamination was also carried. When injected s.c. into Swiss nu/nu mice, LTPA cells formed ductular structures which were destroyed by inflammatory reactions within 3 weeks.

MATERIALS AND METHODS

Establishment of Primary Culture. A single pancreatic tumor, approximately 50 mm long, was discovered at The Jackson Laboratory by Dr. L. C. Stevens during routine autopsy of a 12-month-old LT/Sv female mouse. The tumor was received for culture in a dish of nonsterile 0.9% NaCl solution several hr after excision. All subsequent operations were performed under aseptic conditions. Portions of the tumor were fixed in Bouin's, Bouin-Holland, and Zamboni's fluid for light microscopy and Karnovsky's fluid for electron microscopy. Additional tumor samples, as well as pieces of normal heart, spleen, liver, and kidney, were frozen at −70 ° for later complement-fixation testing for oncornavirus. The remainder of the tumor was carried through 5 washes in CMF-HBSS containing penicillin (400 units/ml) and streptomycin sulfate (400 μg/ml) (Antibiotic Solution A). Sections from the interior of the tumor were then prepared with cataract knives and transferred through 4 additional washes of CMF-HBSS plus antibiotics. Small amounts of these tissue sections were then minced with scissors and cataract knives in a Petri dish containing 20 ml of a 10-mg/ml solution of collagenase (type I; Worthington Biochemicals, Freehold, N.J.), in Hanks' balanced salt solution. The material was transferred to a 50-ml Erlenmeyer flask and shaken vigorously (150 cycles/min) at 37 ° in a reciprocally-shaking water bath (Precision Instruments, Palo Alto, Calif.). Although a series of primary cultures were established at different incubation periods, the following description is limited only to the protocol used to establish the primary culture that gave rise to the cell line, LTPA.

Following 35 min of digestion, undigested pieces of tissue were allowed to settle for 2 min at unit gravity, and 10 ml of the enzyme supernatant fluid were removed into a ml centrifuge tube. The cells were pelleted at 500 × g for 3 min; washed once in 10 ml of medium 199 supplemented with 10% FBS, 5 × 10−7 M hydrocortisone hemisuccinate, and antibiotic Solution A; and inoculated in 5 ml of this medium into a 25-sq cm plastic culture flask (Corning Plastics, Corning, N.Y.). To the remaining 10 ml of original digestion mixture, 10 ml of a solution containing 0.5% trypsin (1:250; Difco Laboratories, Inc., Detroit, Mich.) in CMF-HBSS and 6 ml of a solution containing 0.1% hyaluronidase (Sigma Chemical Co., St. Louis, Mo., type I) in CMF-HBSS were added. After an additional 30-min digestion period, the entire supernatant fluid was removed and, following sedimentation of large tissue pieces at unit gravity, the fluid was centrifuged at 500 × g for 3 min to produce a cell pellet. The pellet was washed and inoculated into a 25-sq cm flask in 5 ml of Medium 199 supplemented with 10% FBS, 5 × 10−7 M hydrocortisone hemisuccinate, and antibiotic Solution A; and inoculated in 5 ml of this medium into a 25-sq cm plastic culture flask (Corning Plastics, Corning, N.Y.). To the remaining 10 ml of original digestion mixture, 10 ml of a solution containing 0.5% trypsin (1:250; Difco Laboratories, Inc., Detroit, Mich.) in CMF-HBSS and 6 ml of a solution containing 0.1% hyaluronidase (Sigma Chemical Co., St. Louis, Mo., type I) in CMF-HBSS were added. After an additional 30-min digestion period, the entire supernatant fluid was removed and, following sedimentation of large tissue pieces at unit gravity, the fluid was centrifuged at 500 × g for 3 min to produce a cell pellet. The pellet was washed and inoculated into a 25-sq cm flask in 5 ml of Medium 199 supplemented as above. This flask was allowed to remain in an incubator for 2 hr to permit precocious attachment of fibroblasts, and then the supernatant fluid containing unattached cells was decanted into another 25-sq cm plastic flask. On the fifth day of culture, the cells were placed on a biweekly feeding schedule of 4 ml of medium 199 (Earle's salts):modified MPNL65/CC (13):F12 without serum (2:2:1, designated 2:2:1 medium). Medium MPNL65/CC was modified from that originally reported in that N-tris(hydroxymethyl)methyl-
glycine was omitted; instead, the 2:2:1 medium was made 15 mM in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer (Sigma). Cultures were briefly gassed with 10% CO₂:90% air to adjust pH to between 7.0 and 7.2 and were maintained at 37°. Medium 199, Medium F12, and FBS were all obtained from Grand Island Biological Co., Grand Island, N.Y.

**Subculturing Procedure.** Subcultures were performed by scraping monolayers with a rubber policeman and then by vigorously shearing the floating sheets of cells in and out of a 10-ml pipet in 5 ml of fresh 2:2:1 medium. Cells maintained in serum-containing media were split 1:2 at weekly intervals and maintained in both 25- and 75-cm² plastic culture flasks. Plating efficiencies were not routinely determined because cells were transferred in sheets, but it was estimated that the plating efficiency based upon single-cell suspensions plated into serum-containing medium was around 60 to 70%. Thirty-eight serial passages were made in 1 subline over an 11-month period. Representative cultures from this subline were selected for frozen storage after the 1st and 28th serial passages. The cultures in 25-cm² plastic flasks were treated for 4 hr in 2:2:1 medium containing Colcemid (1 µg/ml; Grand Island Biological Co.). Detached cells were removed, and Giemsa-stained preparations were made.

**Mycoplasma testing** was performed as described by Barile (1). Living cell cultures were shipped to Microbiological Associates (Bethesda, Md.) for murine antibody production testing for the following viruses: ectromelia, poloma, lymphocytic choriomeningitis, mouse hepatitis, mouse adenovirus, Reo 3, Sendai, GD VII, H-1, K, and pneumonia virus of mice. The presence of budding type C oncornavirus was assessed by labeling nonconfluent cultures with [³H]uridine (3 Ci/mmol, 10 µCi/ml of medium; New England Nuclear) and processing the culture medium for labeled virus as described by Hackett (8). Concentrated virus samples were ultracentrifuged through 15 to 60% linear sucrose gradients at 205,000 × gavenger for 75 min in a SW50.1 rotor (Beckman Instruments, Fullerton, Calif.). Density marker micule beads (1.15 and 1.20 g/ml) were placed in each gradient as references.

**Tests of Transformed and Neoplastic Properties.** Cells were tested for their ability to grow in soft agar according to the method of MacPherson and Montagnier (17). In a test of tumorigenicity, 10⁴ to 10⁶ cells in 0.1-ml volumes were inoculated dorsally and s.c. into newborn LT and LT x C57BL/6J F₁ mice, as well as s.c. and i.p. into adults. Doses of 2.4 × 10⁴ cells in 0.2-ml volumes were also injected i.d. into male and female Swiss nu/nu mice of various ages; these latter mice were all given 450 R of X-rays 24 hr prior to injection (21).

**RESULTS**

**Histopathology of Parent Tumor.** Light microscopic examination of the pancreas revealed a well-differentiated adenocarcinoma of the pancreatic ductal epithelium. The bulk of the tumor was composed of glandular ductal structures lined by a columnar epithelium resembling biliary-pancreatic duct epithelium (Fig. 1). Mitotic figures were seen in this epithelium. The apical (luminal) portions of these cells in many of the ductules stained strongly positive with mucicarmine. The most striking histopathological feature was the presence of numerous polymorphous crystalloid deposits apparently secreted into the luminal spaces (Fig. 1). Intact exocrine pancreatic tissue was largely confined to the periphery of the organ. Many acini exhibited necrotic changes and were surrounded by a mononuclear cell infiltrate. An occasional intact islet of Langerhans was seen in association with exocrine tissue at the tumor periphery. Considerable areas of the tumor were fibrotic.

**Areas of the parent tumor fixed for electron microscopy**
revealed only necrotic cells evincing poor preservation of ultrastructure. On the basis of the presence of electron-dense granules resembling zymogen granules, these cells appeared to be exocrine cells sampled from the periphery of the tumor (Fig. 2). Considerable numbers of intracisternal type A oncornavirus particles were observed in these cells (Fig. 2, inset). No other types of virus were detected at this time. Also appearing in the cytoplasm of and in extracellular spaces adjacent to these presumed exocrine cells were large electron-dense crystalline rods that varied in length and width. They were of uniform electron density and were without recognizable substructure. These crystal-like structures were much smaller than the crystallloid deposits observed by light microscopy in the luminal spaces of ducts.

**Primary Culture and LTPA Cell Line Establishment.** Cells in primary culture initially attached as small epithelioid clusters or as outgrowing sheets from a larger piece of tissue (Figs. 3 to 5). Cell surfaces that were not in contact with other cells showed extensive expansion and ruffling; but as cells became organized into monolayers, they evinced a compacted polygonal structure with centrally positioned nuclei containing 2 to 4 prominent nucleoli. A few fibroblasts were observed at the perimeters of the epithelioid cell monolayers, and occasional clusters of islet of Langerhans cells were scattered throughout the flask (Fig. 4). The mixture of 3 complex media (199:F12:MPNL65/CC, 2:2:1) was used in the hope of establishing a line of islet cells from the tumor, since preliminary experiments with cultured mouse islet cells had indicated that the media in combination maintained islet cells in the absence of serum for 2 weeks. Islet cells, however, remained postmitotic, whereas many dividing cells were observed in the rapidly expanding epithelioid monolayers. No significant levels of insulin were present in the medium changes after the Day 9 of culture. No amylase or trypsinogen activity was detected by 72 hr *in vitro*. By Day 6 of culture, multinucleate cells and the presence of multipolar cell divisions were noted (Fig. 5). Additionally, highly refractile crystals, which were often rhombic, were present in the culture on the day of initiation and remained on the bottom of the flask and in association with the outgrowing monolayers through subsequent medium changes (cf. Figs. 3 to 5).

Large areas of the flask were covered with the epithelioid cell monolayer by the second week of culture. By Day 16 of culture, many of the epithelioid cells in the monolayers began to evince extensive cytopathic changes, especially gross swelling, multinucleation, and the appearance in the cytoplasm of highly refractile vacuolation. By the 35th day *in vitro*, cellular degeneration was widespread throughout the flask; in an attempt to preserve the culture, the defined medium was supplemented with 10% FBS for 48 hr and then the culture was returned to serum-free 2:2:1 medium. On Day 38 a transformation in growth properties was exhibited by a surviving cluster of epithelioid cells in 1 corner of the flask. These cells were actively dividing and exhibited extensive surface membrane activity. Exhibiting a dense perinuclear granulation and the same intracytoplasmic refractile vacuoles observed in the epithelioid cells prior to acquisition of transformed growth characteristics, they spread rapidly and colonized the flask. Isolated clusters of cells extended long cytoplasmic bridges to contact adjacent clusters. Within 1 month of the detection of the initial transformed cluster, the culture had grown to confluence, with piling up of cells in some areas. Upon the first subculture, attachment of cells was observed with 24 hr of plating in serum-free medium. The attachment of the cells to plastic or glass substrata was very tenuous in that brief mechanical agitation in CMF-HBSS was sufficient to detach them. The morphology of the cells remained distinctly epithelial-like throughout serial subcultures (Fig. 6). The cells grew as sheets of cuboidal cells in close contact with one another. Nuclear:cytoplasmic ratios were high, and cells in mitosis were seen throughout the monolayer. The MI dropped only slightly as cell density in the culture vessel increased. (MI = 2.25 in nonconfluent cultures versus 2.15 in confluent monolayers.)

Although the cells in the monolayer had been uniform in appearance prior to the first subculture, within 3 days following subculture at Day 68 *in vitro*, giant cells appeared in the monolayer with increasing frequency. These cells were multinucleate, with nuclei containing variable numbers of nucleoli (Fig. 7). These giant cells often appeared to arise from fusion of adjacent cells. Feulgen-stained preparations suggested that nuclei may also have been fusing. These events were cytopathic; nuclear contents appeared to undergo a crystallization into highly refractive spheroids that persisted in the culture medium long after cytolysis of the cell itself (Fig. 7, arrow). Gaps in the monolayer produced by giant cell cytolysis were lined by cells that arranged themselves in a cuboidal fashion that simulated pancreatic ductal structures. The giant cells were continuously being generated through serial passages and they usually comprised between 1 and 10% of the cells in the monolayer.

Despite the continuous generation of cytopathic cells, the cell line established, designated LTPA, proliferated rapidly and showed some transformed growth properties, namely, growth in soft agar at a low plating efficiency (1.6% when seeded at 10⁶ cells) and failure to show density-dependent inhibition of growth. In the presence of 10% FBS, single cloned cells went through 6 generations in 6 days (generation time, 24 hr). A growth curve for higher-density populations is shown in Chart 1; following lag phase, population-doubling times were at about 24 hr after introduction of a medium change. The inclusion of hydrocortisone in the medium did not affect generation time (Chart 1). MT was calculated to be 46 min with a MI of 2.25 and a generation time (MT + IT (interval time)) of 24 hr in

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\text{MT/(MT + IT) × ln 2 = ln(1 + MI)}
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Hydrocortisone was originally incorporated into the primary culture medium to inhibit fibroblastic outgrowth. Although this steroid was shown not to affect the growth rate of the fibroblast-free LTPA cell line, it was retained as a medium component because its inclusion seemed to produce a more compact, polygonal phenotype of cells in the monolayers.

**Chromosome Analysis of LT Cell Line.** Giemsa-stained chromosome preparations of cultured cells taken shortly...
after the first subculture of the primary flask revealed a modal diploid number of 40 chromosomes with no meta-centric chromosomes present. The cells studied at the 28th serial passage, however, were all aneuploid with chromosome numbers between 50 and 58. Of interest was the presence of large metacentric chromosomes (Fig. 8).

**Virological and Mycoplasmal Screening.** The parent tumor exhibited a high titer of type C protein (M.W., 30,000) group-specific antigen, as did nontumorous spleen, liver, and kidney tissue from the same mouse. Cells taken at the second serial passage and labeled with [3H]uridine released labeled particles into the medium that banded in linear sucrose gradients at a density of 1.16 g/ml characteristic of type C virus (Chart 2). No evidence of labeled mycoplasmas was seen in these gradients. However, when antibiotic-free samples of spent medium sampled after 10 subcultures and onward were plated onto Mycoplasma broth, mycoplasmal contamination was detected. While the mycoplasmal contaminant was not classified serologically, in the 1 instance in which mycoplasmas were observed during electron microscopic examination of monolayers, the morphology closely resembled that of bovine Mycoplasma (M. arginini?).

Murine antibody production testing performed at Microbiological Associates was negative for all virus screened except PV. Positive tests for PV occurred only in response to injections of cellular homogenates treated with receptor-destroying enzyme; the virus was tightly cell associated (M. Collins, personal communication). Incubation of the homogenates in mouse embryo tissue cultures produced cytopathic effect within 1 week. Indirect fluorescent antibody staining of LT monolayers fixed in absolute ethanol revealed positive immunofluorescence for PV in only 1% or less of the cells. Strongest fluorescence was intranuclear, but perinuclear zones of staining were common. Presence of PV was further confirmed by hemagglutination testing at Microbiological Associates.

**Ultrastructural Observations of LTPA Cell Line.** No differences were observed in the ultrastructure of the LTPA cell line maintained in defined or serum-containing media. A typical cell is illustrated in Fig. 9. LTPA cells generally formed close associations with their neighbors, and intracellular spaces were generally permeated by microvillous surface projections. Nuclei were large, were polymorphic, and contained dense heterochromatic areas. The perinuclear cytoplasm was densely packed with fusiform mitochondria. Bundles of filaments, lysosomes, and lipid droplets were commonly seen. Goï complexes and rough endoplasmic reticulum were not well developed. Numerous clusters of free ribosomes (polysomes?) were widely distributed throughout the cytoplasm. In some cells, intracisternal type A particles (Fig. 10) and immature budding type C particles (Fig. 11, inset) were observed. Mature type C particles were not seen, although the biochemical evidence presented in Chart 2 suggested that the cells may have shed mature particles into the culture medium.

The crystallloid structures observed in the parent tumor and the primary culture were not seen in the LTPA cell line. Instead, in approximately 1% or less of the cells examined, characteristic light and dark polyoma icosahedral virions, 450 Å in diameter, were distributed widely throughout the nucleoplasm (Fig. 11). Foci of these virion cores were organized into highly regular crystalline lattice structures (Fig. 12). Large vacuoles were also seen inside the nucleoplasm adjacent to these virions. Apparently, concomitant with virion crystallization, destruction of the cytoplasm occurred, since the viral crystals were usually associated with isolated nuclei in or on the monolayer (Fig. 13). All cytoplasmic polyoma virions were contained within membrane-bound lysosome-like granules; free virions were...
never observed either in the cytoplasm or at either side of the plasma membrane.

**Neoplastic Potential.** Injection of LTPA cells cultured both in serum-free and serum-containing media failed to produce tumors in adult LT or LT × C57BL/6J F₁ mice of either sex after a latent period of 5 to 8 months following injection of 10⁸ to 10⁹ cells s.c., i.p., or i.m. Injection of LTPA cells into neonates failed to produce tumors. However, 2.4 × 10⁶ cells injected i.d. into 3 irradiated 5-month-old female Swiss nu/nu mice did produce local cutaneous nodules within 10 days. These nodules underwent regression and were resorbed within 1 month. Histopathological examination of skin containing these regressed nodules at Day 30 revealed an area of considerable cellular necrosis in the s.c. space. A few duct-like structures, resembling pancreatic ducts, were interspersed throughout this zone (Fig. 14). Of a group of 3 male nu/nu mice (2 to 4 weeks old) receiving an i.p. injection of 1.68 × 10⁶ cells each, one exhibited a reticulum cell sarcoma within the pancreas upon autopsy 1 month later. Germ-free nu/nu mice have been reported to have a high spontaneous incidence of this type of neoplasm (19). No other tumors were found in this group.

**DISCUSSION**

The epithelial cells that showed many properties associated with transformed cell growth after 1 month in primary culture, most probably, were derived from the hyperplastic duct cells seen in the parent adenocarcinoma. Although differentiated islet cells were maintained in the primary culture throughout the first month, they were postmitotic, and insulin levels in the medium declined with increasing time in culture. Differentiated exocrine cells were not maintained in the serum-free medium; assays of the spent medium for amylase and trypsinogen were negative after 72 hr of culture. It is possible, however, that the transformed cell type may have arisen from dedifferentiated acinar cells. This was not considered likely because of the poor ultrastructural condition of the original exocrine cells prior to culture (cf., Fig. 2). The paracrystalline structures observed in what appeared to be necrotic exocrine cells in the parent tumor very closely resembled those structures induced by Forssmann and Metz (7) in exocrine pancreas of rats treated with p-chlorophenylalanine. These exocrine cell structures were intracytoplasmic and quite distinct from those crystals seen by light microscopy in the duct lumina of the parent tumor. These latter structures resembled bile crystals, and similar crystallloid deposits have been observed by Poel et al. (20) in biliary tract mucosal cell adenomas of the mouse.

The detection of PV in the cells shortly after transformed growth properties became evident was of interest in view of the well-known capacity of this virus either to transform cells in culture or to produce cytopathic effects (4). The question of whether the tumor donor was the source of the PV and whether a casual association may have existed between the presence of PV and the pancreatic adenocarcinoma cannot be answered. Although no evidence of a productive PV infection in the LT mouse colony could be found by screening the sera from selected animals for anti-PV antibodies, it seems probable that the LT mouse in which the adenocarcinoma was found was the source of the virus. The LTPA cell line, with its low but chronic incidence of cytopathic effect in 1 to 3% of the cells in a monolayer, seems to represent a stable cell-virus association as has been described in certain lines of cultured rodent cells by others (2, 9, 18). Although immunofluorescent antibody staining showed positive fluorescence around the perinuclear cytoplasm of some cells, ultrastructural examination failed to reveal free PV particles outside the nucleus except for small numbers occasionally seen in lysosome-like structures. The appearance of PV virions in the nucleus was essentially as described by Biberfeld and Ringerz (3). Hare and Morgan (10) have discussed several potential mechanisms whereby PV virus might persist within rapidly dividing host cells in an infectious state but replicate autonomously in only a few. The actual mechanisms responsible for maintaining this carrier state are unresolved, although these workers have excluded a protective role for interferon.

The ability of PV to produce neoplasms when injected into rodents has been demonstrated (22). However, the presence of PV proliferating in a cell line over a long period of time does not necessarily increase the neoplastic potential of the cells (2, 9). Despite the rapid growth rate and aneuploid chromosomal complement of the LTPA cell line, it showed no tumorigenicity when introduced into immunocompetent LT adult mice or into neonates. The presence of a mycoplasmal contaminant, although held in check by antibiotics when the cells were in culture, may have produced necrosis of the cells when the contaminant was injected into mice. Fong and Fong (6) have documented a cellular necrosis similar to that observed around the injected LTPA cells in nude mice when they attempted to produce tumors in Syrian hamsters using a Mycoplasma-infected tumor cell line. It is not known whether the development of glandular duct-like structures observed in immunosuppressed nu/nu mice given injections of LTPA cells reflected a neoplastic potential of these cells or simply the ability to express a differentiated morphology at an ectopic site.

The LTPA cell line represents the first established line from a pancreatic cancer of the mouse and is of interest with regard to its viral components. The presence of intracisternal type A and budding type C particles detected by electron microscopic observation may reflect activation of these oncornaviruses as a result of the PV infection. Harrison and Murphy (11) have demonstrated such an activation in the pancreata of 3-week-old ICR Swiss mice infected with Venezuelan equine encephalitis virus.

**ACKNOWLEDGMENTS**

The authors thank Sue Cook for her technical assistance, Dr. Leroy C. Stevens for providing the tumor, Kathy Triman for doing chromosome analysis, and Dr. David D. Myers and Dr. Hans Meier for assistance in viral screening of this cell line. Special thanks is owed to Dr. Henry C. Outzen for supplying Swiss nu/nu mice.

**REFERENCES**


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Fig. 1. Paraffin section of original pancreatic adenocarcinoma showing the presence of crystalloid structures within luminal spaces. Arrow, mitotic duct cell. & E. × 1,520.

Fig. 2. Ultrastructure of necrotic cell in original tumor, showing presumed zymogen granule (z) and long, intracytoplasmic, crystalloid structures. × 20,885. Inset, intracisternal type A virus particles. × 65,000.

Fig. 3. Outgrowning epithelioid cell cluster, 18 hr in culture. Note large refractile crystal. Phase contrast, × 480.

Fig. 4. Cluster of pancreatic islet cells, Day 6 in culture. Note crystals in the medium. Phase contrast, × 400.

Fig. 5. Outgrowing epithelioid cell monolayer showing mitotic cells. Arrow, a multipolar nuclear division. Day 6 of culture. Phase contrast, × 406.

Fig. 6. Epithelioid appearance of an LTPA cell monolayer at the 8th subculture, 148 days in vitro. × 600.

Fig. 7. Giant multinucleate cell formation within an LTPA cell monolayer at Day 68 of culture. Arrow, a nucleus the contents of which appear to have crystallized. Phase contrast, × 600.

Fig. 8. Giemsa-stained chromosome preparation from a LTPA cell (2n = 56) at the 28th passage. Arrows, long metacentric chromosomes. Oil immersion, × 3,500.

Fig. 9. Electron micrograph of LTPA cell. × 5,900.

Fig. 10. Electron micrograph of LTPA cell showing presence of intracisternal type A particles (arrows). × 22,211.

Fig. 11. Ultrastructural appearance of numerous, individual PV particles within an LTPA cell nucleus. × 11,550. Inset, an immature type C particle budding into the intercellular space between 2 cells. × 42,000.

Fig. 12. Ultrastructural appearance of intranuclear foci of PV crystallization. × 12,855.

Fig. 13. Electron micrograph of a nucleus from a lysed LTPA cell. Note the near-complete crystallization by PV particles of the nucleus. The cytoplasm of another cell surrounds it. × 24,355.

Fig. 14. Light micrograph showing s.c. formation of duct-like structures by LTPA cells injected into Swiss nu/nu mice. × 800.
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*Cancer Res* 1978;38:969-977.

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