Transformation of Renal Cells from a Prosimian by Simian Virus 40 (SV40).* (31224)

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(Introduced by A. W. Frisch)
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The papovavirus SV40 is oncogenic for newborn hamsters(1,2) and can induce *in vitro* transformation in cultured cells derived from several species of animals(3-8). The tumors and transformed cells are both characterized by the presence of a soluble complement-fixing (CF) antigen which is synthesized under the control of the SV40 genome and is distinct from the antigen associated with the virus(9). Recently, another CF antigen associated with cell surface of SV40-transformed cells was reported(10).

This article describes transformation by SV40 of cultured kidney cells from a prosimian, *Lemur mongoz* (mongoose lemur).

Materials and methods. Kidney cells in the 2nd passage from a mongoose lemur were grown in Falcon plastic flasks No. 3017. The monolayer cells were infected with $10^{6.7}$ $TCID_{50}$ of SV40. Since the experiment was designed initially for susceptibility studies, maintenance medium consisting of medium 199 (Earle's BSS and 0.15% $NaHCO_3$) supplemented with 3% calf serum was added to the infected cells after 2 hours of absorption. All media used throughout the following experiment included 100 u/ml of penicillin and 100 μg/ml of streptomycin.

The microplate procedure was used in the CF test as described by Sever(11) and modified by Black *et al*(9) and Huebner *et al* (12). The CF antigen was prepared from a frozen and thawed preparation of 10% cell suspension previously grown as a monolayer in 32-oz Blake bottles. Two aliquot samples, one of which was from a heat inactivated cell-free extract, were centrifuged at 63,768 $\times$ *g* av for 2 hours in a Beckman model L ultracentrifuge. The pellets were resuspended in the saline diluent containing $10^{-4.48}$ M $MgCl_2$ and $10^{-4.18}$ M $CaCl_2$.

Results. A few vacuolated cells were observed 20 days after initial infection and began to increase in numbers from the 30th day. At 40 days growth medium NCTC109 (13) with 20% fetal calf serum was substituted and medium changes were made every 3 to 4 days. Within 60 days nearly all cells in the monolayer appeared to be vacuolated. On day 75, predominantly isolated cells were seen scattered throughout the flask, but in one area measuring 2 mm in diameter, an aggregate of cells was observed to be concentrated (Fig. 1b). Control cells showing granulation without any detectable vacuoles were at this time detaching in sheets.

On the 119th day after infection an outgrowth of cells was observed at the periphery of the "colony." Two weeks later when the outgrowth seemed sufficiently established, the old and portions of the new growth areas were removed with a rubber policeman, and the flask rinsed 4 times with 5-ml volumes of phosphate buffered saline and once with growth medium prior to further incubation. As observed through a phase contrast microscope, the cells were epithelioid with those in the central portion of the mass showing a lower cytoplasm:nucleus ratio (Fig. 1c, d).

On subculturing, these cells proliferated rapidly as evident by pH changes, high mitotic index, and time required for monolayer formation. Contact inhibition was not noticed in cells which were permitted to grow without transferring except for medium changes. Such cells grew into multilayered sheets. On the 2nd transfer, medium 199 supplemented with 20% fetal calf serum was substituted.

As shown in Table I virus titers of the infected cells progressed correspondingly with increase of vacuolated cells. Maximum titer

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was obtained in samples taken when vacuolation of the monolayered cells was most extensive. Virus was detected consistently in BSC-1 cells from 10^{-1} dilution of expended medium of transformed cells in the 2nd, 5th, 9th and 16th passages.

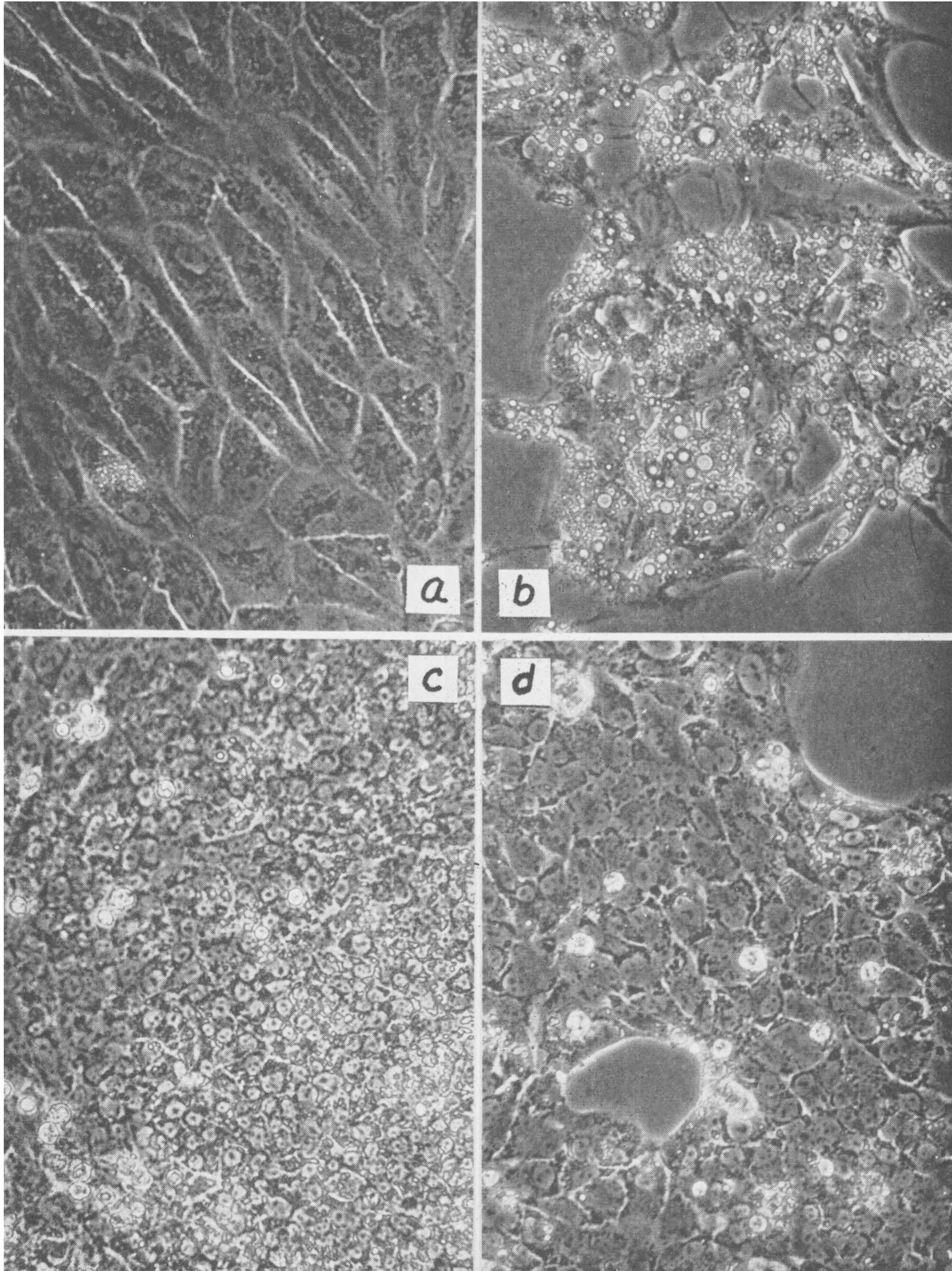

FIG. 1. Kidney cells from *Lemur mongoz*. a) Normal cells. b) SV40-infected cells at 90 days. c) Central growth area of transformed cells. d) Outer growth area of transformed cells. Magnification: 220 ×.

TABLE I. SV40 Titers in Expended Medium of Cultured Kidney Cells from Mongoose Lemur.

Days after infection	Virus titer in BSC-1 cells
20	10^1
32	10^2
44	$10^{5.2}$
57	$10^{7.5}$

Slide preparations stained with hematoxylin and eosin showed characteristics not seen in primary cultured cells. Nearly all nuclei contained 1 to 2 large pleomorphic nucleoli and 3 to 5 small nucleoli. An occasional syncytial cell was also present. Vacuolated cells were not detected but a few cells exhibited an eosinophilic cytoplasmic mass. The significance of these masses is presently not certain. They were also observed in uninfected mongoose lemur kidney cells in the 2nd passage and in BSC-1 cells, although in much lower frequency. In both 48- and 72-hour preparations large numbers of cells in various mitotic stages were seen throughout the slides.

Black *et al*(9) and Rapp *et al*(14) found that the tumor CF antigen was inactivated when incubated at 50-56°C for 30 minutes, whereas viral CF antigen was not affected. Furthermore, high speed centrifugation sedimented the latter antigen. Results given in Table II show that a soluble antigen capable of fixing complement with tumor-bearing hamster antiserum was present in the cell-free extract of the transformed cells. The antigen was sensitive to heat but remained in the supernatant fluid even after ultracentrifugation. The slight reaction in the sediment of the unheated preparation was due to a small volume of supernate left at the bottom of the lusteroid tube. Lysis of the sheep red blood cells did not occur when serum sample from a normal hamster was used.

Discussion. Cytolytic effects of a high multiplicity of SV40 to cultured kidney cells from a mongoose lemur progressed similarly to infected cells of African green monkey kidney except for a marked temporal difference. These cytopathogenic effects by viral inducement were apparent but a few cells may have degenerated nonspecifically. In view of the widespread infection to cells by SV40 resulting in vacuolation and degeneration with loss of most but not all cells, the original parent cell(s) at least must have possessed a certain degree of resistance to the lytic properties of the virus.

The use of growth medium soon after infection or a lower multiplicity of virus might have induced the formation of more foci of transformed cells. On the other hand, even under these conditions, Black and Rowe(15) noted that the transformation of hamster kidney cells occurred at a low frequency.

Prior to the outgrowth of new cells, the concentration of cells may have been maintaining a critical balance between degeneration and virus synthesis. It is possible that a low stage of steady state condition similar to that described for mouse embryo cells infected with polyoma virus(16) was occurring. The group of cells in a sufficiently close proximity could have contributed to the balance by symbiotically providing necessary factor(s) to sustain survival. In any regard, through some mechanism presumably under the influence of the viral genome, one cell was apparently "triggered" to multiply without the growth control mechanism differentiating transformed cells from normal cells.

Recently human diploid cells transformed by SV40 were reported to undergo a "crisis" stage characterized by spontaneous cellular degeneration(17). The time at which these cells entered the stage depended on the "phase" of cultivation that the cells were in-

TABLE II. Titer of Complement Fixing Antigen in SV40-Transformed Cells.

Cell free extract transformed cells	Titer of CF antigen: SV40 hamster tumor antiserum* (4 units)	Titer of CF antigen: Normal hamster serum*
Unheated	16	0
Heated*	0	0
Unheated (ultracentrifuged super.)	16	0
Heated (ultracentrifuged super.)	0	0
Unheated (ultracentrifuged ppt.)	2	0
Heated (ultracentrifuged ppt.)	0	0

* Inactivated 56 C for 30 min.

fected(18). The lemur kidney cells used in this study were infected at phase II. The question of whether these transformed cells will also enter into a crisis stage is under investigation.

Summary. Cultured kidney cells from a prosimian, mongoose lemur were infected with SV40, which induced a typical cytopathic effect after an extensive period of incubation. On the 119th day after infection, transformed cells developed from a focus of cells showing a certain degree of degeneration. The new growth of cells grew rapidly, lacked contact inhibition, formed multilayered cells, and synthesized SV40 and a soluble complement-fixing tumor antigen.

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Occurrence of SV40 Neoplastic and Antigenic Information in Vaccine Strains of Adenovirus Type 3.* (31225)

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Adenovirus type 7 strain LL, which has been employed since 1957 for the production of adenovirus vaccine, was shown to induce tumors in hamsters that contained SV40 complement fixing tumor antigen. This antigen, in turn, elicited antibodies to SV40 T (cell associated) and tumor antigens(1-3). This property of the virus was attributed to incorporation of part of the SV40 genome into the capsid of strain LL during passage of the adenovirus in cultures of rhesus monkey kidney cells infected with SV40 virus(1-3). A similar phenomenon was described by Lewis *et al*(4), as occurring in the JF strain of adenovirus type 3. It is the purpose of this note to record and discuss the oncogenicity of 2 lots of adenovirus type 3 strain JF which were employed as seed stock by 2 separate laboratories for the production of adenovirus vaccines and to corroborate the evidence that SV40 genetic material was encapsidated by the adenovirus particles.

Materials and methods. Viruses. Adenovirus type 3 strain JF was isolated in cultures of rhesus monkey kidney (RhMK) cells from throat washings of a child with pharyngo-

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