IN VITRO
Vol. 8, No. 5, 1973

DEVELOPMENT AND CHARACTERIZATION OF CELL LINES FROM SUBHUMAN PRIMATES*

ROSLYN E. WALLACE AND PAUL J. VASINGTON

Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York 10965

JOHN C. PETRICCIANI, HOPE E. HOPPS, AND DOUGLAS E. LORENZ

Bureau of Biologics, Food and Drug Administration, Rockville, Maryland 20852

ZDENEK KADANKA

University of Toronto, Connaught Medical Research Laboratories, Willowdale, Ontario, Canada

SUMMARY

Seven epithelial cell lines derived from kidney and 20 fibroblastic cell lines deriving from lung, heart, muscle, kidney, and skin tissue of five rhesus and six African green monkey fetuses have been established and propagated in culture. Four epithelial and two fibroblastic cell lines resumed cell multiplication after a period of growth decline, and these lines developed cytogenetic changes and growth characteristics of cells capable of unlimited growth in vitro.

Sixteen of the fibroblastic lines derived from lung, heart, muscle, or skin were characterized by a finite life consisting of a period of active cell multiplication, followed by growth decline, senescence, and cell death. Fibroblasts derived from lung appeared to have the greatest growth potential in terms of total population doublings, and fibroblastic lines from rhesus monkeys were usually capable of more doublings than similar lines from African green monkeys. All fibroblastic lines were predominantly diploid during active growth from passages 1 to 30, but several lines developed karyological changes preceding or during growth decline and senescence.

All lines tested were found sensitive to a number of human viruses. All tests on these cells for microbial agents and for tumorigenicity have been negative, and they have been preserved by freezing without loss of properties.

These cell lines may be useful as standardized substrates in studies requiring nonhuman primate cells.

Of 28 cell lines[1] developed from fetal tissues of rhesus (*Macaca mulatta*) and African green (AG) (*Cercopithecus aethiops*) monkeys, one cell line had characteristics which meet the requirements of populations proposed as substrates for the manufacture of human virus vaccines. This line, derived from fetal lung tissue of a rhesus monkey, is described in the preceding report (1).

Details of development and characteristics of the other 27 cell lines are described here, for, although rejected as candidates for virus vaccine production, they represent a series of pretested, characterized cell lines which may be useful in studies requiring nonhuman primate cells.

MATERIALS AND METHODS

Serological testing of pregnant rhesus and AG monkeys used in this study, culture methods, and procedures for characterizing the cell lines were described in the preceding report (1).

Lung and kidney tissues were dispersed by successive treatment with Tris-buffered saline containing 0.25% trypsin and 0.01% collagenase;

* The research upon which this publication is based was performed pursuant to Contract No. NIH-69-100 with the Division of Biologics Standards of the National Institutes of Health.

[1] Nomenclature for identification of cell lines: DBS, Division of Biologics Standards; F, fetal; Rh, rhesus; C, *Cercopithecus;* L, lung; K, kidney; M, muscle; S, skin; and H, heart.

muscle, heart, and skin were minced and explanted directly.

Results

Serological tests on monkeys. Five rhesus and six AG healthy monkeys with lowest viral antibody levels to foamy virus types I and II and simian cytomegalovirus were selected, and an attempt was made to take fetuses at various stages of gestation. The viral antibody profiles of fetal sera generally matched that of the corresponding maternal serum; all tests for viremia on maternal and fetal sera were negative. Histological examination of formalin-fixed fetal tissues used for culture revealed no pathological findings.

Cell lines: morphology, growth characteristics. Cultures of vigorously multiplying fibroblasts were usually obtained 6 days after seeding with 7×10^6 to 15×10^6 cells from lung or 10 to 15 fragments from skin, muscle, or heart tissue. Primary cultures of lung and skin contained a few epithelial cell colonies which were lost on subsequent subculture.

Near confluent lung cell cultures contained 4×10^6 to 6×10^6 cells and could be subdivided 1:3 to 1:4 each 3 to 4 days during their active growth phase. Cultures were composed of fibroblasts, loosely connected by cytoplasmic processes.

Confluent cultures of skin, heart, and muscle were subdivided 1:2 or 1:3 each 3 to 4 days during an active growth phase. Fibroblasts derived from skin and muscle appeared more slender than lung fibroblasts, whereas fibroblasts derived from heart were more oval and grow in an interlacing network.

Ten fibroblastic lines derived from fetal rhesus monkey lung, skin, heart, or muscle and 15 fibroblastic lines from fetal AG monkey lung, skin, or heart were established in serial culture. Cells derived from lung appeared to have the greatest potential for growth in vitro in that rapid rates of cell multiplication were sustained for longer periods. Sixteen fibroblastic cell lines were maintained through a period of active growth and a subsequent declining growth phase, and these are listed and described in Table 1.

In declining growth phase, cell proliferation rates of fibroblastic lines slowed, and longer periods were required for cultures to become confluent. Cultures in this phase were subdivided 1:2 each 4 to 14 days. In their senescent phase, cultures developed some multinucleated and many vacuolated cells which subsequently ceased to proliferate and degenerated or became detached from the flasks. Five fibroblastic cell lines (FRhL-3, FRhS-3, FRhM-3, FRhS-2, and FCS-1) developed bizarre nuclear morphological changes during growth decline. Lobated, fragmenting, and budding nuclei were observed, and many abnormal mitotic figures were present as many cells disintegrated. The appearance of these cultures was reminiscent of human cells in "crisis" following infection with simian virus 40 (SV_{40}) (2). Cell lines propagated through crisis were eventually lost, due apparently to cell disintegration as a result of the increasing severity of nuclear abnormalities.

One rhesus fibroblastic cell line (DBS-FRhL-1) multiplied actively through 55 culture passages and approximately 93 population doublings. The cells then entered a period of growth decline and senescence, followed by cell death after 92 total passages and 129 population doublings. Cultures, recovered from frozen storage during active growth and propagated as a second and third series through decline and senescence, entered growth decline after the same approximate number of population doublings as the unfrozen cell line. Frozen, recovered DBS-FRhL-1 cells of the third series developed actively multiplying colonies of fibroblastic cells in the 83rd culture passage after a senescent period of 5.5 months. These cells continued to multiply actively in subsequent passages.

One AG monkey fibroblastic cell line (FCL-2) multiplied actively through 47 population doublings and was maintained further during growth decline and senescence until most of the cells degenerated after 67 total population doublings. FCL-2 cells, recovered from frozen storage in active growth phase and propagated as a third series through growth decline and senescence, developed colonies of actively multiplying cells in passage 63 after a senescent period of 6 months. FLC-2 cells in subsequent passages appeared capable of continued, unlimited growth.

The rhesus (DBS-FRhL-1) and AG (FCL-2) lung cell lines which resumed active growth after a long senescence retained the fibroblastic morphology and, at no time in their history, showed the nuclear crisis exhibited by five other fibroblastic cell lines.

Cultures initiated with 7.5×10^6 trypsin-dis-

TABLE 1

FETAL RHESUS AND AFRICAN GREEN MONKEY CELL LINES

Cell Line	Origin		Morphology*	Life Span in Vitro				Notable Cell Changes
				Active growth		Total life		
	Tissue	Fetus		Passages	Population† doublings	Passages	Population doublings	
DBS-FRhL-1	Lung	Rhesus ♯VR-10, male, 350 g	F	55	93	Infinite?	Infinite?	Heteroploid at passage 95
FRhS-2	Skin	Rhesus ♯VR-10, male, 350 g	F	60	118	83	141	Nuclear "crisis" during growth decline
FRhL-3	Lung	Rhesus ♯VR-8, male, 360 g	F	55	90	75	116	Nuclear "crisis" during growth decline
FRhS-3	Skin	Rhesus ♯VR-8, male, 360 g	F	48	67			Nuclear "crisis" during growth decline
FRhM-3	Muscle	Rhesus ♯VR-8, male, 360 g	F	50	58			Nuclear "crisis" during growth decline
FRhL-4	Lung	Rhesus ♯MNR-7, female, 300 g	F	51	82	81	112	Populations with ring chromosome developed during growth decline
DBS-FCL-1	Lung	AG ♯435, female, 135 days	F	35	46	53	60	14 to 55% aneuploidy during passages 31 to 47
FCS-1	Skin	AG ♯435, female, 135 days	F	29	35	66	74	Nuclear "crisis" during growth decline
FCH-1	Heart	AG ♯435, female, 135 days	F	32	40	39	47	
FCL-2	Lung	AG ♯58, male, 141 days	F	40	47	Infinite?	Infinite?	Heteroploid at passage 64
FCL-3	Lung	AG ♯66, male, 147 days	F	18	21	21	24	
FCH-3	Heart	AG ♯66, male, 147 days	F	16	16	26	26	
FCL-5	Lung	AG ♯383, female, 87 days	F	28	36	34	42	
FCH-5	Heart	AG ♯383, female, 87 days	F	24	24			
FCL-6	Lung	AG ♯656, female, 76 days	F	20	23	32	35	
FCS-6	Skin	AG ♯656, female, 76 days	F	16	16	21	21	
FRhK-2	Kidney	Rhesus ♯VR-10	F	5	5			
FRhK-3	Kidney	Rhesus ♯VR-8	E	6	6	6	6	
FRhK-4	Kidney	Rhesus ♯MNR-7, female, 300 g	E	3	3	Infinite?	Infinite?	Heteroploid at passage 12
FRhK-5	Kidney	Rhesus ♯MNR-11	E	5	5	10	10	
FRhK-6	Kidney	Rhesus ♯MNR-13, male, 300 g	E	10	10	12	12	
FCK-1	Kidney	AG ♯435	F	5	5	8	8	
FCK-2	Kidney	AG ♯58	F	5	5	5	5	
FCK-3	Kidney	AG ♯66, male, 147 days	E	5	5	Infinite?	Infinite?	Heteroploid at passage 16
FCK-4	Kidney	AG ♯54, male, 150 days	E	5	5	Infinite?	Infinite?	Heteroploid at passage 13
FCK-5	Kidney	AG ♯383, female, 87 days	E	5	5	Infinite?	Infinite?	Heteroploid at passage
FCK-6	Kidney	AG ♯656, female, 76 days	F	6	6	12	12	12

* F, fibroblastic; E, epithelial.

† See accompanying paper (1) for method of calculation, in many instances estimated from averages of previous determinations. In general, cultures subdivided at 1:2 split ratios averaged 1.0 population doubling per passage; 1:3 split ratios, 1.6 population doublings, and 1:4 split ratios, 2.0 population doublings per passage.

persed cells from rhesus or AG fetal kidney comprised compact colonies of epithelial cells which converged to confluency about the 6th day. Cultures were subdivided 1:2 each 4 to 7 days; growth rates slowed at the 6th to 8th passage, and mixed populations of fibroblastic and epithelial cells were often evident in subsequent passages. Of five lines derived from kidneys of five rhesus fetuses, three developed vacuolated cells and stopped multiplying after 5 to 10 passages, one developed an overgrowth of fibroblasts during the slowing growth phase, and one (FRhK-4) resumed active growth in passage 6.

Of six lines derived from kidneys of six AG fetuses, three developed an overgrowth of fibroblasts at the 6th to 7th passage, and three resumed active epithelial cell growth in passages 7 to 9 after a 3- to 5-month period of growth decline. The latter lines (FCK-3, FCK-4, and FCK-5) developed multinucleate cells in subsequent passages and growth characteristics of cell lines capable of indefinite multiplication. Identification and growth characteristics of the cell line established from kidney tissue are summarized in Table 1.

Cell lines were recovered from storage in liquid nitrogen with 60 to 85% viability and returned to culture without apparent change in growth characteristics.

Tests for tumorigenicity, using 10^6 cells per site of inoculum in both adult and newborn hamsters, were performed on DBS-FRhL-1 cells at passages 10, 20, 52, and 84; on FRhL-3 at passages 20, 29, and 67; on DBS-FCL-1 at passages 28 and 50; and on FCL-2 at passages 34, 53, and 70. All of these tests were negative.

Cytogenetic analysis. All fibroblastic cell lines analyzed from lung, heart, skin, or muscle were predominantly diploid during active growth from passages 1 to 30. Only after the 30th passage were deviations from the diploid state observed.

Cytogenetic analysis of line DBS-FRhL-1 showed that more than 90% of the cells were in the diploid range from passages 10 through 38. Five per cent of the cells were observed with breaks and gaps from passages 10 to 20 and 8.3% from passages 30 to 38. A low level (1.3%) of unstable structural abnormalities was observed in passage 38; these consisted of one dicentric and one ring chromosome. An upward trend of polyploidy occurred after passage 40 and reached levels of 30% by passage 50. In passages 61 to 85, the polyploid levels declined as the number of chromosome breaks and structural abnormalities increased in a predominantly diploid cell population. An apparently stable, abnormally long submetacentric chromosome was observed in 15/206 cells during passage 61 to 85. At passage 85 of the unfrozen cell line, over 90% of the cells were in the diploid range; a polyploid population of 3.0% was observed at this passage, and breaks, gaps, and structural abnormalities totaled 28%. Karyological analysis of DBS-FRhL-1 cells which resumed growth after a senescence of 5.5 months was performed at the 95th culture passage. These cells were 100% aneuploid, with chromosome numbers ranging from 63 to 65 per cell in 40% of the population. The morphology of the chromosomes in all cells examined were characteristic of the rhesus monkey, and typical marker submetacentric chromosomes with prominent secondary constrictions were present (see Fig. 1).

Analysis of rhesus skin FRhS-2 at passage 33 revealed polyploid populations at 14%. Cell multiplication was vigorous at this time, the cultures requiring subdivision at 1:4 each 3 to 4 days. At passage 65, during nuclear crisis, 92% of the cells showed chromosome breaks, gaps, or structural abnormalities such as dicentrics, ring chromosomes, or exchange configurations.

Analysis of rhesus lung FRhL-4 at passages 15, 31, and 37 revealed diploid populations with low levels of polyploidy and less than 2.0% of the cells with chromatid breaks. At passage 45, polyploid populations were 11.2%, and 2/50 cells analyzed showed one ring chromosome in an otherwise normal chromosome complement. A ring chromosome was also observed in 9/50 cells at passage 51 and 17/50 cells at passage 60. The ring was usually present in the cells with the 2N number of chromosomes and appeared to be a consistent marker chromosome in these populations (see Fig. 2*F*). At passage 67, during approaching senescence of the cell line, populations were 100% aneuploid; 6/51 cells were seen with the characteristic ring, and breaks, gaps, or other structural abnormalities were present in 85% of the cells analyzed.

Analysis of rhesus lung FRhL-3 cells showed diploid populations with polyploid levels ranging from 1.3 to 5.0% when examined in passages 10, 20, 30, and 55. At passage 64, when nuclear

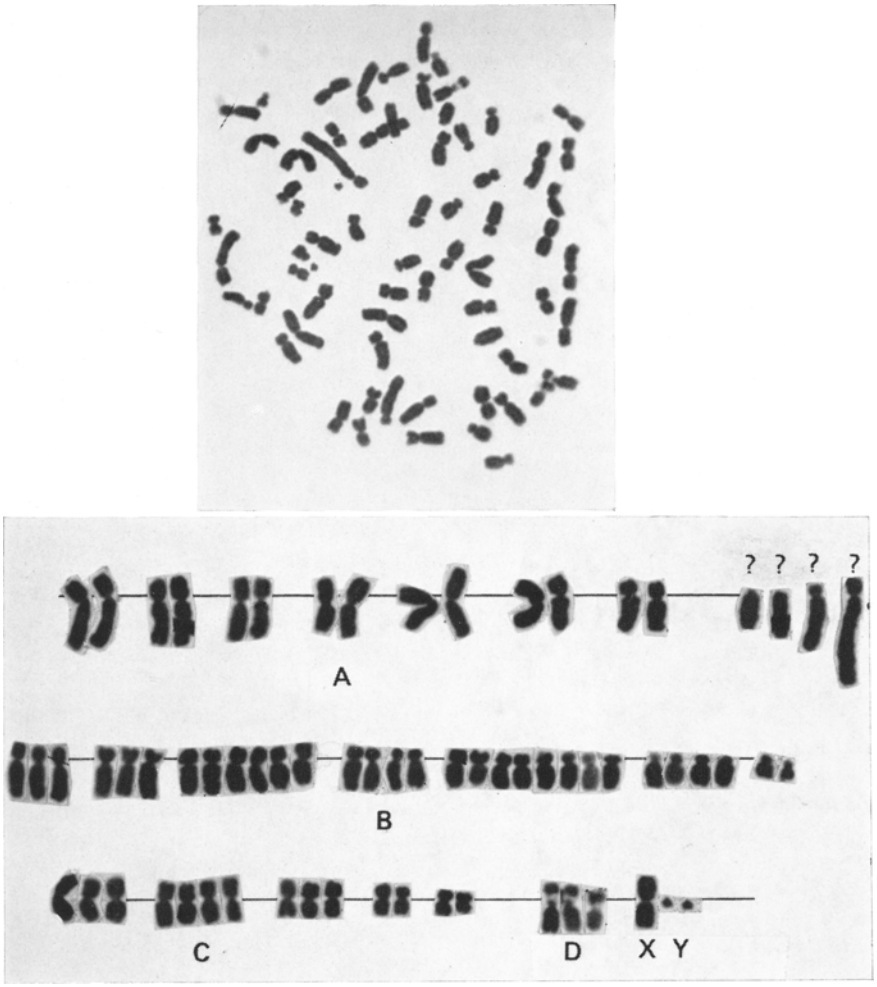

FIG. 1. Aneuploid cell and its karyotype. Rhesus monkey lung line DBS-FRhL-1, passage 95. This cell has 68 chromosomes. Although most extra chromosomes are of the B and C type, one extra chromosome has prominent secondary constrictions of the marker D type, and an extra Y chromosome is present. Four chromosomes are unclassified.

morphological changes were pronounced, 72% of the observed cells contained more than 60 chromosomes, and 60% showed chromosome breaks or structural abnormalities.

Karyological examination of AG lung line DBS-FCL-1 revealed predominantly diploid populations during active growth. Polyploid levels in these populations did not exceed 3.0%, and cells with breaks, gaps, or structural abnormalities ranged from 0 to 5.0% from passages 10 through 48. A recent independent study of DBS-FCL-1, however, has shown that levels of hypo- and hyperploidy may range from 12 to 55% during passages 31 to 47 (3). Because of this finding, DBS-FCL-1 is no longer considered a candidate cell line for use in virus vaccine production (4).

AG lung line FCL-2 comprised diploid populations with less than 3.0% polyploidy when examined during active growth at passages 10, 18, 33, and 40. Aging cultures in passage 57 showed polyploid levels of 11.4%, and 44% of the observed cells showed chromosome breaks, gaps, or structural abnormalities. Populations which resumed growth in passage 63 after a senescence of 6 months were 100% heteroploid at passage 67, with chromosome numbers ranging from 90 to 102 per cell (see Fig. 2*E*).

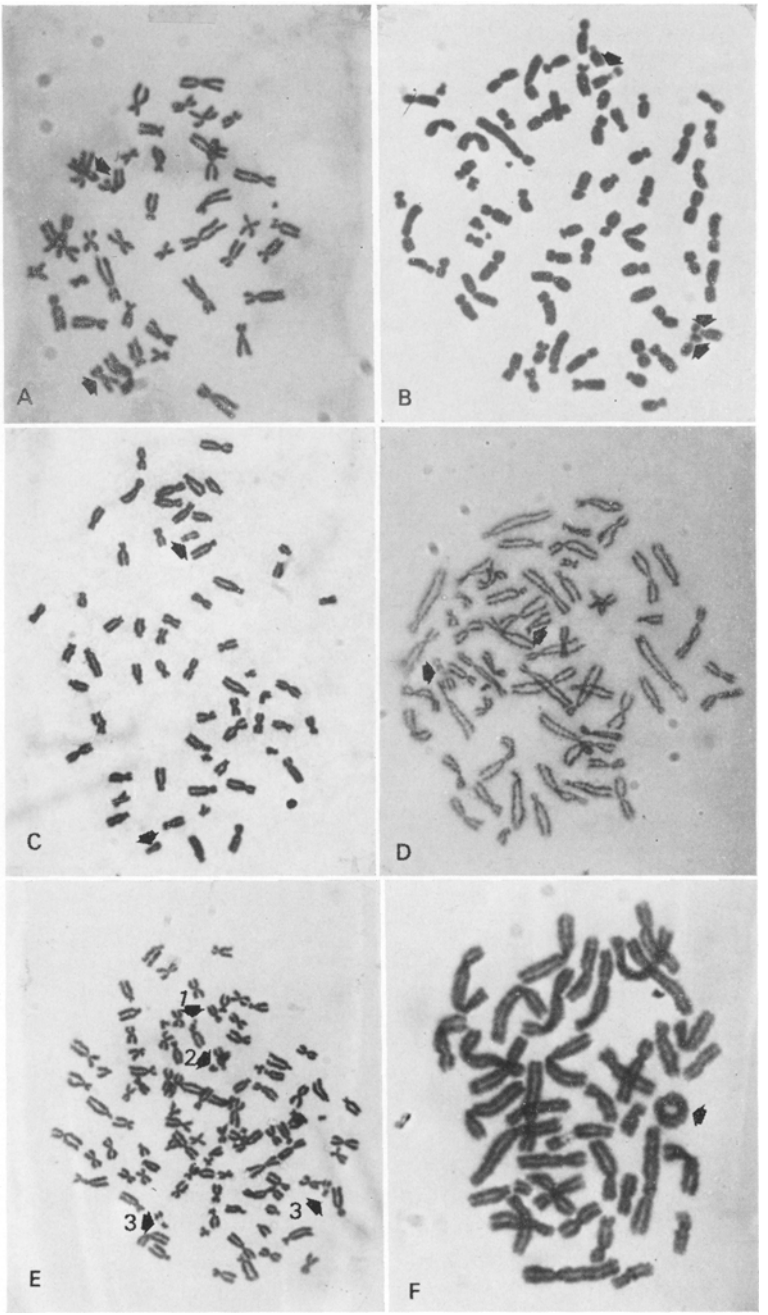

FIG. 2. Metaphase cells showing chromosomes of rhesus (2N = 42) and African green monkey (2N = 60) cell lines. *A* and *B*, DBS-FRhL-1 derived from male rhesus monkey lung. *Arrows* point to marker chromosomes. *A*, diploid, passage 25; *B*, aneuploid, passage 95, with 68 chromosomes. *C*, DBS-FCL-1 derived from female African green monkey lung. Diploid, passage 25. *Arrows* point to one pair telocentric marker chromosomes. *D*, FLC-2 derived from male African green monkey lung. Diploid, passage 33. *Arrows* point to one pair telocentric marker chromosomes. *E*, FCL-2 derived from male African green monkey lung. Aneuploid, passage 64, with 106 chromosomes. *Arrows* point to (*1*) a chromatid break, (*2*) an acentric fragment, and (*3*) one pair telocentric marker chromosomes. *F*, FRhL-4 derived from female rhesus monkey lung. Aneuploid, passage 53, with 42 chromosomes. *Arrow* points to a ring chromosome.

Examination of one rhesus (FRhK-4) and three AG (FCK-3, FCK-4 and FCK-5) kidney epithelial cell lines which resumed active growth after a 3 to 5 month period of growth decline revealed 100% aneuploid populations in passages 12 to 16.

Karyological data on aneuploid AG monkey lines DBS-FCL-1, FCL-2, FCK-3, FCK-4, and FCK-5 are shown in Table 2. Representative photographs of diploid and aneuploid cells in metaphase of rhesus and AG monkey cell lines are shown in Fig. 2.

Viral susceptibility, tests for adventitious agents. In a total of 60 tests performed at approximately each 10th passage level, cell lines derived from rhesus and AG monkey fetal tissues were found susceptible to a number of human viruses. Results of representative tests on rhesus lung line DBS-FRhL-1 and AG lung line DBS-FLC-1 are shown in Table 3. Both lines were comparable to control culture systems in sensitivity to the polioviruses, parainfluenza virus type 3, rhinovirus HGP, rubella, Coxsackie A9, and vaccinia, and the AG lung line was also sensitive to herpes simplex virus.

All tests for mycoplasmas, bacteria, and fungi

TABLE 2

KARYOLOGICAL DATA ON ANEUPLOID AFRICAN GREEN MONKEY CELL LINES (2N = 60)

Cell Line	Culture Passage	No. Chromosomes per Cell							Total Examined	Polyploidy (%)	Breaks and Gaps (%)	Structural Abnormalities (%)	Comments
		<57	58	59	60	61	62	>63					
DBS-FCL-1	14			4	44	1			49	2/311 (0.6)	5/49 (10.0)	0/49 (0.0)	Diploid
	22–24	8	13	14	744	8	10	3	860	92/3900 (2.3)	46/1300 (3.5)	15/1300 (1.1)	Diploid
	25–28	5	7	5	372	7	3	1	400	71/3000 (2.3)	34/1000 (3.4)	21/1000 (2.1)	Diploid
	31	1	2	5	23	3	3	12	50	3/340 (0.9)	3/50 (6.0)	0/50 (0.0)	Aneuploid
	36			5	34	5	5	1	50	1/333 (0.3)	2/50 (4.0)	0/50 (0.0)	Aneuploid
FCL-2	67	1					1	48	50	120/313 (38.0)	2/50 (4.0)	3/50 (6.0)	Aneuploid
FCK-3	16	1						110	111				Aneuploid
FCK-4	13							117	117				Aneuploid
FCK-5	12							106	106				Aneuploid

TABLE 3

VIRUS SUSCEPTIBILITY OF RHESUS AND AFRICAN GREEN MONKEY CELL LINES

Culture Passage	Poliovirus			Adenovirus		Parainfluenza		Herpes Simplex	Mumps	Rhinovirus HGP	Rubella	Coxsackie A9	Vaccinia	Measles
	I	II	III	3	7	2	3							
DBS-FCL-1														
10	6.2*	6.2	6.4	3.2	3.2	0.7	6.9	>4.2	4.7	5.7	4.4	6.9	8.4	4.4
	(5.9)†	(6.1)	(7.0)	(4.7)	(4.3)	(3.4)	(6.2)	(7.1)	(5.5)	(6.0)	(5.5)	(6.2)	(7.0)	(6.5)
31	4.4	4.4	6.4	2.9	2.4	1.2	3.4	6.2	3.2	5.9	2.9	7.2	8.2	4.2
	(5.7)	(6.4)	(7.2)	(3.4)	(3.4)	(4.2)	(3.4)	(4.9)	(3.2)			(7.2)	(8.2)	(5.9)
40	4.4	4.4	7.2	2.9	3.2	1.2	3.2	5.4	3.2	5.9	4.2	6.4	7.4	3.7
	(5.7)	(6.4)	(7.2)	(3.4)	(3.4)	(4.2)	(3.4)	(4.9)	(3.2)			(7.2)	(8.2)	(5.9)
DBS-FRhL-1														
10	5.7	6.2	7.4	2.2	2.2	3.2	6.4	4.2	4.2	4.2	4.2	>7.2	8.2	3.4
	(5.7)	(6.2)	(6.7)	(5.4)	(4.7)	(3.4)	(6.2)	(7.2)	(5.2)	(6.0)	(4.2)	(6.2)	(8.2)	(6.2)
20	5.4	6.9	7.2	3.2	2.9	3.9	6.4	4.2	5.4	5.4	4.2	6.4	8.4	6.2
	(6.0)	(6.2)	(7.2)	(4.4)	(4.0)	(4.2)	(6.4)	(6.7)	(5.5)	(6.0)	(4.2)	(7.7)	(8.2)	(6.4)
30	6.2	6.2	7.2	2.9	2.9	3.2	6.4	1.2	5.2	5.2		8.4	8.2	

* Reciprocal $\log_{10}$ of the $TCID_{50}$ per ml.

† Figures in parentheses are titers obtained in control culture systems.

and all in vitro and in vivo tests on these cells lines for viral agents or their antigens have been negative.

Discussion

Of 16 fibroblastic cell lines derived from fetal lung, heart, skin, and muscle tissue of rhesus and AG monkeys and maintained through a period of active growth followed by growth decline and senescence, 6 were established from three rhesus fetuses and 10 from five AG monkey fetuses. Cell lines from lung tissues appeared to have the greatest growth potential in terms of total doublings, and fibroblastic cell lines derived from rhesus monkeys were usually capable of more doublings than similar lines from AG monkeys. Rhesus monkey fibroblasts were maintained in active growth phase during 6 to 8 months and 44 to 60 culture passages; AG monkey fibroblasts maintained active cell multiplication during 3 to 6 months and 16 to 40 passages. In this study, cell lines from tissues of the youngest AG fetuses did not have a longer in vitro life span than those derived from tissues of older AG fetuses.

All fibroblastic cell lines analyzed (from lung, heart, skin, or muscle) during passages 1 to 30 were predominantly diploid and showed few morphological changes over that seen in the primary cultures. At the beginning of growth decline, four cell lines derived from lung, skin, or muscle of two rhesus fetuses (FRhL-3, FRhS-3, FRhM-3, and FRhS-2) and one cell line derived from skin of one AG fetus (FCS-1) developed morphological changes characterized by budding or lobated nuclei, micronuclei formation, and a random growth pattern. These aberrant nuclear changes increased in severity in succeeding passages and involved progressively the entire cell population. Cytogenetic analysis of FRhL-3 and FRhS-2 cells during this period revealed chromosome abnormalities and a shift toward aneuploidy. Cultures were eventually composed of only unstable, nonviable cell types, which could not be propagated further.

The other fibroblastic cell lines entered growth decline without notable cell morphological change. These cultures were characterized by a slowing growth rate followed by a senescent phase during which multiplication ceased, and cells developed prominent cytoplasmic vacuoles and degenerated. Some binucleated cells were present as were a few with budding nuclei. Two fibroblastic cell lines (DBS-FRhL-1 and FCL-2) resumed active growth after a senescence of 5.5 to 6.0 months, and these cells were heteroploid in subsequent passages.

The rise in numbers of polyploid cells in rhesus lung line DBS-FRhL-1 in passages 40 to 50, their apparent decline, and the subsequent reappearance of a dominant diploid karyotype from passage 61 would seem an unusual pattern of genetic change in cultured cells. Observations on shifts from polyploidy to heteroploidy and back to diploidy have been observed in cell lines developed from marmoset monkeys (5).

Seven epithelioid and four fibroblastic cell lines were established from kidney tissue of fetal rhesus and AG monkeys. Four of the seven epithelioid lines showed morphological and chromosomal evidence consistent with indefinite growth potential in culture. There was no positive correlation between such changes and maternal antibody status, in that other animals having similar antibody patterns failed to give rise to morphologically and cytogenetically aberrant cell populations.

Although the presence of an undetectable viral agent in cultures undergoing aberrant nuclear changes remains a possibility, repeated attempts to demonstrate viral contaminants in these cultures were unsuccessful. It is of interest that nuclear budding and fragmentation, which occur in low incidence in senescent cultures of the rhesus and AG fetal lines described here, has also been reported in senescent cultures of human diploid cells (6) and in cells of several human tissues of nonpathologic nature (7). Additional studies of monkey cell lines which eventually die with apparently "normal" cytoplasmic changes associated with senescence, and those which develop increasing nuclear aberrations, may lead to a better understanding of factors influencing nuclear stability and the capacity for unlimited cell multiplication in vitro.

REFERENCES

1. Wallace, R. E., P. J. Vasington, J. C. Petricciani, H. E. Hopps, D. E. Lorenz, Z. Kadanka. 1972. Development of a diploid cell line from fetal rhesus monkey lung for virus vaccine production. In Vitro 8: 323–332.
2. Shein, H. M., J. F. Enders, L. Palmer, and E. Grogan. 1964. Further studies on SV_{40}-induced transformation in human renal cell cultures. I. Eventual failure of subcultivation despite a

continuing high rate of cell division. Proc. Soc. Exp. Biol. Med. 115: 618–621.
3. Hsu, T. C., Principal investigator; J. E. K. Cooper, Project investigator. Research Contract NIH-70-2247 with University of Texas.
4. Petricciani, J. C., H. E. Hopps, and D. E. Lorenz. 1971. Subhuman primate diploid cells: possible substrates in vaccine production. Science 174: 1025–1027.
5. Marczynska, B., G. Treu-Sarnat, and F. Deinhardt. 1970. Characteristics of long-term marmoset cell cultures spontaneously altered or transformed by Rous sarcoma virus. J. Nat. Cancer Inst. 44: 545–572.
6. Hayflick, L., and P. S. Moorhead. 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25: 585–621.
7. Elston, R. N. 1963. Nuclear budding and micronuclei formation in human bone marrow, skin and fascia lata cells in vitro and in oral mucosa cells in vivo. Acta Pathol. Microbiol. Scand. 50: 195–199.

The skilled technical assistance of Mrs. Edna Schuler, Miss Mary Fox, Mrs. Lois Larroy, and Mr. W. J. Boss is gratefully acknowledged. We are also indebted to Dr. George R. Sharpless and his group in Quality Control for tests of tumorigenicity, viral sensitivity, and tests for adventitious agents, and to Dr. R. J. Vallancourt for performing the caesarian sections. We also thank Dr. T. C. Hsu and Dr. J. E. K. Cooper at the University of Texas for their help in the cytogenetic analyses.