DEVELOPMENT OF A DIPLOID CELL LINE FROM FETAL RHESUS MONKEY LUNG FOR VIRUS VACCINE PRODUCTION*

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SUMMARY

The primary goal of this study was to develop and characterize diploid cell lines from fetal tissues of subhuman primates for use in virus vaccine production. Cell lines have been established from fetal tissues of rhesus and African green monkeys, and these have been characterized according to the general criteria recommended by the International Cell Committee for Microbiological Standardization. Of these cell lines, DBS-FR_hL-2, developed from lung tissue of a rhesus monkey fetus, has been found to meet the requirements of populations proposed as substrates for virus vaccines.

Characterization studies show that DBS-FR_hL-2 cells have a finite life of more than 50 population doublings in vitro and maintain the diploid karyotype through an active growth phase. The cells are nontumorigenic, and tests have not revealed the presence of adventitious agents. They are susceptible to a number of human viruses and can be preserved by freezing without change in virus susceptibility, cytogenetic, or growth characteristics. These results indicate the need for further evaluation of this rhesus monkey diploid cell line for acceptability as an alternate substrate in the manufacture of human virus vaccines.

Hayflick and Moorhead (1) have shown that human diploid cells (HDC) of fetal origin can be serially cultured through a limited number of passages, producing a theoretical limit of 10²² cells for each cell line¹ capable of 50 population doublings. When frozen and stored in liquid nitrogen in early and later subcultures, such lines can provide a plentiful if not inexhaustible supply of cells. Further studies by Hayflick and other investigators (2, 3) have shown that HDC

are karyotypically and morphologically stable during serial culture, are free of detectable viral contaminants, are susceptible to a broad spectrum of viruses, and can be preserved by freezing without loss of properties. One such line (WI-38) is now used as an alternate to primary monkey kidney cells as substrate in the manufacture of live, oral poliovirus vaccine. The numerous advantages of serially cultured cell lines as standardized, pretested cell seed in vaccine production have been reviewed (4).

The experiences with HDC derived from human fetal tissue suggested that similar tissues of other animal species also be explored for their potential usefulness in the development of diploid cell lines. Subhuman primate diploid cell lines offer the advantage of an immense, positive

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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.

clinical experience with viral vaccines produced in primary cell cultures deriving from rhesus (*Macaca mulatta*) and African green (AG) (*Cercopithecus aethiops*) monkeys; an additional advantage is the availability of animals for in vivo tumorigenicity testing.

Previous reports on the serial culture of cells from kidney tissues of an AG monkey (5), rhesus monkey fetus (6), and a marmoset monkey (7) suggested the feasibility of establishing diploid cell lines from tissues of subhuman primates.

We have developed 28 cell lines from fetal tissues of rhesus and AG monkeys, and these have been characterized according to the general criteria recommended by the International Cell Committee of the Permanent Section for Microbiological Standardization (2, 3). One of these cell lines, DBS-FR_hL-2, has been found to have culture characteristics similar to those described for HDC and to meet the requirements proposed for acceptability as an alternate substrate for virus vaccine production. This cell line is described here. Details of the development and characteristics of the 27 other cell lines are described in an accompanying report (8) for, although rejected as candidates for virus vaccine production, they may be useful in studies requiring standardized cell lines from nonhuman primates. Preliminary results of these studies have been presented (9, 10).

MATERIALS AND METHODS

Serological tests on monkeys. Pregnant rhesus monkeys were obtained from Primate Imports Corp., Port Washington, Long Island, and from Vita Private, Ltd., Delhi, India. The latter were isolated and held in quarantine for 6 weeks after arrival. AG monkeys were selected from the breeding colony maintained by the Division of Biologics Standards at Bionetics Research Laboratories in Kensington, Maryland.

Monkeys were skin-tested for tuberculosis, examined for malarial and internal parasites, and bled from the femoral artery before removal of the fetus. Sera were tested for antibodies to simian virus 40 (SV₄₀); foamy viruses types I and II; simian cytomegalovirus (CMV); poliovirus types I, II, and III; rubella virus; and measles virus. Antibodies to CMV were determined by the indirect fluorescent-antibody test of Thiel and Smith (11), using human diploid

cells, strain Led-130 (12), previously infected with CMV and fixed with methanol. Rubella and measles hemagglutination-inhibiting (HI) antibodies were determined in tests essentially as described by Enders (13), using pigeon erythrocytes for rubella and rhesus or AG monkey erythrocytes for measles virus. Foamy virus and SV₄₀ complement-fixing antibodies were determined by the Micro Laboratory Branch Complement Fixation test (14). Tests for neutralizing antibodies to poliovirus were performed by using the metabolic inhibition test described by Schmidt (15). Tests for viremia were performed by inoculating 0.1 ml of serum into each of three tube cultures of primary AG monkey kidney (AGMK), rabbit kidney (RbK), and Led-130 cells. These cultures were observed microscopically for viral cytopathic effects (CPE) during 14 days incubation at 37°C.

Fetuses were taken by cesarian section and bled by cardiac puncture; heart, kidneys, lung, skin, and muscle were removed. A portion of each tissue was fixed in formalin for histological examination; remaining tissues were placed in 2-oz jars containing culture medium with antibiotics and delivered to the laboratory where they were prepared for culture the same day. The DBS-FR_hL-2 cell line described in this report was derived from lung tissue of a rhesus monkey fetus.

Culture methods. Culture medium used for initiating and propagating the cells was Eagle's minimum essential medium (MEM) containing nonessential amino acids (16), vitamins, and glutamine at twice the usual concentrations and bovine fetal serum at 10% concentration. Both MEM and bovine fetal serum had been pre-tested for growth support of human Led-130 cell cultures, and the serum was also tested for bacteria, mycoplasmas, and for bovine viruses. A single lot of MEM, purchased in bulk as powdered medium from Grand Island Biological Co., Grand Island, New York, and two pooled lots of bovine fetal serum from Reheis Chemical Co., Chicago, Illinois, were used throughout these studies. Penicillin (100 I.U. per ml), streptomycin (50 µg per ml), and neomycin (20 µg per ml) were incorporated into the medium only during initial explantation of tissues.

Other media used in comparative studies were CMRL 1969 (17), NCTC 135 (18), RPMI 1640 (19), Eagle's Basal Medium (20), McCoy's 5a

(21) as modified by Iwakata and Grace (22), 199 (23) and lactalbumin hydrolysate medium (LAPAGT) (12).

Cultures were prepared and maintained in an area isolated from work with infectious agents and other cultured cell lines. Fetal lung tissue was minced and dispersed by three successive treatments with Tris-buffered saline (24) containing 0.25% trypsin and 0.01% collagenase. The dissociated cells were passed through a stainless steel sieve, sedimented by centrifugation, and rinsed with Tris-buffered saline. Seven million cells were seeded into each 75 cm² plastic flask with 25 ml of culture medium, and flasks were incubated at 37°C with slightly loose caps in a humidified incubator provided with 5% CO₂ in air. Cultures received a medium change or were subcultured by dispersal of monolayers with 0.01% crystalline trypsin each 3 to 4 days. Split ratios were 1:2 to 1:4, depending on population densities; efforts were made to seed subcultures with the minimum numbers of cells required to produce near confluency in 3 to 4 days. Aliquots of cell suspensions were preserved in a 2.0% solution of citric acid containing crystal violet at 0.1% concentration for determinations of population densities by cell count. These provided an estimate of population doublings over the 3- to 4-day growth period as well as total doublings during in vitro life. Culture passages in this report refer to flask passages at split ratios varying from 1:2 to 1:4; population doublings during the 3- to 4-day growth periods were determined by the formula

$$[\log_{10} \text{ of (number of cells at } T_2) / \text{(number of cells at } T_0)] / \log_{10} \text{ of 2.0}$$

Population doubling times were determined by the general procedures of Harris (25).

Cells at various passages were suspended in medium containing 7.5% glycerol or dimethylsulfoxide, sealed into ampoules in 1.0-ml volumes, and stored in liquid nitrogen by methods previously described (12). Cell suspensions were thawed, and viability was determined by direct count of living and dead cells in the presence of eosin (12).

Tests for adventitious agents, tumorigenicity. The cells were tested at each 10th passage for the presence of adventitious agents generally as specified by United States Public Health Service regulations for safety testing of biological prod-

ucts (26). This included direct tests on the cells for hemadsorbing agents using guinea pig red cells, tests for nuclear inclusions using acridine orange stain, and fluorescent-antibody tests for specific viral antigens of SV₄₀, CMV, and simian virus SV₈₀. The cells were also tested for positive immunofluorescent reactions to viral antibodies present in maternal serum. Cells and spent media were tested for bacteria, fungi, and mycoplasmas in suitable media and for the presence of viruses by inoculation into cultures of primary AGMK, rhesus monkey kidney (RMK), RbK, and human amnion, as well as cultures of BSC-1 (5) and Led-130 (12). Cell harvests from tests in primary AGMK were subcultured once into other primary AGMK cultures. In vivo tests were also performed by inoculation of cells and spent medium into embryonated hens' eggs, adult mice, suckling mice, and rabbits. Complement fixation and fluorescent-antibody tests for SV₄₀ tumor "T" antigen were also performed, using serum from hamsters bearing large, SV₄₀-induced tumors and methods described by Huebner et al. (27).

Cells were tested for tumorigenicity in the adult Syrian hamster cheek pouch by the technique of Handler and Foley (28) and in suckling hamsters treated with antithymocyte serum by methods described elsewhere (29). Human epidermoid carcinoma cells, strain KB (30), were used as a positive control in all tests.

Cytogenetic analysis. Permanent slides of cells in metaphase were prepared at various passages by the air-drying technique (31). Karyotypic constructions were made from photographic enlargements of 4 × 5 inch Polaroid negatives. Chromosomes were paired according to the arrangement given for *M. mulatta* and *C. aethiops* by Hsu and Benirschke (32).

Virus susceptibility tests. Viruses used were: the polioviruses type I LSc, type II p712, and type III Leon, Sabin vaccine strains; human adenoviruses type 3, strain J. F. and type 7, strain L. L. isolated by Dr. R. J. Huebner, National Institute of Allergy and Infectious Diseases; rhinovirus type 2 HGP, Salisbury strain; rubella, strain M33 HPV-77 obtained from Dr. P. Parkman, Division of Biologics Standards; vaccinia, commercial calf lymph vaccine propagated on chorioallantoic membrane of embryonated hens' eggs; measles, Edmonston strain obtained from Dr. J. Enders, Children's Hospital,

Boston; human parainfluenza types 2 and 3 obtained from Dr. R. Chanock, National Institute of Allergy and Infectious Diseases; mumps, Angela strain isolated in these laboratories by Dr. C. P. Cerini; Coxsackie A₉ and herpes simplex HF obtained from the American Type Culture Collection.

Tests were performed on 48-hr tube cultures. Ten-fold dilutions of each virus were inoculated in 0.1- to 0.2-ml amounts to each of three tubes. Viruses were also titrated simultaneously in control tissue culture systems: the polioviruses, adenoviruses, parainfluenzas, Coxsackie A₉, and vaccinia in primary cultures of RMK; mumps in primary cultures of AGMK; herpes simplex in cultures of AGMK cell line BSC-1 (5); rhinovirus in cultures of Lcd-130 (12); and rubella in cultures of AGMK or rabbit kidney, strain RK₁₃ (33). Cultures were incubated 7 to 14 days and read microscopically for viral CPE; TCID₅₀ titers were calculated by the method of Reed and Muench (34).

RESULTS

Serological tests on monkeys. Monkeys were screened for viral antibodies with the hope of selecting animals free of latent viral infections which may be present in primate tissues (35), and these results have been reported (10).

Although animals free of overt illness and viremia could be selected, no monkeys were free of antibodies to all viruses tested. The healthy pregnant rhesus monkey used in this study had low levels of serum antibodies to SV₄₀ (1:8), foamy virus type I (1:32), and CMV (1:160). The fetus removed from this monkey had antibodies to the same viruses at slightly lower levels.

Tests for viremia on maternal and fetal sera were negative, and histological examination of formalin-fixed tissues from the fetus revealed no pathological findings.

Growth characteristics of cultures. The DBS-FR_hL-2 cell line originated from lung tissue of a 248-g male rhesus monkey fetus.

Cultures of vigorously multiplying fibroblasts were obtained 6 days after seeding with 7.0×10^6 dispersed lung cells. Primary cultures contained a few epithelial cell colonies which were lost on subsequent subculture. During the first few subcultivations, flasks were seeded with inocula ranging from 0.5×10^6 to 6.0×10^6 cells

to determine the minimum number of cells required to produce near confluent monolayers in 3 to 4 days. Inocula of 1.0×10^6 cells per flask produced subcultures containing approximately 6.0×10^6 cells in 3 to 4 days during passages 1 to 8. In later passages during active growth of the cell line, an inoculum of 1.3×10^6 cells produced near confluent monolayers of 4×10^6 to 5×10^6 cells in 3 to 4 days. From passage 8, cultures were regularly subdivided 1:3 each 3 to 4 days during an active growth phase of 44 total culture passages and approximately 67 population doublings. During this time, the population doubling time of the cells averaged 33 hr.

Cultures in active growth were composed of oval to spindle-shaped fibroblasts, loosely connected by cytoplasmic processes (see Fig. 1). In densely populated areas, the cells appeared to grow in an orderly orientation suggesting some inhibition of movement upon contact with other cells.

After 44 passages and 6 months in culture, the growth rate of the cells slowed noticeably, and the cultures were subdivided 1:2 each 4 to 10 days during the next 3 months. The cells ceased to multiply beyond passage 51, after approximately 74 total population doublings. Cultures from passages 48 to 51 were composed of some multinucleate and many enlarged and/or vacuolated cells which subsequently ceased to proliferate and degenerated or became detached from the flasks. Senescent cultures in passages 50 and 51 received medium changes once or twice each week during a 3-month period in which they were observed for cell morphological changes or resumption of cell division. The cultures were discarded when all cells had degenerated or become detached from the flasks.

Cells frozen at various passages have been recovered from storage in liquid nitrogen with cell viability ranging from 60 to 85% and returned to culture without apparent change in growth characteristics. In experiments on freeze survival, highest viability was obtained when no more than 4×10^6 cells per ampule were frozen, when dimethylsulfoxide was the cryoprotective agent, and when a controlled freezing rate of about 1°C per min was maintained during the critical phase from 0°C to -25°C.

DBS-FR_hL-2 cultures have been recovered from frozen storage in passages 15, 25, 40, and 43 and propagated through their remaining

Fig. 1. Fetal rhesus monkey diploid cells DBS-FRhl-2, passage 26. Giemsa stain. $\times 220$.

finite life. Fifteen to 20 cultures of each series were maintained through growth decline and senescence and observed for changes in cell morphology or growth properties. Each series entered growth decline after 35 to 44 total passages and ceased cell multiplication after 60 to 73 population doublings.

Tests for tumorigenicity, using 2.5×10^5 to 10^6 cells per site of inoculum in both adult and suckling hamsters, were performed at passages 20, 24, 29, 31, 40, and 47. All tests were negative. Human epidermoid carcinoma (KB) cells, which were included as controls in each test and inoculated at 2.5×10^6 cells per site, produced progressively growing tumors in both adult and suckling hamsters.

Cytogenetic analysis. DBS-FRhl-2 cells were found to be predominantly diploid with polyploid levels below 2.0% at all passages examined during their active growth phase (see Table 1).

As shown, over-all hypodiploidy and hyperdiploidy were 6.3 and 1.3%, respectively, during passages 22 through 40. An increase in polyploidy to 11.2% and chromosome structural abnormalities to 2.8% was observed only in aging cultures at passages 45 to 46. The structural abnormalities observed were unstable dicentric forms. A photograph and karyotype of a DBS-FRhl-2 cells in passage 27 is shown in Fig. 2.

Growth of diploid cells in various culture media. To determine whether a monkey diploid cell line established in MEM could be propagated satisfactorily in tissue culture media of different formulation, comparative studies were made using some of the conventional culture media: CMRL 1969, NCTC 135, RPMI 1640, Eagle's BME, McCoy's 5a, 199, and lactalbumin hydrolysate-LAPAGT medium. All media were supplemented with bovine fetal serum at 10% concentration.

TABLE 1
KARYOLOGICAL DATA ON RHESUS MONKEY DIPLOID CELLS, DBS-FR_hL-2 (2N = 42)

Culture Passage	Hypodiploidy (%)	Hyperdiploidy (%)	Polyploidy (%)	Breaks and Gaps (%)	Structural Abnormalities (%)
22-25	48/1000 (4.8)	13/1000 (1.3)	53/3300 (1.6)	50/1100 (4.5)	7/1100 (0.6)
26-31	56/700 (8.0)	10/700 (1.4)	50/3300 (1.5)	37/1100 (3.4)	21/1100 (1.9)
32-40	35/500 (7.0)	6/500 (1.2)	64/2700 (2.4)	20/900 (2.2)	17/900 (1.5)
45-46	45/106 (42.0)	3/106 (2.8)	69/611 (11.2)	6/106 (5.6)	3/106 (2.8)*
Over-all 22-40	139/2200 (6.3)	29/2200 (1.3)	167/9300 (1.8)	107/3100 (3.4)	45/3100 (1.4)

* Three dicentrics.

DBS-FR_hL-2 cells were harvested by trypsin dispersal from cultures which had been propagated through 9 to 12 passages in MEM. Culture flasks were inoculated each with 1.3×10^6 cells and grouped, three flasks per group, to receive the test media. Cultures were incubated and observed during 39 days; they received a medium renewal each 3 to 4 days and were subdivided when confluent at split ratios of 1:2 or 1:3 depending on the population density.

Cultures in each group were prepared for cytogenetic analysis at the end of the observation period. Results are summarized in Table 2.

Cells in MEM, CMRL 1969, NCTC 135, and RPMI 1640 multiplied actively through 10 passages (13 to 15.8 population doublings) and required subculture at split ratios of 1:3 each 3 to 4 days. Cells in BME also multiplied satisfactorily through nine passages (10.2 population doublings). Cultures were composed of healthy, ac-

Fig. 2. Male rhesus monkey diploid cell and its karyotype. Rhesus monkey lung line DBS-FR_hL-2, passage 27.

TABLE 2
EFFECT OF CULTURE MEDIUM ON GROWTH AND
POLYPOIDY OF RHESUS MONKEY DIPLOID
CELLS, DBS-FR_hL-2

Medium*	Growth during 39 Days		Condition of Cells in Final Passage	Per cent† Poly- poids
	Cul- ture pas- sages	Popu- lation dou- blings		
MEM	10	15.8	Good	0.6
CMRL 1969	10	15.7	Good	1.9
NCTC 135	10	13.4	Good	0.6
RPMI 1640	10	13.0	Good	2.2
BME	9	10.2	Good	1.9
McCoy's 5a	9	9.6	Poor, granu- lar	0.3
199	7	7.6	Poor, aged	0.6
LAPAGT	5	5.0	Poor, aged	10.9

* All media were supplemented with bovine fetal serum at 10% concentration.

† Observations were based on at least 300 cells per sample.

tively proliferating cells after 39 days of continued growth in BME, MEM, CMRL 1969, NCTC 135, and RPMI 1640. Cell multiplication slowed after 9 passages in McCoy's 5a and ceased after five to seven passages in LAPAGT and 199 media; cells propagated in LAPAGT and 199 media also developed cellular changes associated with senescence.

Cytogenetic examination of these cultures revealed no significant chromosome changes in cells propagated in MEM, CMRL 1969, NCTC 135, BME, or RPMI 1640 medium. Polyploidy ranged from 0.3 to 2.2% of 300 cells observed in each of the above media, whereas cells propa-

gated in LAPAGT medium developed a polyploid level of 10.9%. The frequency of chromatid and chromosome breaks in LAPAGT and 199 media was two to three times that observed in the other test media.

Viral susceptibility, tests for adventitious agents. The DBS-FR_hL-2 line was found susceptible to a number of human viruses. Results of representative tests are shown in Table 3. The cells were comparable to control culture systems in sensitivity to the polioviruses, parainfluenza type 3, rhinovirus HGP, rubella, Coxsackie A9, and vaccinia and were insensitive to the other viruses tested (human adenovirus types 3 and 7, parainfluenza virus type 2, mumps and measles). No change in viral sensitivity was noted with continued culture passage.

To investigate rubella and poliovirus production in this diploid cell line, 75 cm² flask cultures of DBS-FR_hL-2 cells in passage 38 were infected with 10^{5.0} TCID₅₀ of poliovirus type I or 10^{4.0} TCID₅₀ of rubella virus. Cultures infected with poliovirus were placed at 34°C, and 1-ml samples were removed for viral assay at intervals during 72 hr of incubation. Rubella virus-infected cultures were incubated at 32 or 36°C and received a medium change each 2 to 4 days.

Cultures produced peak titers of 10^{7.7} to 10^{8.2} TCID₅₀ per ml of poliovirus after 48 hr when about 75% of the cells were destroyed by virus. These titers were sustained through the remaining period of incubation. Rubella virus titers of culture fluids removed at 2- to 4-day intervals ranged from 3.6 to 6.0 TCID₅₀ per ml during 35 to 39 days at 36°C and 71 to 76 days at 32°C. No significant difference in titers was

TABLE 3
VIRUS SUSCEPTIBILITY OF RHESUS MONKEY DIPLOID CELLS, DBS-FR_hL-2

Cul- ture Pas- sage	Poliovirus			Adenovirus		Parainfluenza		Herpes Sim- plex	Mumps	Rhino- virus HGP	Ru- bella	Cox- sackie A9	Vac- cinia	Mea- sles
	I	II	III	3	7	2	3							
14	4.4* (5.7)†	5.2 (6.2)	6.9 (6.4)	2.9 (4.2)	1.7 (2.4)	4.4 (4.4)	3.2 (4.2)	3.2 (4.2)	3.2 (4.2)	5.9 (6.2)	4.2 (3.5)	7.2 (7.4)	8.4 (8.0)	2.2 (5.2)
20	6.2 (5.7)	6.4 (6.7)	7.4 (7.7)	1.2 (4.4)	1.2 (2.2)	2.2 (4.1)	6.9 (5.9)	1.2 (5.2)	3.9 (5.2)	4.2 (5.2)	3.2	7.2 (8.2)	8.9 (8.4)	2.9 (5.2)
30	6.0 (5.7)	6.9 (6.7)	6.9 (7.7)	2.4 (4.4)	1.2 (2.2)	2.2 (4.1)	6.9 (5.9)	1.2 (5.2)	2.2 (5.2)	5.2 (5.2)	4.2	6.9 (8.2)	8.9 (8.4)	4.2 (5.2)

* Reciprocal log₁₀ of the TCID₅₀ per ml.

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

noted in cultures maintained at 32 or 36°C. Cellular degeneration, noted after 35 days in rubella-infected cultures held at 36°C, became more extensive resulting in death of the cultures after 50 days at 36°C and after 76 days at 32°C.

Pilot lots of poliovirus and rubella virus vaccines have been produced in cultures of DBS-FR_hL-2 propagated and maintained under conditions currently used in the manufacture of poliovirus vaccine. Viral titers of polio and rubella virus vaccines produced under these conditions compared favorably with titers of these vaccines produced in primary cultures of AGMK. Detailed results of these experiments will be reported elsewhere.

All tests for mycoplasmas, bacteria, and fungi and all in vitro and in vivo tests on these cells for viral agents or their antigens have been negative.

DISCUSSION

On the recommendations of the 1967 Cell Culture Conference (4), studies were undertaken to develop nonhuman diploid cell lines which could be used in the manufacture of virus vaccines. Twenty-eight cell lines were developed from fetal tissues of rhesus and AG monkeys, and our studies show that one of these, DBS-FR_hL-2, deriving from lung tissue of a rhesus monkey fetus, has characteristics which meet the requirements of populations proposed as substrates for virus vaccines (2, 3). The other lines which were rejected as candidates for virus vaccine production are described in the accompanying report (8).

A critical evaluation of the cytogenetic characteristics of cell lines derived from rhesus or AG monkeys requires karyological data on primary cultures from both species. Although the diploid number and karyotype of both species have been established, no information on the types and frequency of chromosome aberrations or polyploidy could be found in the literature. A separate study to establish the cytogenetic characteristics of normal rhesus and AG monkey cells was begun and will be reported elsewhere (36).

The DBS-FR_hL-2 cell line described in this report maintains a stable cell morphology and a diploid karyotype during an active growth phase of approximately 67 population doublings. At the end of this time, the cells enter a declining

growth period followed by senescent changes which end in cell death.

These cells were nontumorigenic when tested at various culture passages by inoculation intradermally into the cheek pouch of cortisone-treated weanling hamsters and subcutaneously in suckling hamsters treated with antithymocyte serum. More recent tests have been performed in rhesus monkeys treated with antithymocyte serum globulin with negative results (37).

Viral susceptibility studies indicate that DBS-FR_hL-2 cells maintain the sensitivity of primary cell cultures of rhesus monkey kidney to a number of human viruses, and results of tests on poliovirus and rubella virus production show DBS-FR_hL-2 cells capable of producing these viruses in titers comparable to that usually obtained in primary cell cultures of AGMK.

DBS-FR_hL-2 cells which have been frozen in medium containing glycerol or dimethylsulfoxide and stored in liquid nitrogen for periods up to 3 years have been recovered with 60 to 85% viability. Cells recovered from the frozen state have shown no changes in virus susceptibility or in cytogenetic or growth characteristics.

Repeated tests on this cell line for viral and other adventitious agents have been negative.

Rhesus monkey diploid cell line DBS-FR_hL-2 has cytogenetic, growth, and virus susceptibility characteristics which suggest additional studies to evaluate further its acceptability as a substrate in the manufacture of human virus vaccines. Preliminary work in a number of other laboratories on DBS-FR_hL-2 cells has been consistent with the results reported here.

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