This enzyme has an unusually high K_m (Michaelis constant) [$3 \times 10^{-4} M$ (*19*)] for tryptophan, and therefore its activity in vivo probably depends on the concentration of tryptophan. If our measurements of tryptophan concentrations in whole brain are indicative of those actually within serotonin-producing cells, then the increase in brain tryptophan produced by insulin would be of sufficient magnitude to cause a considerable acceleration in serotonin synthesis. In support of this hypothesis, we have shown (*2*) that small increases in tryptophan concentration in brain which occur after the injection of 12.5 mg of tryptophan per kilogram of body weight also cause significant elevations in brain serotonin (*2*).

Since almost any food that the rat might consume would probably elicit insulin secretion and thereby elevate plasma tryptophan, our observations suggest the sequential relation between food consumption and brain serotonin content described in Fig. 2. Carbohydrates, proteins, and, probably, fats (*20*) elicit insulin secretion in various mammalian species; dietary proteins would also be expected to raise plasma tryptophan by a direct contribution (*21*). It remains to be determined whether plasma and brain tryptophan, and brain serotonin, are also elevated in rats consuming diets other than pure carbohydrate.

Serotonin-containing neurons apparently participate in the control of a variety of behavioral and neurovisceral functions, including sleep, thermoregulation, motor activity, food consumption, and the secretion of hormones from the anterior pituitary gland. The increase in brain serotonin that occurs in rats given tryptophan appears to be highly localized to these serotonin-containing neurons (*22*). If the insulin-induced changes in brain serotonin content are, in fact, associated with alterations in the input-output characteristics of serotonin-containing neurons, then the sequence described in Fig. 2 may represent an important component of the systems by which the rat brain integrates information about the metabolic state of the animal into its control of homeostasis and behavior.

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Subhuman Primate Diploid Cells: Possible Substrates for Production of Virus Vaccines

Abstract. *Results of a program for development of new cell lines suggest that it it possible to establish cell lines from both rhesus and African green monkeys which are comparable to diploid lines of human origin, and that these monkey lines should be candidates for use in the production of virus vaccines.*

Most of the virus vaccines licensed for human use in the United States are prepared from primary cell cultures derived from monkeys, chicks, ducks, or rabbits (*1*). All of these tissue sources, except for monkeys, come from closed breeding colonies that are free of specific pathogens. Monkeys whose tissues will be used for vaccine production are held in quarantine for at least 6 weeks, but cross infection among these animals can occur, and their use poses many practical problems as well as certain theoretical risks.

The clinical experience with virus vaccines produced from monkey kidney cell cultures has been overwhelmingly successful. Nevertheless, it would be better to produce vaccines in a cell substrate which had been extensively studied and characterized as being free of any known microbial agent. The suggestion that vaccines be produced in a well-characterized cell system such as WI-38, a diploid cell line of human origin, instead of in primary cultures was first made in 1961 (*2*), and this recommendation has been offered repeatedly since then (*3*). This report describes the results of a program for development of diploid cell lines from subhuman primates. In these results, several cell lines appear promising for use in production of vaccines.

The published data on WI-38 cells has indicated the general safety and economic practicability of usage of this diploid cell line. However, there have been reservations about the use of parenterally administered vaccines made from such cells. Because of these theoretical objections to the use of human cells in vaccine production, the Division of Biologics Standards (*4*) felt that alternatives to WI-38 should be explored. Priority was given to de-

velopment of cell lines derived from rhesus monkeys (*Macaca mulatta*) and African green monkeys (*Cercopithecus aethiops*), since there was extensive clincal experience with virus vaccines produced from cell cultures of these species with no evidence of untoward reactions.

Diploid cell lines derived from fetal lung tissue have been developed for each species (*5*). Results have been sufficiently encouraging for us to pursue detailed characterization of these cell lines and to make them available for independent evaluation (*6*).

Selection of animals for the project was based on screening of pregnant rhesus and African green monkeys for the presence of (i) overt illness, (ii) parasites, (iii) detectable viremia, and (iv) antibodies to nine selected viruses including SV40, cytomegalovirus, and simian foamy types 1 and 2. While the first three criteria could be met, none of the 52 pregnant animals tested was free of antibodies to all of the selected viruses, and the animals chosen for the project had low antibody titers to simian foamy type 1 (1:16 to 1:64), simian type 2 (1:4 to 1:64); and cytomegalovirus (1:20 to 1:160).

After delivery of the fetus by Cesarean section, heart, kidney, lung, skin, and muscle tissues were removed, and cultures were established. Cells were grown in Eagle medium containing vitamins and glutamine at twice the usual concentration, nonessential amino acids, and 10 percent fetal bovine serum (not inactivated). Antibiotics were employed only during the initial preparation of the cultures, and the cultures were grown at 37°C in humidified air containing 5 percent CO_2 and fed at 3- to 4-day intervals. Cells were subcultivated by treatment of healthy confluent monolayers with trypsin, and cultures were divided at 1:2 to 1:4 split ratios, depending on the growth rate. In all, 23 lines of fibroblast cells were periodically assessed from three major standpoints: (i) growth potential, (ii) susceptibility to viruses, and (iii) cytogenetic (chromosomal) stability. Two cell lines of each species have appeared promising in these three respects and have been selected for detailed study. A summary of the biologic characteristics of two of these cell lines is presented in Table 1.

Cell line DBS-FRhL-2 (*7*) was derived from lung tissue from a 248-g male rhesus monkey fetus on 29 April 1969. During the first 44 passages the cells multiplied at a rapid unchanging rate, and population doubling time was 30 hours. During this period 67 doublings occurred, and cultures were divided at a 1:3 ratio. The cells entered a declining growth phase around the time of passage 45, and cultures were divided at a 1:2 ratio every 3 to 10 days until passage 50, when senescence was complete. The DBS-FRhL-2 line grows as a uniform monolayer of fibroblasts during its active growth period, and the cells show a clear, nonvacuolated cytoplasm with hematoxylin-eosin or Giemsa stains.

At passages 1 to 5, ampuls of these cells were prepared and stored in liquid nitrogen to provide a seed stock. (This represents the equivalent of 700 ampuls at passage 5.) After thawing, approximately 60 percent of the cells were viable, and the reestablished cultures showed no alteration in growth characteristics. Cytogenetic studies revealed a modal 2N chromosome number of 42 at passages 1 to 36 with polyploidy ranging from 0.3 to 1.3 percent. There was no evidence of an upward trend in polyploidy or in gross structural abnormalities after successive passages. Extensive tests for adventitious agents were performed according to federal regulations (*1*) on cells in continuous culture as well as on batches from liquid nitrogen. These have included inoculation of the cells into a variety of laboratory animals and cell cultures, examination of thin sections by electron microscopy, and fluorescent antibody studies. No microbial agents (bacteria, fungi, mycoplasma, or viruses) have been recovered from DBS-FRhL-2 thus far. Tests for tumorigenicity employing cheek pouches from Syrian hamsters treated with cortisone (*8*) as well as newborn Syrian hamsters treated with antilymphocyte serum (*9*) have been uniformly negative. Additional studies involving transplantation of cultured cells into newborn animals of the same species from which cells were derived are now in progress. The potential of such experiments for screening for tumorigenicity is obvious. This system allows a latitude of testing that does not exist with cultures of human diploid cells.

Studies of susceptibility to viruses were performed for several passages between passages 10 and 36 and indicate that DBS-FRhL-2 is sensitive to many viruses including poliovirus types 1, 2, and 3, Coxsackie A9, rubella, parainfluenza type 3, rhinovirus HGP, vaccinia, and mumps. Time of appearance of cytopathological effect or viral

Table 1. Biologic characteristics of cell lines derived from lungs of male monkey fetuses. Abbreviations are as follows: R, resistant (virus titers less than those in control cultures); S, sensitive (virus titers similar to those in control cultures).

	DBS-FRhL-2	DBS-FCL-1
	Monkey	
	Rhesus (*Macaca mulatta*)	African green (*Cercopithecus aethiops*)
	Passage history	
Life-span	50 passages	53 passages
Split ratio	1:3, passages 1–44 1:2, passages 45–50	1:3, passages 1–35 1:2, passages 36–53
	Cell morphology	
	Fibroblast-like	Fibroblast-like (epithelial through passage 5)
	Cytogenetics	
2N number	42	60
Diploid period	Through passage 36	Through passage 34
Polyploidy	0.3–1.3% (all passages)	0.4–6% (all passages)
	Virus growth	
Adenovirus		
Types 3, 7	R	S
Type 4	R	R
Coxsackie A9	S	S
Cytomegalovirus	R	R
Herpes simplex	R	S
Influenza A2	R	R
Measles	R	R
Mumps	S	S
Parainfluenza		
Types 1, 2	R	R
Type 3	S	S
Polio virus		
Types 1, 2, 3	S	S
Rabies	R	R
Rhinovirus HGP	S	S
Rubella	S	S
Vaccinia	S	S

titers, or both, were similar to those in appropriate control cultures (*10*) tested simultaneously. No change in sensitivity to viruses was noted for the various passages tested.

When DBS-FRhL-2 cultures were inoculated with adenovirus types 3, 4, or 7, parainfluenza types 1 or 2, influenza A2, herpes simplex, measles, cytomegalovirus, or rabies, vital titers were less than those observed in control cultures.

A second cell line, DBS-FCL-1 (*7*) was derived from lung tissue of a male fetus of *Cercopithecus aethiops* on 18 November 1969. Cultures were prepared and maintained under conditions identical to those for DBS-FRhL-2. During passages 1 through 5, epithelial cells predominated. After passage 6, however, the population was comprised largely of fibroblasts. Cells grew rapidly for 35 passages, with a population doubling time of 33 hours, and were transferred every 3 to 4 days with division at a 1:3 ratio. Around the time of passage 36 a decrease in growth rate was observed, and the cells were subcultured at 3- to 10-day intervals with division at a reduced 1:2 ratio. After passage 53 no further multiplication of the cells was noted. At an early passage, a seed stock of this cell line was prepared and stored in liquid nitrogen in a manner similar to that for DBS-FRhL-2. As with DBS-FRhL-2, a low frequency of polyploidy (1.3 to 6.0 percent) was observed between passages 1 and 34. Tests for the presence of adventitious agents and for tumorigenicity, as described for DBS-FRhL-2, have been negative.

The spectrum of susceptibility to viruses of DBS-FCL-1 is similar to that of DBS-FRhL-2. Sensitivity was demonstrated to poliovirus types 1, 2, and 3, adenovirus type 3, parainfluenza type 3, herpes simplex, mumps, rhinovirus HGP, rubella, Coxsackie A9, and vaccinia.

In order for these cell lines to be economically feasible as substrates for vaccine production, they must provide (i) adequate cell population, (ii) rapid growth rate, and (iii) yields of virus sufficient for vaccine production. An adequate population is assured by the ability of both cell lines to progress through at least 50 doublings before senescence. Thus, growth potential of these lines is equal to or greater than that reported for human diploid cells (*11*) and rhesus monkey diploid cells (*12*). The doubling time of both cell lines (approximately 30 hours) compares favorably with other values found for primate lung cells (*2*, *13*). The stability of these cells during extended periods of storage is another factor assuring their adequacy for production of virus. No changes have been recognized in either cell line after 6 to 12 months of storage in liquid nitrogen.

The results of this program for development of new cell lines suggest that it is possible to develop populations of cells from subhuman primates which meet basic requirements for use in the production of human biologics. However, further evaluation by other independent investigators will be necessary to increase the level of confidence in the safety of these cells.

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5. Contract NIH-69-100 with Lederle Laboratories; P. J. Vasington, project director; R. E. Wallace, principal investigator.
6. Samples of DBS diploid cell lines are available to all investigators by contacting J. C. Petricciani. A more complete report on development and characterization of these cell lines is in preparation.
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Limulus Lateral Eye: Properties of Receptor Units in the Unexcised Eye

Abstract. *Single receptor units in the compound eye of the horseshoe crab were illuminated, and their impulse discharges were recorded without removing the eye from the animal. The receptors were spontaneously active in darkness and responded without saturation over a light intensity range of 10^{10} to 1. When the eye was excised, the receptors did not discharge in darkness and had an intensity range of 10^{5} to 1, as is usually found. Experiments show that these and other differences result from cutting off the blood supply to the eye when it is excised. In addition, the range and shape of the intensity characteristic suggest that more than one receptor mechanism encodes light intensity in this eye.*

The lateral eye of the horseshoe crab, *Limulus polyphemus*, has been studied for many years (*1*). In most experiments the eye was removed from the animal and placed in a recording chamber (*2*). Although the preparation is well suited for experimentation, the sensitivity to light of the receptor units (ommatidia) often declines steadily after the eye is excised. To try to overcome this problem, we recorded from the optic nerve fibers without removing the eye from the animal.

Here we describe a method for recording from the intact *Limulus* eye. We show that responses from single ommatidia can be recorded for periods up to 30 hours and that the sensitivity of the eye remains stable for at least one week. We describe some response characteristics of ommatidia in the intact preparation and show how these characteristics differ from those of the excised eye.

Our method was to clamp an adult *Limulus* to a rigid platform that was placed in an aquarium filled with recirculated artificial seawater (*3*). The dorsal surface of the animal, including the lateral eyes, remained above the