the sensor output is 0.02 mV per degree Kelvin, which is negligible. Our experimental measurements over the temperature range of 297 to 315 K and a *p*H range of nominally 6 to 8 show that the temperature effect on the sensor output is within experimental error and negligible.

In addition to the pure Pd wire used in this study, we also investigated a Pd-Ag alloy wire (60 percent Pd and 40 percent Ag). This alloy is stronger than pure Pd and might be more suitable for certain applications. Our results showed that the *p*H electrode characteristics of this alloy are identical to those of pure Pd.

Palladium oxide can dissolve in a medium containing Cl^-. Coating the electrode with a cation-permeable polymer material, Nafion latex, to minimize the dissolution of the oxide was investigated. The coating proved to be effective and did not alter the sensitivity of the electrode, but it did significantly increase the response time. Without this coating, the fabricated Pd-PdO electrode could be reused repeatedly in a normal saline solution over a period of 6 days without any deterioration in performance. Coated electrodes operated for as long as 10 days.

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Human Rotavirus Type 2: Cultivation in vitro

Abstract. *A strain of type 2 human rotavirus (Wa) was grown to relatively high titer through 14 passages in primary cultures of African green monkey kidney (AGMK) cells. This passage series was initiated with virus that had been passaged 11 times serially in newborn gnotobiotic piglets. In contrast, virus present in the stool of patient Wa as well as virus from the first, second, or third passage in piglets could not be propagated successfully in African green monkey kidney cells. Prior to each passage in cell culture, the virus was treated with trypsin and the inoculated cultures were centrifuged at low speed. Cultivation of a type 2 human rotavirus should aid attempts to characterize this virus and to develop a means of immunoprophylaxis for a serious diarrheal disease of human infants.*

Rotaviruses of the family Reoviridae are now recognized as major pathogens causing acute diarrheal disease in the young of a number of species including man (*1*, *2*). Only a few animal rotaviruses have been adapted to grow to high titer in cell culture (*1*–*3*). Numerous attempts to propagate human rotaviruses to high titer in vitro have failed (*4*–*8*). In this report we describe the successful adaptation of a human rotavirus strain that grows efficiently in primary cultures of African green monkey kidney (AGMK) cells.

We obtained 42 fecal specimens from pediatric patients with diarrheal disease; the patients were previously shown to be infected with rotavirus by electron microscopic examination of their feces (*9*–*11*). The specimens were tested for their efficiency in initiating infection in AGMK cells. A 0.5-ml portion of a 2 to 10 percent stool filtrate was mixed with 0.5 ml of trypsin (20 μg/ml) (Sigma) and incubated for 1 hour at 38°C. The mixture was then inoculated into a culture well containing approximately 10^6 AGMK cells. Plates containing six such wells were centrifuged at 1400*g* in an IEC centrifuge (model PRJ) for 1 hour at 37°C, after which the inoculum was removed. This technique of centrifugation had previously been shown to increase the efficiency with which various rotaviruses initiated infection of cells in culture (*5*, *12*, *13*). Plates were washed once and refed with L-15 medium containing a solution of sucrose, phosphate, and glutamate (*14*) as well as glutamine and antibiotics. The cells were incubated for 48 hours at 37°C and then fixed with cold (−20°C) methanol and stained for examination by direct fluorescence microscopy by using fluorescein-conjugated goat antiserum (immunoglobulin G fraction) to human rotavirus. The percentage of antigen-containing cells was estimated. Thirty-one of the fecal suspensions were positive by this technique, but the proportion of cells exhibiting viral antigens was low in most instances, 1 percent or less. Only five specimens induced antigens in 10 percent or more of cells. These five strains were previously determined to be serotype 2 by the enzyme-linked immunosorbent assay (ELISA) (*10*).

To promote the growth of viruses that induced antigens in 10 percent or more of cells and hence increase the probability of the emergence of a cell culture–adapted mutant, we administered orally 1 to 2 ml of a 2 percent bacteria-free fecal filtrate containing type 2 rotavirus from patients S or Wa to four 26-hour-old gnotobiotic piglets (*15*, *16*). The piglets developed mild, transient diarrhea. Intestinal contents for subsequent passage were collected from individual animals killed 46 to 48 hours after inoculation. Examination of intestinal contents from the passage in piglets revealed rotavirus by electron microscopy, ELISA, and quantitative fluorescence microscopy (*12*). A piglet inoculated with Wa strain of human rotavirus had a titer of $10^{5.7}$ fluorescent foci (FF) per milliliter of intestinal contents. Immunofluorescence staining of jejunal impressions (*15*) revealed that 2 to 3 percent of epithelial cells were infected with rotavirus. Because infection by the Wa strain was slightly more extensive than that seen with the S strain, the former was used for subsequent passages.

Ten additional passages in gnotobiotic piglets (6 to 144 hours old) were performed serially, 1 ml of a mixture of contents from the small and large intestine being used as the inoculum except during the second passage when mixed intestinal contents were diluted 1:5 and the third passage when an aspirate from the large intestine was used as the inoculum. At each passage the virus administered orally consisted of gut contents collected at necropsy from piglets killed 19 to 48 hours after they were inoculated with virus. At each passage, infection was documented by the detection of viral antigen in gut contents. During passage 2 in piglets the quantity of virus in intestinal contents was $10^{6.8}$ FF per milliliter and remained at approximately this level during the subsequent nine passages. Infection was also confirmed by detection of rotavirus by electron microscopy of intestinal contents and direct fluorescence microscopy of intestinal impressions. During passage

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Table 1. Serologic relation between tissue culture-adapted Wa strain of rotavirus and rotaviruses of simian, bovine, and porcine origin. Homologous values are underlined; values for serum samples containing antibody against SA-11 and OSU rotaviruses represent geometric mean values of two determinations. An additional test (without centrifugation) with the Wa strain and the UK, SA-11, OSU, and paired Wa serum samples confirmed the relationships shown here.

Immunizing rotavirus	Serum		Reciprocal of antibody titer with indicated virus			
	Animal	Type	UK*	SA-11*	OSU*	Wa†
Bovine, UK strain	Guinea pig	Hyperimmune	7,606	< 160	< 160	< 160
Bovine, NCDV strain	Guinea pig	Hyperimmune	≥ 40,960	694	778	< 160
Simian, SA-11 strain	Guinea pig	Hyperimmune	< 20	≥ 40,960	< 160	640
Porcine, OSU strain	Gnotobiotic piglet	Convalescent	< 20	937	1,691	20
Human type 2 Wa strain	Gnotobiotic piglet	Preinfection	< 20	23	< 20	80
Human type 2 Wa strain	Gnotobiotic piglet	Convalescent	51	343	< 20	1,280

*Sixty percent plaque reduction. †Sixty percent reduction of fluorescent foci.

10, 20 to 80 percent of jejunal epithelial cells contained viral antigens. Moderate diarrhea developed during some of the passages, while inapparent infection occurred during other passages. There was no indication that virulence increased with passage.

We attempted to initiate serial passage of the Wa strain in AGMK cells using stool filtrate derived from patient Wa and intestinal aspirates collected at passages 1, 2, 3, and 11 in piglets. A standardized technique was used, but serial passage in culture was achieved only with passage 11 material from piglets. Inocula were first treated with trypsin, because this procedure enhances significantly the infectivity of some animal rotaviruses, that is, bovine and porcine rotaviruses, for tissue culture (*3, 17*). One milliliter of diluted ($10^{-0.7}$, 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4}) trypsin-treated virus was inoculated onto primary AGMK cell monolayers in six-well plates and maintained with Eagle's minimum essential medium, gentamicin, and glutamine. These plates were centrifuged at 1400*g* at 37°C for 1 hour and then 2 ml of medium was added. At 24 to 48 hours after inoculation, cells were fixed with cold methanol and stained for examination by direct fluorescence microscopy as before.

During passage 1 in AGMK a $10^{-0.7}$ or 10^{-1} dilution of piglet-derived virus induced the production of viral antigens in approximately 50 percent of AGMK cells; these antigens were localized chiefly in the cytoplasm. With higher dilutions of virus fewer cells were positive and it was possible to determine the viral titer ($10^{5.6}$ FF per milliliter) of the inoculum by counting fluorescent foci.

Passage 2 of virus was carried out after 24 hours with concentrated cells that exhibited maximum expression of antigen being used as the inoculum. After passage 2, such cells were suspended in their tissue culture medium and passaged at 24- to 96-hour intervals. In this manner the virus was passaged 14 times yielding titers ranging from 10^3 FF per milliliter (passage 4) to $10^{6.6}$ FF per milliliter (passage 10). At passage 14 the virus titer was $10^{5.2}$ FF per milliliter. During passage the Wa strain became contaminated with SV5 virus, an indigenous simian virus. Before passage 12 SV5 was eliminated by treating the suspension with 20 percent ether for 30 minutes. The Wa strain grew less well in cultures of human, porcine, or bovine embryonic kidney than they did in AGMK cells.

When examined with the electron microscope, most of the virions at passage 8 appeared to be complete, possessing a double capsid. This was in contrast to another type 2 human rotavirus that grew poorly in human embryonic kidney cell culture; this virus when grown in vitro was exclusively incomplete, lacking the outer capsid (*4*).

Finally, we performed studies to establish the identity of the Wa tissue culture-adapted virus. Previous experience indicated the ease with which cultures could become contaminated with other animal rotaviruses well adapted to tissue culture that were also under study in the laboratory. The identity of the culture-adapted Wa strain was investigated by co-electrophoresing its viral RNA with that of other rotaviruses whose RNA gel patterns had been characterized previously (*18*). The RNA from the Wa isolate after eight passages in AGMK cultures was compared with RNA from the following rotaviruses: human rotavirus strains D and L; bovine rotavirus strains UK and NCDV; porcine rotavirus strains OSU and EE; simian rotavirus SA-11; and O agent (*18*). The RNA gel pattern of the Wa strain differed from each of the animal rotavirus RNA patterns in the mobility of three to seven genome segments. The Wa strain RNA differed from the D strain of type 2 human rotavirus in the mobility of only one segment (No. 5), and its pattern was identical to that of the L strain of type 2 human rotavirus. The last pattern represents one of three previously recognized human rotavirus RNA gel patterns (*18*).

The Wa strain at the 11th culture passage was also compared to bovine, porcine, and simian rotaviruses by neutralization in tissue culture. Antibody assays for the animal rotaviruses were performed by plaque reduction. Bovine and simian rotaviruses were treated with trypsin (10 μg/ml, final concentration) prior to being incubated with diluted serum (1 hour at 37°C) and inoculated onto CV-1 cells (a line derived from AGMK). The porcine rotavirus plaque reduction test was performed in MA104 cells (a line derived from embryonic rhesus monkey kidney) by a procedure in which the agar overlay contained pancreatin (0.15 percent of 25 percent pancreatin 4 × N.F.; Gibco) and DEAE dextran (100 μg/ml) (*19*). The Wa strain did not produce plaques reproducibly in AGMK cells and for this reason neutralization was assayed by the technique of reduction of immunofluorescent foci similar to that described by Thouless and co-workers (*12*).

The bovine, simian, and porcine rotaviruses were distinct from the Wa culture-adapted strain when tested by virus neutralization (Table 1). However there was a one-way antigenic relationship between the simian virus and the Wa isolate; this is consistent with a previous observation made by the immune electron microscopy technique (*20*). There was also a one-way antigenic relationship between the porcine and simian rotaviruses. The Wa culture-adapted virus was typed by ELISA and identified as serotype 2, the same type as the rotavirus present in the stool of patient Wa. Thus the tissue-grown Wa strain appears to be type 2 human rotavirus and not a tissue culture-adapted animal rotavirus contaminant acquired in the laboratory.

Cultivation of type 2 human rotavirus in tissue culture should facilitate a more

detailed examination of its properties and permit us to manipulate its genome with the intent of developing attenuated mutants for use in the prevention of a serious diarrheal disease of human infants.

Note added in proof: After submission of this manuscript, we developed a plaque assay for the Wa strain of human rotavirus. The relationship of the Wa strain to the cultivable animal rotaviruses shown in Table 1 was confirmed with this assay for measurement of plaque reduction antibody.

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Retesting the Commitment Theory of Cellular Aging

Abstract. *The commitment theory of human fibroblast aging predicts that 55 percent of cells will be nondividing at the middle to late stages of the replicative life-span; in the present study, however, fewer than 10 percent were nondividing. The fact that no immortal diploid cells have yet been reported is also at odds with the theory. Available data on the variable life-span of clones and mass cultures, the dependence of longevity on population size, and the predominance of certain cell types at termination of a culture are compatible with simpler theories, which support the idea that the limited replicative life-span of diploid fibroblasts is a valid model for organismic aging.*

Kirkwood and Holliday (*1*) and Holliday *et al.* (*2*) proposed a commitment theory to explain the finite life-span in culture of human diploid fibroblasts (HDF). They asserted that all cells initially belong to a class of uncommitted or immortal cells. At each division a fraction of the cells enters the committed or mortal class, which, after additional divisions, ceases to divide. As the culture expands, the uncommitted population accounts for a diminishing fraction of the total, and by passage 20 there is a good chance of losing the uncommitted population altogether if the number of cells retained at each subcultivation does not greatly exceed 10^6 (*2*). They concluded that the finite replicative life-span of HDF is an artifact of the relatively small number of cells in culture. We argue that the commitment theory of cellular aging is untenable as proposed; even if it were correct in principle, the finite life-span of HDF would still reflect the age-dependent decline of cellular function in vivo.

We emphasize that we have tested the Kirkwood-Holliday model and its assumptions only and are proposing neither an altered version of their commitment theory nor a new model of our own. The main assumptions of their model, then, are (i) initially there is one uncommitted cell (U), and at each generation (g) the probability (P) that an uncommitted cell becomes committed is .275; (ii) all cells attach after each subcultivation and double at a uniform rate; and (iii) committed cells (C) become nondividers (D) after an additional 60 divisions (M).

Equations for the fraction of the population in each class have been derived for the case in which uncommitted cells are lost by dilution at generation 43 (*3*). Solutions to these equations were determined and the theoretical fractions of dividing and nondividing cells were plotted as a function of the percentage of life-span completed (Fig. 1). Kirkwood and Holliday (*1*) determined the relationship between generations and passage level in vitro based on 20 to 25 cell doublings in vivo and during the phase of explantation. This assumption is tenuous, since little is known about the history of cells in vivo or during emigration from the explant. The relationship between passage and generation is further complicated by asynchronous cell division and plating efficiencies of less than 100 percent (*4*, *5*). Nonetheless, the striking feature of the theoretical curve (Fig. 1b) is the sharp rise in the fraction of nondividing cells at $g = 60$ (50 percent of the life-span completed) from 0 to 55 percent. This change must correspond to the 45 percent decline in growth rate reported by Kirkwood and Holliday (*1*). Although the abrupt transition is an artifact of a simplified model wherein cells divide synchronously, it is fundamental to the commitment theory, since it was used to determine P and M (*2*).

Rather than measure growth rate, which is influenced by several variables, we measured the fraction of nondividing cells directly by labeling with thymidine (*6*) to determine whether the proposed transition existed. The fraction of labeled nuclei was 80 to 90 percent throughout the middle to late interval of passage (Fig. 1a); the commitment theory predicts only 45 percent for MRC-5 cells, which is clearly inconsistent with our data or data on any of the 12 fibroblast strains tested so far (*6*, *7*). The decline in growth rate at passage 42, during what was ostensibly a single experiment (*1*), must have been caused by other factors, such as a serum change or alteration in culture conditions.

Since the fraction of nondividing cells is less than 10 percent for MRC-5 and other cells (including WI-38 and IMR-90)

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