while if the increase occurs in the nutrient membrane doubt is cast on the assumption that Cl^- and HCO_3^- moves across the nutrient membrane as free ions because improbably high emf's would be demanded (5). Further work needs to be done before an analysis of the implications of this finding for the mechanisms of ion transport would be profitable.

Conclusion. The addition of Ba^{++} to the fluid bathing the nutrient side of the frog gastric mucosa resulted in a marked increase in resistance and relatively little change in the H^+ rate and the PD. Addition of Ba^{++} in comparable and higher concentrations to the secretory fluid had no effect.

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Serologic Screening of Rhesus and Grivet Monkeys for SV_{40} and the Foamy Viruses (32662)

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The spontaneous appearance of virus cytopathic effect (CPE) in many cultures has been a critical problem in the use of monkey kidney cell cultures for virus vaccine production or research purposes. Although a number of viruses have been isolated and identified, their detection in monkey kidney cell cultures from this laboratory has been limited mainly to two virus CPE groups (1). Group one includes the syncytial CPE, induced primarily by measles virus; SV_5 (parainfluenza 5 as it is now classified); and the foamy viruses. The vacuolating CPE comprises the second group of which SV_{40} is the causative agent. In order of frequency of occurrence, the foamy viruses and SV_{40} comprise the bulk of viruses isolated from monkey kidney cell cultures.

The serological studies of Ruckle (2) and the fluorescent antibody studies of Carski (3) indicate a direct correlation between the absence of antibody to foamy virus in monkeys and the absence of this virus in their kidney cell cultures. With these criteria, by use of serological techniques it should be possible to select monkeys free of foamy viruses for tissue culture production.

This paper describes in detail the development and routine application, over an extended period, of a complement fixation-antibody screening test, which has been used for the rapid and effective selection of monkeys for production of tissue cultures free of the foamy and SV_{40} viruses.

Materials and Methods. Monkeys. Both rhesus, *Maccaca mulatta,* and grivet, *Cercopithecus aethiops,* monkeys, which are used for poliovirus vaccine production, were tested in this study. These monkeys were trapped and housed by a procedure described by Vickers (4), and shipped to this laboratory. Upon arrival, the monkeys were isolated in single-cage units and test-bled, and sera were taken for testing. At the end of a 6-week quarantine period, selected monkeys were bled again and killed, and their kidneys were removed aseptically for tissue culture preparation.

Tissue cultures. Kidney cell cultures derived from both rhesus (RMK) and grivet monkeys (GMK), and from rabbits (RbK), were prepared by the method of Youngner (5). The growth media consisted of lactalbumin hy-

drolyzate (0.5%) in Earle's balanced salt solution with the addition of sodium bicarbonate (0.11%), 100 μg/ml each of streptomycin and neomycin, and 2% calf serum for the monkey tissue and 10% for the rabbit cell cultures. The maintenance media consisted of the same basic medium except that the sodium bicarbonate level was increased to 0.16%; the calf serum was omitted from the media for the monkey kidney cultures and reduced to 5% for the rabbit kidney cultures (RbK).

Virus isolation. The RMK and GMK cell cultures were tested for adventitious viruses according to Public Health Service requirements (6), as illustrated by Bittle *et al.* (1). The procedures for isolation and identification of the foamy viruses have been previously reported (7).

Complement fixation (CF) antigens. The foamy virus (FV) CF antigens were prepared by seeding a concentrated RbK cell suspension with the virus and adsorbing at 37°C for 1 hour. The virus-cell mixture was then diluted to a cell concentration of 200,000 viable cells/ml and a final concentration of 100-5000 TCD_{50} of virus/ml. Each Blake bottle was then seeded with 50 ml of the diluted virus-cell suspension and incubated at 37°C. Monolayers developed quickly; a CPE could be demonstrated within 5 days and complete cell-sheet destruction by 10 days. The fluids and cells were then harvested after two freeze-thaw cycles in dry ice and alcohol, and the harvest was clarified by centrifugation at 2000 rpm for 15 minutes. The antigens were then dispensed in ampules and stored at —60°C. Fluids and cells from normal (RbK) control bottles were also processed as described, and were used as normal tissue-control antigens. All antigens were pretested by CF checkerboard titrations against positive and negative reference sera for potency, specificity, and anticomplementary activity.

The SV_{40} CF antigen was prepared by seeding established monolayers of $LLCMK_2$ cell line in Blake bottles with 5 ml of the virus (about 10^8 TCD_{50}/ml). The cultures were incubated at 37°C, and the fluids and cells were harvested, processed, and tested as previously described, when at least 75% of the cell sheet was destroyed. Normal control tissue antigens were also prepared. The $LLCMK_2$ cell line was employed for production of SV_{40} CF antigen, instead of primary GMK cultures, because higher CF potency and specificity were obtained.

Complement fixation test. The micro LB_{50} CF method (8) was used primarily in this study. Test sera collected from each monkey before and after the 6-week holding period were heat-inactivated in a 60°C water bath for 30 minutes. Antibody was titrated by using constant quantities of antigen, complement, and doubling dilutions of serum. Appropriate controls, including negative and positive reference sera to each antigen and normal tissue antigens, were incorporated in each test. For routine screening of a large number of monkeys, only 1:4 and 1:8 dilutions of sera were tested. Test sera were considered antibody-negative only when no specific CF reactions were obtained in either test dilution.

Results. Development of the CF screening test. Tables I and II summarize the bases for the selection and use of the foamy and SV_{40} viral antigens for the CF screening of both rhesus and grivet monkeys. Thirty-seven per cent of the rhesus monkey sera, when tested with a Type I foamy virus originally isolated from RMK tissue culture, were found to be antibody-positive. The same sera, when tested with serotypes II and III foamy viruses isolated from spontaneously degenerating GMK cultures, were relatively nonreactive, even though foamy virus was detected in the kidney cell cultures from 45% of these rhesus monkeys. With grivet monkeys, the sera from 51 and 38% of the monkeys, respectively, were found to be antibody-positive when tested with types II and III foamy virus antigens. The same monkeys, when tested with type I antigen, were found to contain only 8% antibody-positive monkeys, although foamy virus was detected in the kidney cell cultures from 36% of these grivet monkeys. The serological results from this study would tend to support the observation previously reported (7) that, from a substantial number of monkeys tested, only type I of the three foamy virus serotypes was isolated from rhesus monkeys, and both types II and III from grivet monkeys. The use of an SV_{40} anti-

TABLE I. Incidence of CF Antibody to Foamy Virus in Sera[a] of Rhesus and Grivet Monkeys, and of CPE in Cultures of Their Kidney Tissue.

Monkey species	Number	Sera with antibody to:			Cultures with CPE
		Type I	Type II	Type III	
Rhesus	65	24	1	0	29
	100%	37%	2%	—	45%
Grivet	65	5	33	25	23
	100%	8%	51%	38%	36%

[a] Considered positive when CF dilutions of 1:4 or greater was $\geq 2+$.

body-screening test appears to be applicable in testing only rhesus monkeys, since no antibody was detected in the grivet monkeys. This observation was not surprising since no definite indication of natural SV_{40} infection in grivet monkeys has been observed.

TABLE II. Incidence of CF Antibody to SV_{40} in Sera[a] of Rhesus and Grivet Monkeys and of Recovery of Virus from Cultures of Their Kidney Tissue.

Monkey species	Total no. monkeys	Sera with antibody to SV_{40}	Cultures with SV_{40}
Rhesus	100	16	14
Grivet	100	0	0

[a] Considered positive when CF dilutions of 1:4 or greater was $\geq 2+$.

An attempt was made to compare the CF properties of the three foamy virus serotypes by hyperimmunization of rabbits and guinea pigs, and cross-checking the sera by CF with the three antigens. No results could be obtained due to poor antibody response and nonspecific reactions. Indirect evidence, however, based on the serological testing of a large number of both rhesus and grivet monkeys, indicates the existence of at least two CF antigenic groups, with type I foamy virus comprising one group and types II and III, the other. Therefore, it was found necessary to screen rhesus monkeys only with SV_{40} and type I foamy virus antigens, and grivet monkeys with either the types II or III foamy viruses. Solely on the basis of CF antigenicity and ease in handling, the type II antigen was selected for routine CF antibody screening.

Application and efficiency of the CF screening test. Approximately 3000 rhesus and 2500 grivet monkeys have been tested for CF antibody. Table III shows the number of rhesus monkeys tested over a 4-year period, and the incidence of foamy I and SV_{40} antibodies; Table IV, the number of grivet monkeys tested during a 3-year period, and the incidence of foamy type II antibody. The serologic results from monkeys bled and tested before the 6-week holding period indicate a high prevalence of antibody to their respective test antigens,

TABLE IV. Complement Fixation Results for Foamy Virus II Antibodies in Grivet Monkeys.

Year	Foamy virus II		
	No. tested	No. positive	% Positive
1963	1967	941	47.9
1964	3667	2450	66.8
1965	1079	685	63.5

TABLE III. Complement Fixation Results for Foamy I and SV_{40} Antibodies in Rhesus Monkeys.

Year	Foamy I			SV_{40}		
	No. tested	No. positive	% Positive	No. tested	No. positive	% Positive
1962	601	389	64.7	77	16	20.7
1963	391	171	43.7	398	57	14.3
1964	956	327	34.2	908	104	11.5
1965	1131	669	59.2	1173	209	17.8

TABLE V. Efficiency of Complement Fixation (CF) Antibody Test in the Detection of SV_{40} and Foamy-Virus-Free Monkeys.

Monkey type	CF antigen serotype	No. monkeys tested	Percentage screened correctly
Rhesus	SV_{40}	407	80.5
Rhesus	FV I	508	97.8
Grivet	FV II	972	86.5

TABLE VI. Incidence of CF Antibody to Foamy Viruses I, II, and SV_{40} in Sera[a] of Vervet Monkeys.

	No. monkeys with antibody to		
Number tested	Foamy I	Foamy II	SV_{40}
50	0	28	0

[a] Considered positive when CF at dilutions of 1:4 or greater was $\geq 2+$.

and closely coincide with the frequency of virus detection in the kidney cell cultures. No definite indication of any seasonal variation could be determined from this study.

Data obtained over a 2-year period with both rhesus and grivet monkeys indicate that the CF test was 80–98% effective in selecting monkeys free of SV_{40} and the foamy viruses (Table V). These results were based on monkeys that were antibody-negative on at least two bleedings spaced approximately 6 weeks apart, and on the absence of any detectable virus in their kidney cell cultures. However, efficiency of the screening test in detecting SV_{40} and foamy virus-contaminated kidneys has been found to be much lower. Approximately 45% of the GMK or RMK kidney cell cultures obtained from either foamy or SV_{40} antibody-positive monkeys were free of these viruses. This low correlation with the foamy viruses, as an example, is attributed to the fact that the virus is not yet detectable in the kidneys, although the monkey is most likely infected. If the presence of antibody is compared with recovery of virus from throat swabs, the correlation is much better (9).

A study was performed to determine if the incidence of foamy and SV_{40} CF antibody in the South African vervet monkey, *Cercopithecus pygerythrus centralis*, paralleled the results obtained with grivet or rhesus monkeys. The results summarized in Table VI indicate that the antibody spectrum is almost identical with that of the grivet monkeys. No antibody to SV_{40} or type I foamy virus, but a high incidence of antibody to type II foamy virus, was detected.

The relationship between the size (weight) of a monkey and the presence of foamy CF antibody was investigated. The results summarized in Table VII show that, with grivet monkeys, the incidence of foamy virus antibody is lower in monkeys weighing 4 pounds or less, or weighing over 8 pounds. Therefore, cell cultures prepared from monkeys in these two weight ranges should stand a better chance of being free of foamy virus contamination. Our experience supports this observation.

Discussion. Since its inception, the use of monkey kidney tissue culture has been plagued by the spontaneous appearance of a number of simian viruses. The presence of these viruses has rendered the tissue in many instances unusable for virus vaccine production or research purposes. The problem has been further aggravated by the fact that, although every precaution was taken in the selection, transportation, and maintenance of these monkeys, virus infection was still evident. There was no procedure by which one

TABLE VII. Relationship Between Size (Weight) of Grivet Monkeys and Presence of Foamy II CF Antibodies.

	No. and percentage of foamy CF-positive monkeys						
No. tested	<3 pounds	3–4 pounds	4–5 pounds	5–6 pounds	6–7 pounds	7–8 pounds	Over 8 pounds
216	0/43[a]	5/85	14/34	7/13	9/19	7/16	1/6
	0%	6%	41%	54%	47%	44%	18%

[a] Number positive over number tested.

could determine if these latent viruses were in the kidneys prior to preparation of cell cultures. To obtain an adequate supply of virus-free cell cultures, it was therefore necessary to harvest the kidneys from more than twice the required number of monkeys.

The results of this study show that a large proportion of apparently normal, healthy monkeys are infected with either SV_{40} or the foamy viruses. Serologic and virus-isolation studies have shown that Indian rhesus monkeys are heavily infected with a foamy virus and that this virus is exclusively the serotype I, or the SA 1 virus as denoted by Malherbe and Harwin (10). The incidence of SV_{40} in rhesus monkeys has been found to be considerably lower than that of the foamy viruses. Even though the incidence of foamy viruses is similar in both grivet and rhesus monkeys, the grivet has been used for virus vaccine production because of its freedom from the oncogenic SV_{40} virus. Serologic and virus-isolation studies have shown that East African Grivet monkeys are relatively free of foamy virus type I and SV_{40}, but are heavily infected with foamy virus serotypes II and III.

Although a number of other viruses, mainly measles and SV_5, have been detected in these monkeys and in their kidney cell cultures, the frequency of detection has been reduced by proper housing, maintenance of isolation, and establishment of a 6-week-quarantine holding period on all newly acquired monkeys. Specifically with monkey measles, where infection is acquired from man, a lengthy quarantine period prior to kidney harvest has reduced the prevalence of this virus in the kidney cultures (11). The incidence of SV_5 virus in the kidney cell cultures of both rhesus and grivet monkeys has been relatively low (1). Whether the monkeys were naturally free of infection or the quarantine period reduced the prevalence of this virus remains to be determined. With SV_{40} and the foamy viruses, a quarantine period does not appear to be effective. Long-term persistence of SV_{40} antibody has been reported by Ashkenazi and Melnick (12), and our studies indicate that the same is true with the foamy viruses. Monkeys that were antibody-positive and were shedding the virus have been held in isolation for as long as 6 months, with no loss in antibody and with recovery of the virus from throat swabs and kidneys.

The utilization of a complement fixation-antibody screening test has been found effective for the detection of monkeys free of SC_{40} and the foamy viruses. This procedure eliminates virus carriers, and results in a significant reduction in the occurrence of these viruses in the kidney cell cultures. The results of this study indicate that the screening test is effective only when the appropriate viral antigens are used; that is, SV_{40} and foamy virus type I are used to screen rhesus monkeys, and either foamy virus types II or III for testing grivet and possibly vervet monkeys. The test was most effective if at least two bleedings were tested; the first bleeding was taken when the monkey is first obtained, and the second, just before kidney harvest. If both bleedings are antibody-negative, the test is 80–95% effective in providing cell cultures free of these viruses.

It has been found that the kidney cell cultures from approximately 50% of the SV_{40} and foamy virus CF-positive monkeys are free of these viruses. The screening test in this aspect would appear somewhat wasteful in discarding clean monkeys. However, from an economic standpoint, it has been found advantageous to serologically prescreen the monkeys prior to purchase, and select for tissue culture production only those monkeys that are antibody-negative. As long as the supply of serologically negative monkeys is available, we are able to regularly obtain a high percentage of monkeys free of these viruses for the production of kidney cell cultures.

Summary. Progress toward the elimination of SV_{40} and the foamy viruses from primary cell cultures of monkey kidney has been achieved by a complement fixation-antibody screening test. Data obtained over a 2-year period with a large number of rhesus and grivet monkeys indicate that the test was 85–98% effective in selecting monkeys free of foamy virus contamination, and approximately 80% effective in selecting rhesus monkeys free of SV_{40}. Valid results with the foamy virus CF tests were obtained only when rhesus monkeys were screened with a serotype I foamy virus antigen, and grivet monkeys

with serotype II or III foamy virus antigens. Analysis of serologic and virus-isolation data obtained over a 4-year period revealed that approximately 50% of the rhesus monkey sera tested were CF-positive for type I foamy virus and 16% were positive for SV_{40}. With grivet monkeys, approximately 60% were CF-positive for type II foamy virus, and none were positive for SV_{40}.

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Efficacy of Interferon in the Treatment of Mice with Established Friend Virus Leukemia* (32663)

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On the basis of the possible virus etiology of human leukemia, treatment of this disease might logically be considered in terms of virus inhibition, perhaps by employing the principles of viral interference. Such an approach was attempted in an earlier study (1) in which the sequential inoculations of six virus types into a patient with acute myelogenous leukemia were followed by transient clinical and hematological remissions. Since the mechanisms involved in the production of these remissions could not be determined with certainty, a murine system was developed in which the effects of nontumor viruses on virus-induced leukemia could be more readily investigated.

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In earlier studies (2,3) it had been found that Sendai virus (parainfluenza 1) inoculated into mice *prior* to Friend leukemia virus could markedly inhibit the replication of and the splenomegalic response to Friend virus and prolong the life of leukemic mice. Since human leukemia, be it of virus etiology or otherwise, presents as an established disease, it was of interest to study the effects of Sendai virus in mice with *established* Friend virus leukemia.

The present communication describes experiments in which the life of mice with established Friend virus leukemia was prolonged following inoculation of Sendai virus. As evidence suggesting that interferon is the mechanism involved in this prolongation of life, it will be shown that the repeated administration of interferon or an injection of Statolon (an interferon-inducing substance