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Latent Virus Infections in Primate Tissues with Special Reference to Simian Viruses

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INTRODUCTION

In recent years, there have been increasing numbers of reports concerning the recognition of latent virus infections in tissues of primates as well as nonprimates. The original isolation of adenovirus from adenoid tissues (87) and the subsequent recovery of adenovirus in tonsils (26), cytomegalovirus in adenoids (86), and several other virus types in kidney tissues (8, 22, 63) are well-documented instances of latent virus infections in tissues of man. Various methods have been used for the detection of hidden viruses in human tissues and these methods have been reviewed by Melnick et al. (68, 70).

Latent virus infections have also been recognized in cell cultures derived from tissues of nonprimates. These include the isolation of cytomegalovirus from the salivary glands of guinea pigs and mice (38, 94), of herpesvirus from dog (95) and horse (51) kidney cell cultures, of adenovirus from avian (16) and swine (11) kidney tissues, and of many others which are beyond the scope of this review. Furthermore, certain viral agents have even been recovered from germ-free animals (4, 79). Thus, one cannot be assured that the organs of any animal species are microbiologically sterile.

While searching for the viral etiology of human Kuru disease, Gajdusek and associates (30, 83) isolated 47 strains of viruses from a total of nine chimpanzees which had been experimentally inoculated with Kuru 1 to 3 years previously. Some of these isolates were identified as adenovirus, reovirus, and foamy virus. All virus isolates were obtained from tissue explants from chimpanzee organs, such as brain, spinal cord, spleen, and kidney. It became evident that some of these animals had been latently infected by these viruses prior to the inoculations of Kuru.

Simian tissues have been of special interest, since monkey kidney cell cultures are commonly used in the studies of animal viruses and in the preparation of virus vaccines for human use. As a result of the extensive use of primate cell cultures,

a great number of simian viruses have been recovered from a variety of monkeys, baboons, and marmosets (9, 12, 14, 15, 24, 25, 29, 40, 41, 47, 52, 56–58, 60, 65, 66, 72, 88, 89, 98, 99, 100). These indigenous viruses have caused considerable frustration and economic loss to workers in terms of contaminated virus stocks and rejected cultures. In this paper, I have reviewed the available data, although in many instances the information is rather limited regarding latent virus infections in primate tissues with special reference to viruses isolated from the monkey kidney tissues of apparently healthy animals. A brief description of the classification of simian viruses and some of the physicochemical and biological properties of the major virus groups is also included. In addition, attempts have been made to elucidate the origin of virus infections in primates, the mechanism of viral persistence in kidney tissues, and the problems in the control of virus infections in primate cell cultures.

CLASSIFICATION OF SIMIAN VIRUSES

Simian viruses are, by defintion, a group of viruses isolated from tissues or excreta of primates other than man. The isolation of virus-like agents from monkey kidney tissue cultures was first reported by Rustigian et al. in 1955 (89). Subsequently, as a result of the extensive use of monkey kidney cell cultures, especially in the preparation of virus vaccines, a great number of simian viruses have been recovered as endogenous tissue contaminants by Hull and associates (56–58). Since such a large number of viral agents have been isolated from monkeys, Hull et al. have designated all viruses isolated from simian origin as "SV" with serial numbers, irrespective of their properties or taxonomic order (56). In the meantime, Malherbe and Harwin of South Africa isolated several viral agents from vervet monkeys and designated them "SA" with serial numbers, indicating simian viruses isolated from South African monkeys (65, 66).

On the basis of certain biological properties, especially cytopathic effect (CPE), simian viruses were originally divided into four groups by Hull et al. (57). Other biological properties, including plaque morphology, host-cell susceptibility, and hemagglutinin production, have also been used for grouping these viruses (54, 81).

More recently, the physicochemical properties of certain simian viruses have been studied. Infectious deoxyribonucleic acid (DNA) was obtained from SV40-infected cultures soon after the virus was discovered (32), and infectious ribonucleic acid (RNA) has been successfully extracted from several strains of simian enterovirus (39). During our study characterizing the simian viruses, size determination by membrane filtration was emphasized (6). It was shown that SV5, measles, and foamy virus did not pass through a membrane filter having a 100 nm limiting pore diameter; thus, these were considered large viruses. SV11 and SV12, which passed through the 100-nm filter with some reduction in infectivity titer, were in the medium range; SV16 and SV40 passed through both 100- and 50-nm filters and were considered small viruses. Thus, the use of membrane filtration has facilitated the grouping of unknown viruses (44).

The use of electron microscopy has led to highly detailed studies of the morphological structure and the size of some simian viruses, especially SV40 (31, 34). The fine structure of other simian viruses has been described. These include simian adenoviruses (2, 28), enteroviruses (3, 39), myxoviruses (19), foamy virus (61), and Yaba poxvirus (21). Based upon physicochemical properties, such as nucleic acid type and ether sensitivity, and on the results obtained by ultrafiltration and electron microscopy, the classification of simian viruses has been proposed (45) in a manner similar to that described for other animal viruses (1, 44). This classification includes the DNA and RNA viruses, as shown in Table 1. In the DNA virus group, there are four major subgroups: the simian papovavirus, adenovirus, herpesvirus, and poxvirus. Similarly, there are four major subgroups among the RNA viruses: the simian picornavirus, reovirus, myxovirus, and pseudomyxovirus (monkey measles and foamy agents).

BRIEF DESCRIPTIONS OF THE MAJOR SIMIAN VIRUS GROUPS

Recognition and characterization of simian viruses in cell cultures are of practical importance, since monkey tissues often harbor a variety of viruses. Some of the physicochemical properties of the major simian virus groups are summarized in Table 1. Methods used for recognition of each simian virus group have been reported by various investigators and are described briefly below. Some of the simian viruses, for example, the poxvirus, were not recognized as latent virus infections in primate tissues. But, for completeness in listing the simian virus groups, the poxvirus group is included. For a detailed discussion on the classification of the major groups of animal viruses, the reader is referred to Andrewes (1) and to Melnick and McCombs (71).

DNA Viruses

Papovavirus groups. SV40 or the vacuolating virus of monkeys was found to be a small DNA, ether-resistant virus (52). This virus was first

isolated from kidney cell cultures derived from rhesus and cynomolgus monkeys by Sweet and Hilleman (98) and was isolated from patas monkeys in our laboratory (52). Subsequently, SV40 was also isolated from kidney tissues of African green monkeys (47). In rhesus monkey cells, SV40 produced intranuclear inclusions, although CPE generally was not apparent (52). In patas, African green monkey, and baboon cell cultures, SV40 induced extensive vacuolations in the cytoplasm of infected cells (Fig. 1, B1). Intranuclear inclusions of a basophilic Feulgen-positive nature were observed in infected monkey cells regardless of monkey species (Fig. 1, B2). Nonsimian cells were generally resistant to SV40 infection (52). However, transformation has been observed in human (64, 93) and hamster cells (13) infected with SV40. Since the discovery that SV40 produced tumors in hamsters (23), this virus type has been used by hundreds of investigators as a model for oncogenic studies.

Adenovirus group. A total of 18 simian adenovirus types have been reported (81). Most of these were isolated from rhesus and cynomolgus monkey kidney cell cultures by Hull and co-workers during 1954–1956. Additional types have been obtained from green monkeys (66), patas monkeys (101), and chimpanzees (83). Clusters of rounding cells were commonly seen throughout the cell sheet of infected cultures (Fig. 1, C1). In hematoxylin-eosin stained preparations, characteristic basophilic inclusions were seen in the nuclei of infected cells (Fig. 1, C2). Morphologically, simian adenoviruses were found to resemble human adenovirus. They were 80 nm in diameter and icosahedral in shape (2, 28). All simian adenoviruses share a complement-fixing antigen with human adenovirus strains, but no serological cross between human and simian adenovirus types was noted when viruses were tested by neutralization methods (56, 57). As with human adenoviruses, most simian adenovirus-infected culture fluids showed hemagglutination with rhesus monkey or rat erythrocytes, or with both. On the basis of these hemagglutinating properties, simian adenoviruses were divided into four subgroups by Rapoza (81). Recently, Hull and associates (55) reported that several simian adenovirus types were capable of producing tumors in infant hamsters. Among the different types of simian adenoviruses tested, SV20 and SA7 appeared to be most oncogenic.

Herpesvirus group. In monkeys of the Old World species, herpes B or *Herpesvirus simiae* produced a naturally occurring mild infection. However, most human cases of B virus infection have been fatal. Hull and Nash (59) showed that 10% of newly caught rhesus monkeys had antibodies to the B virus, and the percentage rose to 60 to 70% when the monkeys were confined in "gang-cages." Other studies indicated that 100% of the experimental monkeys showed B virus antibody rise when the monkeys were housed together over a period of 6 weeks (67).

Herpes T or *Herpesvirus tamarinus*, a new member of the herpesvirus group, was isolated from marmoset, a New World monkey obtained from South America (41, 72). Although herpes T was highly pathogenic to marmosets, it did not produce disease when inoculated into rhesus or green monkeys or baboons (72).

Both herpes B and T grew readily in cultures of monkeys, human, and rabbit cells and produced extensive cellular degeneration (Fig. 1, D1). The type A eosinophilic inclusions observed in herpes B-infected monkey cells resembled those produced by herpes simplex virus-infected human cells. Pinpoint pocks were produced on the chorioallantoic membrane of embryonated eggs. Following inoculation with virus B or T strains, these pocks resembled those produced by other members of the herpesvirus group.

Cytomegalovirus was isolated from kidney cell cultures prepared from African green monkeys (12). The virus underwent a long latent period before the appearance of CPE. The production of intranuclear DNA inclusions in the monkey cells (Fig. 1, D2) confirmed its presence.

Poxvirus group. Monkey pox was described by von Magnus et al. during an epidemic among captive cynomolgus monkeys (106) where it appeared to exist as a silent infection. Another poxvirus, the Yaba virus, was obtained from subcutaneous tumors of rhesus monkeys (76). The replication of Yaba virus in tissue culture was first described by Yohn et al. (109), who demonstrated cytopathology in an infected green monkey cell line, BSC-1.

RNA Viruses

Picornavirus group. Originally, the name ECMO (enteric cytopathic monkey orphan) or enteroviruses of monkeys was used to describe this group of viruses. Most of the virus strains were isolated from the stools of monkeys; the others were isolated from kidney tissues (39, 40, 56). The CPE produced in monkey kidney cells (Fig. 2, A) was similar to that produced by the enteroviruses isolated from man. However, no antigenic relationship has been demonstrated between the monkey and human strains tested (56). Plaque morphology and host cell spectrum of susceptibility have provided additional bases for subdividing these viruses into two groups (54).

Studies of the physicochemical properties of

TABLE 1. *Physicochemical properties of simian viruses and natural occurrence of major simian virus groups*

Virus group	Virus strain or type[a]	Physicochemical properties				Methods of recognition[c]	Natural occurrence			References
		Nucleic acid type	Size determination (nm)		Ether treatment		Monkey species	Source of isolation	Antibody study (AB)	
			Filtration[b]	Other methods						
Papovavirus	SV40	DNA	<50 (S)	30–40	Resistant	CPE of vacuolating cells in fluid culture, intracytoplasmic vacuoles, and intranuclear basophilic inclusions in H&E stained preparation	Rhesus, cynomolgus, patas, and African green	Kidney cell tures	SV40 AB was found in 18% young juveniles and 100% adults of free living rhesus	6, 9, 31, 32, 34, 47, 52, 92, 96, 98
Adenovirus	SV1, SV11, SV15, SV17, SV20, SV23, SV25, SV27, SV30–34, SV36–39, SA7	DNA	50–100 (M)	70–80	Resistant	CPE of rounding cells in fluid culture cluster, intranuclear basophilic inclusions in H&E stained preparations	Rhesus, cynomolgus, African green, and patas	Kidney cell cultures and stools	(Limited studies), SV1, SV15, SV39, antibodies were found in captive monkeys but not in their native state	2, 6, 56–58, 62, 65, 66, 101
Herpesvirus	Herpes B, SA8	DNA	ND[d]	125	Sensitive	Rapid CPE in fluid culture, type A intranuclear eosinophilic inclusions in H & E stained preparation	Rhesus, cynomolgus, African green	Kidney cell culture, central nervous system	Herpes B AB was found in 10–20% of newly caught rhesus but rose to 100% in laboratory animals. In free living rhesus high prevalence in adults but absent in juveniles	59, 67, 69, 108

	Herpes T		ND	110	Sensitive	CPE in fluid culture, type A intranuclear inclusions in H&E preparation, plaques under agar medium	Marmosets	Liver, kidney, lung, spleen,	Herpes T AB; none was found in old world monkeys but commonly found in New World monkeys	41, 42, 72
	Cytomegalo-, SA6		ND		Sensitive	Delayed CPE in localized areas in fluid culture, type A, intranuclear inclusions in H&E stain preparation plaques under agar medium	African green monkey	Kidney cell culture	AB is commonly found in laboratory green monkeys but none was found in newly captured rhesus	12, 66
Poxvirus[e]	Monkey pox	DNA	ND	ND		ND	Cynomolgus	Skin lesion		106
	Yaba virus		ND	250–280	Resistant	CPE in BSC-1 cell line, intracytoplasmic eosinophilic inclusions in H&E stained preparation	Rhesus in Yaba, Nigeria	Subcutaneous tumor		21, 76, 109
Picornavirus (enterovirus)	SV2, SV6, SV16, SV18, SV19, SV26, SV29, SV35, SV42–49, SA4, SA5, SA11	RNA	<50 (S)	32–38	Resistant	CPE of small rounding cells in fluid culture, plaques under agar medium	Rhesus, cynomolgus, African green	Kidney cell culture, stool, or rectal swab	(Limited studies), SV19 and SV49 AB frequently found in captive monkeys, baboons and chimpanzees	6, 39, 40, 54, 56–58, 62, 65, 66

[a] SV = simian virus (56); SA = South African virus (66); Panvirus = chimpanzee virus (30, 83).
[b] Size determination by membrane filtration: <50 nm = small; 50–100 nm = medium; > 100 nm = large (44).
[c] H&E = hematoxylin and eosin.
[d] ND = not done.
[e] This virus group is not considered as a latent virus infection but is listed here for the completeness of the major simian virus groups; for details the readers are referred to the reference cited.

TABLE 1—*Continued*

Virus group	Virus strain or type[a]	Physicochemical properties				Methods of recognition[c]	Natural occurrence			References
		Nucleic acid type	Size determination (nm)		Ether treatment		Monkey species	Source of isolation	Antibody study (AB)	
			Filtration	Other methods						
Reovirus	Type 1 SV12	RNA	50–100 (M)	60–65	Resistant	CPE cytoplasmic granulation in fluid culture, intracytoplasmic eosinophilic inclusions in H&E stained preparation	Rhesus	Kidney cell cultures	(Limited studies) SV12 AB frequently found in captive chimpanzees	6, 56–58, 62
	Type 1 SA3						African green	Kidney cell culture		66
	Type 2 SV59						Monkey	Lung		58
	Panvirus 3, 4						Chimpanzee	Brain		83
Myxovirus	SV5	RNA	>100 (L)	130	Sensitive	No CPE, hemadsorption with guinea pig erythrocytes	Rhesus, cynomolgus, patas, African green	Kidney cell culture	SV5 antibody rarely found in newly caught monkeys but commonly found in captive monkeys	6, 18, 24, 45, 47, 56, 62, 92, 99, 110
	SV41		ND	180–250	Sensitive	No CPE, hemadsorption with guinea pig erythrocytes	Cynomolgus	Kidney cell culture	(Not known)	75
	Para-3		>100 (L)	140–250	Sensitive	No CPE, hemadsorption with guinea pig erythrocytes	Patas	Lung, throat swabs	Parainfluenza 3 antibody rarely found in newly caught monkeys but found in captive monkeys	10, 17, 20, 45, 92

Pseudomyxovirus	Measles	RNA	>100 (L)	150	Sensitive	CPE foamy type in fluid cultures, multinucleated syncitial cells with eosinophilic cytoplasmic and intranuclear inclusions in H&E preparation	Rhesus, African green	Kidney cell culture	Measles antibody rarely found in newly caught monkeys but commonly found in captive monkeys	9, 47, 66, 73, 88
	Foamy virus types 1, 2, 3, 4		>100 (L)	100–300	Sensitive	CPE foamy type in fluid cultures, multinucleated syncytial cells absence of inclusions in H&E stained preparation	Rhesus, cynomolgus African green	Kidney cell culture	Foamy type 1 AB commonly found in rhesus; foamy types 2 and 3 AB commonly found in African green monkeys	9, 15, 60, 61, 66, 89, 96
	Panvirus 1 and 2						Chimpanzee			83

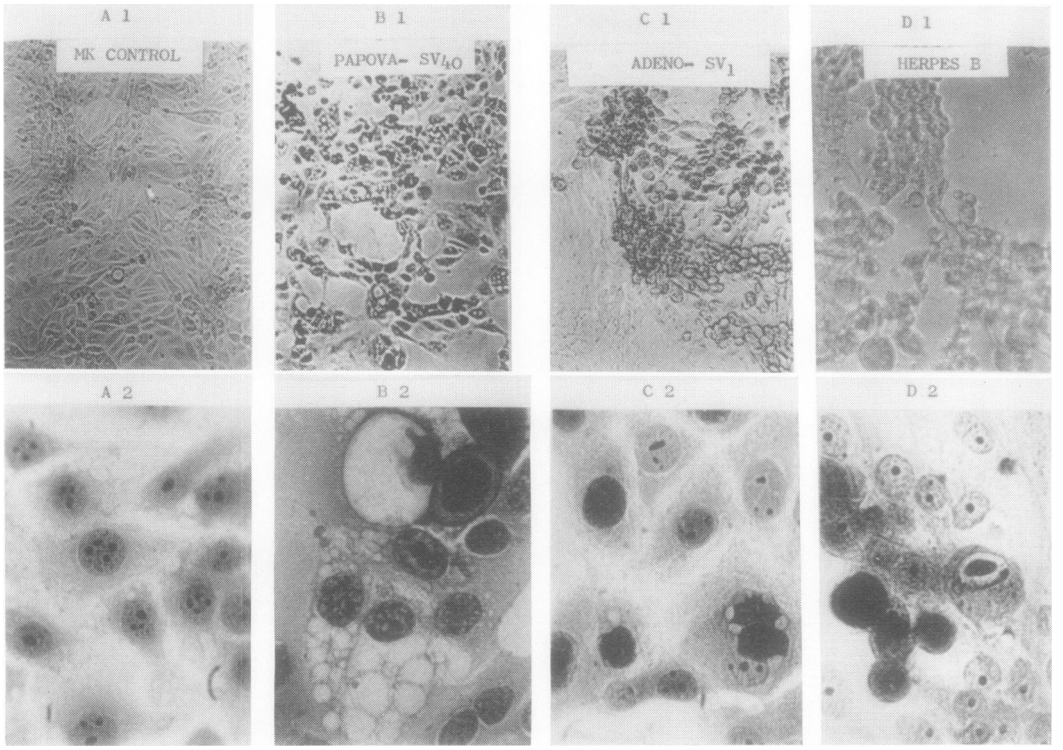

FIG. 1. *Simian DNA viruses. Top row: Monkey kidney cells in fluid cultures, × 100. Bottom row: Hematoxylin-eosin stained preparations of monkey kidney cells, × 970. (A1 and 2) Rhesus monkey kidney cell culture control. (B1 and 2) SV40-infected patas monkey kidney cells. (C1 and 2) SV1-infected rhesus monkey kidney cells. (D1) Herpes B-infected rhesus monkey cells. (D2) Cytomegalovirus-infected rhesus monkey cells. Note intranuclear inclusions in B2, C2, and D2. From reference 45.*

some 15 strains of simian enteroviruses revealed that all were small RNA viruses, resistant to ether and acid *p*H (39). Properties such as plaque morphology, inhibition by guanidine and hydroxybenzyl benzimidazole, rates of thermal inactivation and host-cell spectrum have been used to subdivide enteroviruses further (39).

Reovirus group. Reovirus type 1 includes SV12, which was isolated from rhesus monkey kidney cells by Hull et al. (56) and SA3, which was isolated from African green monkey kidney cells by Malherbe and Harwin (66). Infected cells with very granular and distinct cytoplasmic eosinophilic inclusions were apparent in hematoxylin-eosin stained preparations (Fig. 2, B1 and B2). SV59, a reovirus type 2, was isolated from the lung tissue of a monkey with respiratory illness (58). Two additional strains, Panviruses 3 and 4, isolated from the brain of chimpanzees were identified as reovirus type 2 (83).

Myxovirus group. SV5 is a common contaminant of certain lots of monkey kidney cells (24, 47, 56, 99, 110). The number of SV5 isolates from tissue cultures varied from laboratory to laboratory, ranging from 5 to 50% of the cultures examined. A seasonal incidence was noted, with a higher percentage of virus isolations occurring from December to April than from May to October (24). As with other myxoviruses, CPE was not a distinct phenomenon that readily permitted identification of this particular virus group. However, SV5 infection could easily be determined by the hemadsorption of guinea pig erythrocytes onto the infected cell sheet (Fig. 2, E), as described by Vogel and Shelokov (105). On the other hand, erythrocytes treated with receptor-destroying enzymes (RDE) were unable to adsorb onto SV5-infected cultures, a feature common to the myxovirus group.

There has been only one report concerning the isolation of another myxovirus, SV41, from cynomolgus monkeys (75). Serologically, SV41 was related to SV5 but they were not identical. Apparently SV41 is not as common or as widely distributed as SV5.

Parainfluenza 3 virus was isolated from the

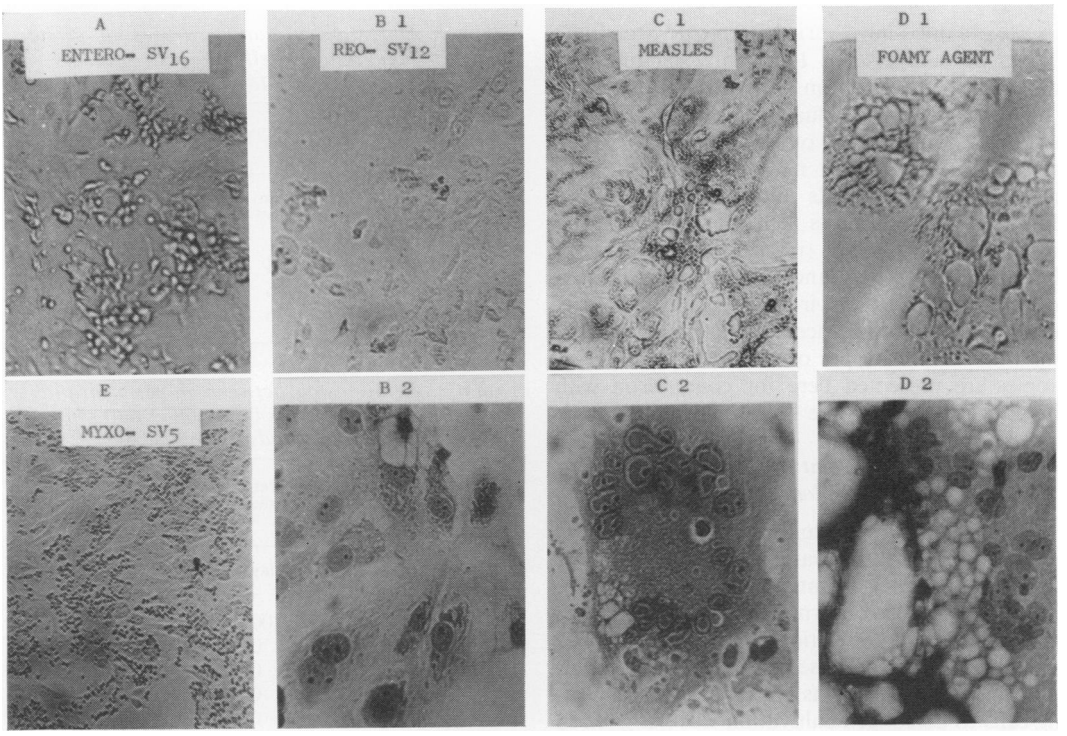

FIG. 2. *Simian RNA viruses. Top row: CPE in monkey cell fluid cultures, × 100. Bottom row: Hematoxylin-eosin stained preparations of infected monkey kidney cells, × 400 (except E). (A) SV16-infected rhesus monkey kidney cells. (B1 and 2) SV12-infected rhesus monkey kidney cells showing intracytoplasmic inclusions. (C1 and 2) Monkey measles virus-infected green monkey kidney cells showing intranuclear inclusions in the nuclei of a syncytical cell. (D1 and 2) Foamy agent-infected rhesus monkey kidney cells; note absence of intranuclear inclusions. (E) SV5-infected rhesus monkey cells showing adsorption of guinea pig erythrocytes, × 100. From reference 45.*

lung tissues of patas monkeys during a severe outbreak of respiratory disease in these animals (20). The data on parainfluenza 3 and SV5 antibody distributions in monkeys will be discussed in the later sections.

Pseudomyxovirus group. This name has been adopted here for the group of viruses which resemble the myxoviruses morphologically, but can be easily distinguished by certain biological properties. Since agglutination by the pseudomyxoviruses was not affected by RDE treatment of erythrocytes, this method has been applied for differentiation of the myxovirus and pseudomyxovirus groups. Measles and foamy viruses are included in the latter.

Monkey measles virus, originally known as minia (monkey-intranuclear-inclusion-agent) virus (88), produced multinucleated syncytial cells in infected cultures (Fig. 2, C1). In hematoxylin-eosin stained preparations, eosinophilic inclusions located in the nuclei as well as in the cytoplasm could be readily seen (Fig. 2, C2). The epidemiology of measles virus in monkeys will be discussed later.

Foamy virus, usually observed in aged monkey kidney cell cultures (89), has four serological types (97), with additional types, for example, the chimpanzee foamy virus (83). In fluid cultures, CPE occurred in the form of large vacuoles (Fig. 2, D1) which were indistinguishable from the syncytial cells produced by monkey measles virus. However, the complete absence of eosinophilic inclusions in the nuclei of foamy virus-infected cells (Fig. 2, D2) immediately distinguished them from cells infected with measles virus. Recently, Stiles showed that most of the rhesus monkeys were infected with foamy virus type 1, whereas green monkeys were mostly infected with foamy virus types 2 and 3 (96).

EPIDEMIOLOGICAL ASPECTS OF LATENT VIRUS INFECTIONS IN SIMIAN TISSUES

Various investigators have reported that monkey cells often harbor a variety of viruses, but the

results obtained have varied from study to study. It has become evident that many factors may influence the recognition of endogenous agents. These variables may include: (i) the frequency and nature of contact with other animals in nature, (ii) the frequency and nature of contact with man and other animals at capture and during shipment, (iii) the stress factors imposed during capture and shipment, (iv) the period and conditions of quarantine, and (v) the methods used for detection of latent viruses. Any of these factors could have influenced the number of virus isolations and the types of viruses obtained. Examples are presented here for comparison with the data available in the literature.

Detection of Latent Infections in Cell Cultures by Prolonged Cultivation

Primary monkey cultures have been used by many laboratories for various purposes, but for most investigators latent virus infections have not been a major concern. The question arose as to why these latent infections were not recognized in the routine use of these cultures. In an attempt to answer these questions, an experiment subjecting cells to prolonged cultivation was designed to investigate the presence of latent viruses; the results were then compared with those from cultures which were kept under observation for shorter periods of time (Table 2). Of the 120 lots of cultures examined, about 3% showed virus infections when examined 2 to 3 weeks after the cells were planted, the usual duration of most virological studies. However, when the same lots of cultures were examined after prolonged cultivation, i.e., 4 to 8 weeks after the cells were planted, it was noted that the percentage of isolations increased 10-fold. Thus, the longer the cultures were kept, the higher the percentage of virus isolations obtained (46). A similar situation was noted in the detection of latent virus infections of the human respiratory tract (26), of chimpanzee tissues (83), and of those experimentally induced in monkeys (5, 17, 74).

The reason for this increase in the number of latent viruses recovered after prolonged cultivation was thought possibly to be due to the presence of a minute amount of infectious virus in the original tissue cell, which became evident only after virus multiplication had occurred and a significant amount of virus had been produced. This interpretation was supported by the following experiment. Freshly prepared monkey kidney cell suspensions containing 3×10^5 cells per ml were divided into portions of 100 ml. Each portion was infected with a serial 10-fold dilution of a myxovirus with multiplicities per cell ranging from 10 to 0.0000001.

TABLE 2. *Detection of latent viruses in primary monkey kidney cell cultures (total, 120 lots)*

No. of weeks after cells planted	No. showing virus infections		Percentage showing virus infections	
	SV40	Foamy virus	SV40	Foamy virus
2–3	3	4	2.5	3.3
3–4	15	16	12.5	13.4
4–8	36	36	30.0	30.0

TABLE 3. *Relationship between in-put multiplicity and appearance of virus infection as determined by hemadsorption*

Virus in-put multiplicity per cell	Percentage of cultures showing hemadsorption at indicated no. of days after cells planted[a]			
	3 days	14 days	27 days	38 days
10	100	100	ND	ND
0.01	0	92	100	ND
0.0001	0	64	78	100
0.000001	0	0	0	16
Control, no virus	0	0	0	0

[a] ND = not done.

These infected cell suspensions were seeded into tubes. Each tube contained 3×10^5 cells. At selected time intervals, 20 to 40 tubes from each group were tested for the presence of the myxovirus infection by the hemadsorption technique (105). As shown in Table 3, with an input multiplicity of 10, 100% of the cultures showed infection on the third day after planting. When a multiplicity of 0.01 was used, 92% of the cell cultures showed infection 14 days after planting, but 100% showed infection on day 27. When the input multiplicity was 0.000001, infection could not be recognized until day 38, and even then only 16% of the cultures showed infection. Thus, quantitative factors appeared to have an important bearing on the recovery of certain types of latent viruses in cell cultures.

Incidence of Virus Infections in "Normal" Monkey Kidney Cell Cultures

To study the incidence of latent virus infections in monkey cell cultures, we conducted three longitudinal survey studies during the years 1963–1968. Cultures were prepared from monkey kidney tissues obtained from apparently healthy animals. Details of these studies have been described previously (45, 47). In general, a set of 20 to 40-tube cultures, each containing an 11 ×

22 mm cover slip, was prepared from each lot of freshly trypsinized monkey kidney cell suspension. These cultures were kept incubated at 37 C for observation as long as the cells were in good condition, usually 4 to 8 weeks. All cultures were examined twice weekly, and the culture medium was replaced as needed. Both CPE in fluid cultures and cytopathology in hematoxylin-eosin stained preparations were used for recognition of the possible presence of viral agents. The hemadsorption method using guinea pig erythrocytes was utilized for the detection of myxoviruses. Identification of each isolate was made by the neutralization test or histopathology of the infected cells, or by both methods. Much to our surprise, an unusually high percentage of cultures that were considered "normal" showed virus infection.

The first study, in which monkey kidney cell cultures which had been prepared in the New Haven Laboratory were used, started in October 1963 and ended in July 1965. A total of 191 lots of cultures were examined; of these, 126 lots were from rhesus monkeys and 65 lots were taken from green monkey cells. For each month, the percentage of virus infection with one or two kinds of virus was greater than 50% (47). All monkeys used in this study were housed in the same quarters and were purchased from primate importers. They were used *without* any special "conditioning."

The second study was conducted in New York City from February 1966 to June 1967. The kidney cell cultures were prepared from monkeys which had been shipped directly from the field to the laboratory where they underwent thorough "conditioning." Green monkeys and rhesus monkeys were housed in separate quarters (same animal caretaker for both rhesus and green monkeys), were kept in separate cages, and were quarantined for 30 to 90 days before sacrifice for kidney tissues. A total of 539 lots of cultures were examined; of these, 287 were from rhesus monkeys obtained from India, and 252 were taken from African green monkeys from Ethiopia. A segment of this study is presented in Fig. 3. Again, the average rate of virus isolation for each month was about 50% regardless of the monkey species used or the month during which the tissues were obtained.

From September 1967 to January 1968, a third study was conducted in the West Haven Laboratory. It was noted that the percentage of virus isolations in each month was less than 5%. The monkeys used for the third study were transported directly to the laboratory upon arrival at the airport. All monkeys used in the second study, however, had been kept at the airport for a few

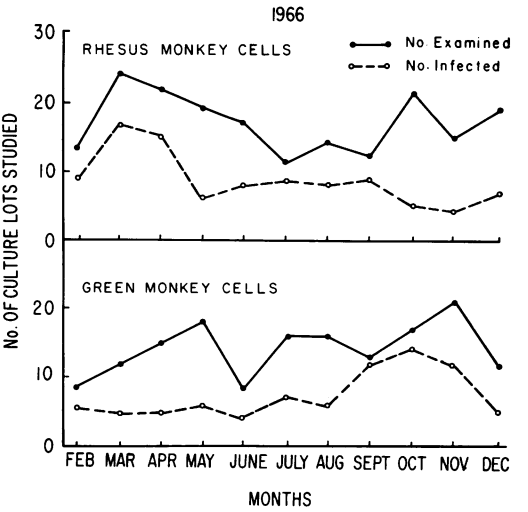

FIG. 3. *Incidence of virus infections in 344 lots of "normal" rhesus and green monkey kidney cell cultures (February to December 1966, New York).*

hours to a few days before shipment to the laboratory. It is possible that the grouping together of the monkeys at the airport may have helped to spread endemic virus infections in the animals used in the second study.

Comparison of Virus Types Recovered from Tissues of Monkeys With or Without Quarantine

The early studies of Hull and co-workers, undertaken in Indianapolis in 1954–1955, showed that reovirus and adenovirus were the agents most frequently encountered (56, 58). However, there was a conspicuous absence of adenovirus and reovirus infection during our 5-year survey, as determined by lack of virus isolation and by serological studies (47). The methods used for virus isolations in our study differed from those used by Hull et al. (56). This may account in part for the difference in the virus types isolated by the two laboratories. Other epidemiological factors encountered by the two laboratories may also be responsible for the differences in prevalence of virus types. This is illustrated in Table 4 by the results obtained in two of our studies conducted in 1964 and in 1966 (45). Although the same techniques were used for recognition of viruses in both studies, it is apparent that SV5, one of the most prevalent virus types isolated in the 1964 study, was not present in any of the 344 culture lots examined in 1966. In the second study, over 5,000 culture tubes were tested exclusively for hemadsorption with guinea pig erythrocytes, a technique similar to that used in 1964. Negative results were obtained consist-

TABLE 4. *Comparison of virus type obtained from tissues of monkeys with or without quarantine*[a]

Determination	Monkeys without quarantine, New Haven, 1964		Monkeys with 30–90 days quarantine, New York, 1966	
	Rhesus	Green	Rhesus	Green
Total no. of culture lots examined	64	34	187	157
Virus types isolated (%)				
SV5	25	30	0	0
Measles	12	3	0	0
Foamy virus	29	30	37	14
SV40	17	14	13	36
Mixed infections	25	30	6	3

[a] From reference 45.

ently. (It might be pointed out here that SV5 was used in the laboratory for other experiments while the second survey study was being conducted. There has been, however, no evidence of SV5 cross-contamination of survey cultures in the laboratory.) Neither SV5 nor measles virus was isolated in cultures from the kidney tissues of monkeys conditioned for 30 to 90 days.

Additional variations were noted in regard to foamy agents and SV40, which occurred with equal frequency in both monkey species in 1964. There was, however, a distinctly different percentage of virus recovery in 1966 (Table 4). Apparently, heavy cross-infections had occurred in 1964 among the animals when both species of monkeys were housed together. In the 1966 study, the green monkeys and rhesus monkeys were housed in separate quarters, although the same individual took care of both monkey species. It was clear that cross-contaminiation between the two monkey species was minimized, since foamy agents appeared more frequently in the rhesus cultures and SV40 occurred more frequently in the green monkey cultures. Other investigators indicated that green monkeys, if not exposed to rhesus monkeys, were usually free from SV40 infections (5, 9, 74). It was possible that in our 1966 study the same animal caretaker could have brought SV40 from the rhesus monkey quarters to the green monkey quarters, even though all possible precautions were taken. However, a recent report indicated that SV40 "T" and "V" antigens could be detected in kidney cells derived from African green monkeys obtained directly from the field (78). It is not clear at the present time whether the African green monkeys are actually free from SV40 infection.

Mixed Infections

Mixed infections with two or three virus types have occasionally occurred in both rhesus and green monkey cells (Table 4). It was not possible to recognize the presence of two or more virus types in the fluid cultures unless cell culture preparations were fixed and stained. Figure 4 shows a hematoxylin-eosin stained preparation of a mixed infection. Two distinct types of intranuclear inclusions were observed in a multinucleated giant cell. Subsequently, it was established that the eosinophilic inclusions were due to measles virus, and the basophilic inclusions were due to SV40 (47).

When SV40 and measles virus intranuclear inclusions occurred in separate cells, or when a single cell showed inclusions of the two viruses at different sites, the type of mixed virus infection was not difficult to recognize. In hematoxylin-eosin stained preparations, SV40 intranuclear inclusions were basophilic and purplish-blue in color, whereas measles intranuclear inclusions were eosinophilic and appeared pinkish. However, it was difficult to recognize a doubly infected nucleus containing inclusions of both virus types. Recognition of those nuclei doubly infected with SV40 and measles virus was achieved by utilizing the modified method of acridine orange staining with phosphate buffer at *p*H 8.0 instead of at a low *p*H (48). The doubly infected nuclei appeared reddish-brown, and SV40 inclusions were brilliant green. Nuclei doubly infected with SV40 and measles virus have been further identified by electron microscopy (49). Mixed infections of SV40 and foamy agents, SV5 and foamy agents, and SV5 and measles virus were also observed (47). In addition, experimentally induced double infections of reovirus and SV40 were obtained. Single cells doubly infected with SV12, a reovirus type 1, and SV40 are shown in Fig. 5 (50). Bright pinkish eosinophilic inclusions of reovirus in the cytoplasm and purplish-blue basophilic inclusions of SV40 in the nucleus could be easily recognized. Other investigators have reported mixed infections of SV40 and an adenovirus, and SV40 and herpes simplex virus (77, 80). Since adeno-SV40 hybrids were obtained in monkey cultures doubly infected with adenovirus type 7 and SV40 (82, 84), other hybrids could conceivably occur in multiple infected cultures.

Separation of mixed infections has been accomplished by membrane filtration. For example, measles virus was in the large size range, whereas SV40 was in the small virus group (Table 1). Thus, a mixture of SV40 and measles virus can be conveniently separated by filtration with mem-

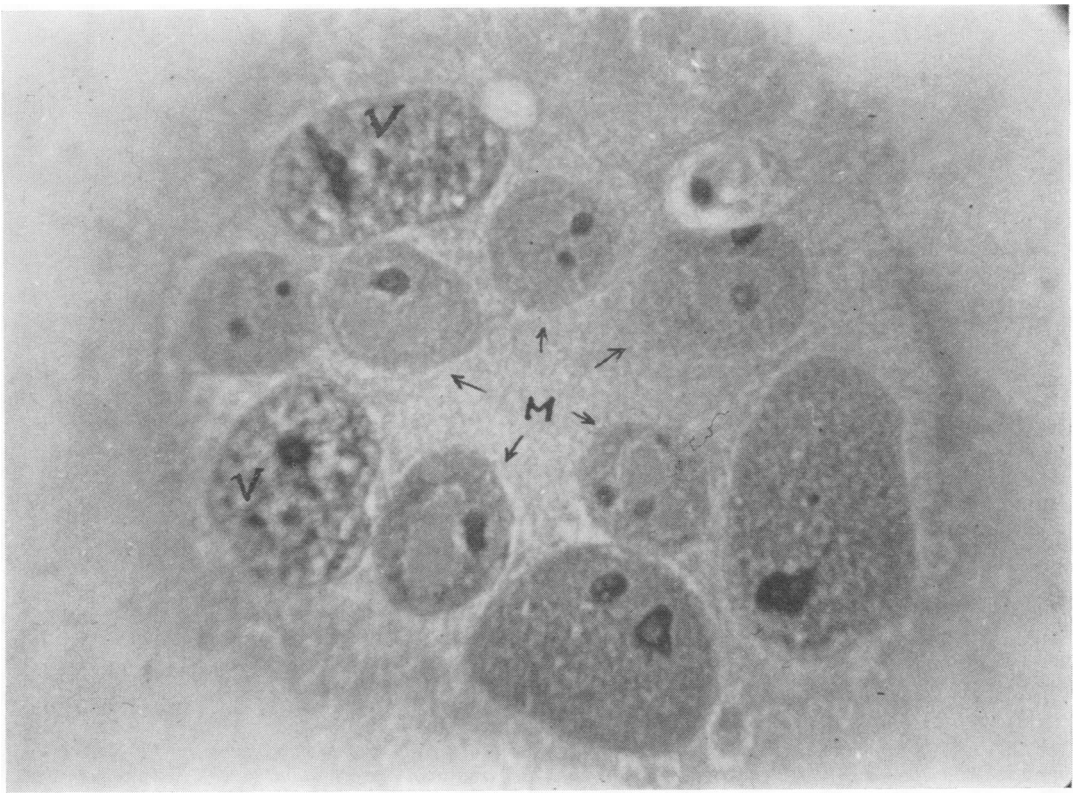

FIG. 4. *Mixed infection with measles (M) and SV40 (V) viruses, × 970. Note the distinct difference of intranuclear inclusions produced by measles virus (nuclei surrounding M) and inclusions produced by SV40 in the two nuclei (V), 27 days after seeding, hematoxylin-eosin stained preparation. From reference 47.*

branes of 100 and 50 nm limiting pore diameters. SV40 passed through filters of both 100 and 50 nm, but the measles virus was retained by both. However, this method was not satisfactory when the mixture consisted of SV5 and measles virus, both of which are large viruses. Consequently, the plaque purification method or limiting dilution method had to be used to separate viruses of similar sizes.

Distribution of Virus Types According to Conditions of Monkey Shipment

During the 5-year survey, it was noted that in most instances monkeys from the same shipment tended to acquire the same virus types. A few examples are presented in Table 5. Of a total of over 50 shipments of monkeys examined, only one group of 14 rhesus monkeys, shipped on September 17, 1966, showed no virus infection during the study period. In general, SV5 and measles virus could be isolated only from the kidney tissues of monkeys that had been quarantined at the local quarters for less than a month, whereas SV40 and foamy virus were recoverable in kidneys of monkeys even after 60 to 72 days in quarantine. In one instance, measles virus was isolated from one kidney of a monkey but not from the other kidney removed only 9 days later (47). Apparently, measles virus does not persist long in the kidney tissues of monkeys. Meyer et al. (73) showed that measles virus was transmitted to monkeys that had been in contact with infected human beings, and it is now recognized that measles infection in monkeys occurs only when the animals are exposed to man. These data suggest that certain virus infections in monkeys may be viruses of their own, but others may be viruses of man acquired by monkeys soon after contact with man. Some virus infections may persist for relatively short periods, as in the case of measles, but others, such as SV40 or foamy virus, may persist for longer periods of time.

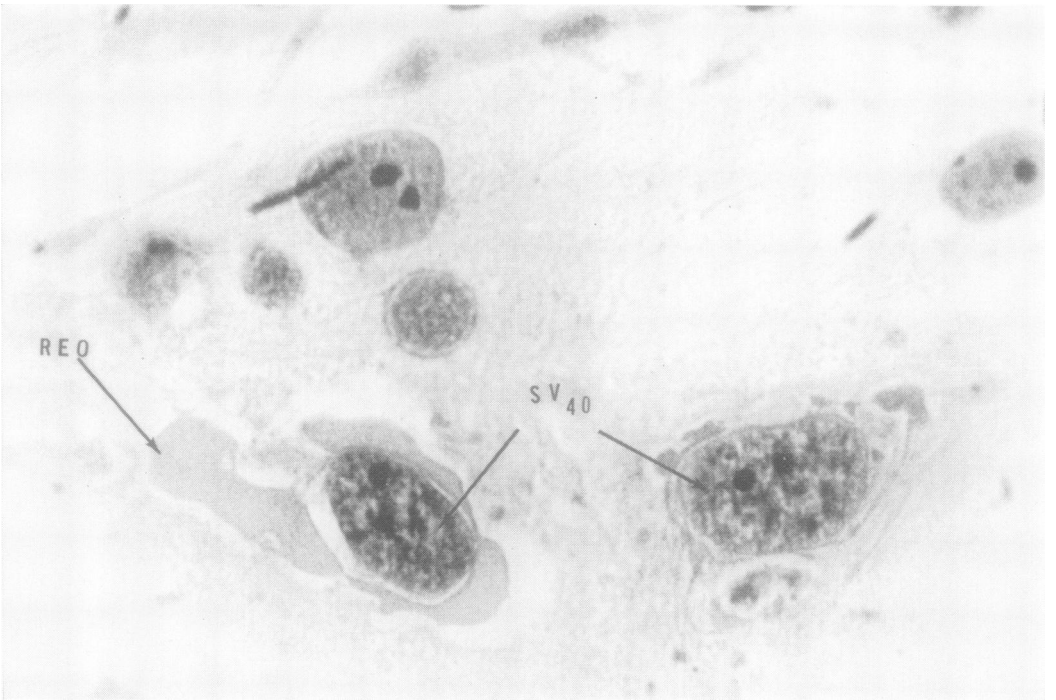

FIG. 5. *Doubly infected patas monkey kidney cells showing reovirus cytoplasmic inclusions (Reo) and SV40 intranuclear inclusions (SV40) in the same cell. Hematoxylin-eosin stained preparation, × 970. From reference 50.*

Acquisition of Parainfluenza Virus Antibody in Monkeys During Captivity

In the early studies of Schultz and Habel, it was noted that SA virus antibody was present in sera obtained from normal monkeys (90). We reported that monkeys were free from DA virus antibodies while in their natural habitat but developed antibodies after capture (53). These findings were confirmed by recent studies (62, 92, 99) which showed that SV5 antibody was absent in free-living monkeys and was acquired after contact with man. [SV5, SA, and DA viruses are serologically identical; it was suggested at the VIIIth International Congress of Microbiology in Montreal, Canada (1962) that SV5-SA-DA group of viruses be designated as parainfluenza 5 virus.] In the preceding sections, it was noted that SV5 was one of the most prevalent virus types found in the cultures prepared from kidney tissues of monkeys without quarantine, but SV5 was found to be completely absent in cultures prepared from tissues of monkeys which were quarantined in the local quarters for 30 days or longer. It was considered possible that the conditioned animals could have acquired SV5 infection during transit or soon after arrival and then became immune during the quarantine period. This interpretation was supported by the results obtained from the serological testing of 351 paired sera collected from the monkeys of the 1966 study during the period of quarantine (Table 6). The initial sera, SI, of all monkeys were taken at the time of arrival at the laboratory, and the second sera, SII, were collected 30 to 90 days later, at which time the kidneys were used for tissue cultures. The tests revealed that 45% of the 190 rhesus monkeys and 56% of the 161 green monkeys showed antibody conversion to SV5. The percentage of monkeys that showed a rise from a negative antibody titer ($\leqq$ 1:5) to a positive antibody titer (1:20 or greater) appeared to be related to the number of days the monkeys were quarantined. A similar result was obtained when the same paired sera were tested against parainfluenza 3 virus (Table 6). Of the 168 rhesus monkeys studied, 26% showed parainfluenza 3 virus antibody conversion, and 41% of the 141 green monkeys showed conversions to parainfluenza 3. The prevalence of parainfluenza 3 antibodies in captive monkeys has been noted by other investigators (10, 17, 20, 92). However, there was no evidence that parainfluenza types 1 or 2 virus infections had oc-

TABLE 5. *Distribution of virus types in rhsuse monkeys according to shipment and duration in captivity*

Date of arrival	Laboratory studied	Total no. of monkeys in each shipment	No. of days monkeys in quarantine	No. of monkeys studied	Virus types isolated
10/7/63	N.H.	36	9	6	0
			16	7	0
			23	9	0
			30	8	SV5
			37	6	0
3/3/64	N.H.	25	1	5	0
			8	8	0
			22	6	Foamy agents + SV5
			36	6	Foamy Agents + SV5
11/19/64	N.H.	22	5	8	Measles
			12	9	SV5 + foamy
			20	5	SV5 + foamy
2/10/66	N.Y.	22	38	6	Foamy agents
			45	6	Foamy agents
			52	6	Foamy agents
			60	4	Foamy agents
9/17/66	N.Y.	14	36	6	0
			43	3	0
			50	5	0
10/28/66	N.Y.	16	36	2	SV40
			45	6	SV40
			65	4	SV40
			72	4	SV40

curred in these monkeys, or in those tested by Shah et al. (92) or by Kalter et al. (62). Because of the complete absence of parainfluenza 2 antibody in monkeys and the high prevalence of SV5 antibody in this animal species, the antigenic relationship between these two viruses as reported previously (18) requires reconsideration.

POSSIBLE ORIGIN OF VIRUS INFECTION IN KIDNEY TISSUES

Model Study on Viremia and Viruria in Monkeys

As noted above and from the reports of other investigators, a variety of viruses have been isolated from the kidney tissues of apparently healthy animals. It is not known, however, how often and to what extent the kidney is infected. Several reports have been concerned with the

TABLE 6. *Proportion of monkey sera showing antibody conversion to parainfluenza viruses*[a]

Parainfluenza virus type tested	Rhesus		Green	
	No. of monkeys tested	Percentage showing antibody conversion[b]	No. of monkeys tested	Percentage showing antibody conversion
1	96	0	97	0
2	96	0	97	0
3	168	26	141	41
5 (SV5)	190	45	161	56

[a] Modified from reference 45.

[b] SI taken on the day of arrival showed a neutralizing antibody titer of 1:5 or less and SII taken on the day of sacrifice showed a neutralizing antibody titer of 1:20 or greater.

isolation of virus from human urine (33, 35–37, 85, 102–104, 107), but the origins of the viruria are obscure. In an attempt to demonstrate that viruria follows viremia, monkeys were experimentally inoculated intravenously with poliovirus type 1. Virus was recovered from the urine 30 min later (50). The titers of poliovirus recovered in the serum fell rapidly during the first 7 hr of sampling. The titers of poliovirus in the urine, although lower than those in the serum, also fell rapidly and in unison with the titers of the blood samples. Similar results have been obtained by Schultz and Neva with poliovirus injected intravenously into mice and rats (91). Although poliovirus is not one of the viruses involved in the latent infections discussed in the present paper, these model studies indicated that, once viremia was established with a sustained level of virus in the blood, viruria followed immediately. However, the degree of virus multiplication in the kidney cells and the persistence of the virus in this organ probably depend upon the properties of the different virus types.

Experimentally Induced Latent Infection by SV40

Meyer et al. (74) showed that intranasal inoculation of SV40 into monkeys resulted in the multiplication of this virus in the nasopharynx and digestive tract (Fig. 6). Viremia occurred in the inoculated monkeys and also in an uninoculated cage mate. Moreover, SV40 was recovered from cell cultures derived from kidney tissues of these monkeys 4 to 5 months after initial infection. Ashkenazi and Melnick showed that SV40 could be recovered in the urine of all monkeys inoculated intracerebrally, intracutaneously, or directly into the kidneys (5). In addition, they found that SV40 persisted in the kidney tissues 6 to 8 weeks after virus administration, regardless of the route

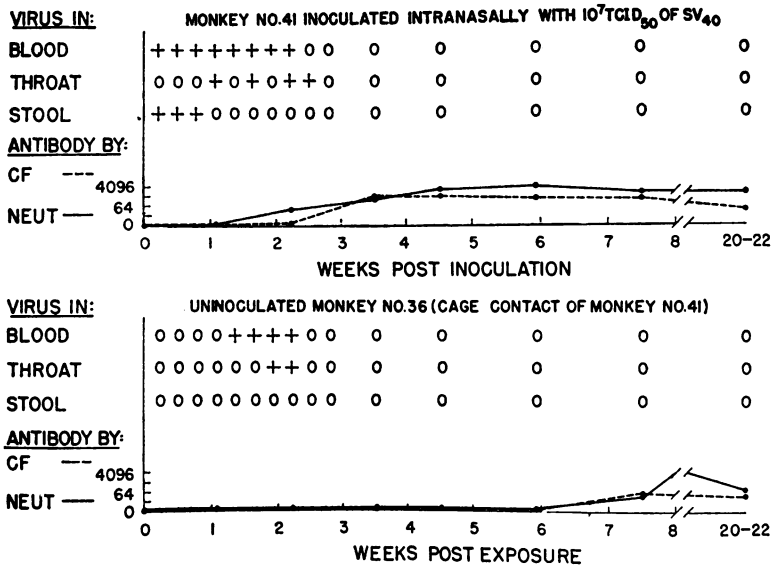

FIG. 6. *Experimental and contact infection of green monkeys with SV40. Reproduced by permission from reference 74.*

of inoculation. The recovery of SV40 from the kidney tissues, however, succeeded only when the tissue cells were cultivated for a prolonged period of time but SV40 was not recovered from test cultures inoculated with the minced kidney tissue suspensions (5).

Persistent Infection of the SV5 Group of Viruses in Monkeys after Intranasal Inoculation

Intranasal inoculation of the DA virus strain into monkeys with no preexisting DA antibodies resulted in the multiplication of this virus in the nasopharynx (17). Virus was recovered daily from throat swabs for 3 to 7 days after virus inoculation. The infected monkeys were kept for long-term observation. No clinical manifestations of illness were noted during the period of study. In several instances, one kidney from the same monkey was removed on two separate occasions and virus isolations were attempted. In one case, virus was isolated from the kidney tissue 16 weeks after inoculation. Again, the persistent infection by the virus of the kidney tissues was demonstrated only by prolonged cultivation of the cells in cultures prepared from the kidney. Direct inoculation of the minced kidney tissue suspensions into test cultures did not result in virus isolation (17).

LATENT VIRUS INFECTIONS IN HUMAN KIDNEY TISSUES

There have been several reports of virus isolation from human urine. These have included adenovirus types 1, 4, and 7 (36, 37), cytomegalovirus (85), mumps virus (104), WB myxovirus (33), measles virus (35), rubella virus (107), and coxsackie B virus (103). Viruria in man has been discussed by Utz (102), and the mechanism of viruria has been studied by Flanagan and Schultz (27). However, there are only a few reports concerning the isolation of virus from human kidney tissues (7, 8, 22, 63).

Human embryonic kidney (HEK) cell cultures are available commercially and are being used by many laboratories for the propagation of a variety of viruses. However, the possibility of latent virus infection in the HEK cell cultures has not been given much attention. Human kidney cell cultures have been used in our laboratory since 1959. The methods used for the preparation of the human kidney cell cultures have been described previously (43). Not until recently was a survey of latent virus infections in human kidney tissues initiated. Recognition and techniques for isolation were similar to those described earlier for the monkey cell cultures. During a 3-year period, November 1964 to December 1967, 124 lots of human kidney cell cultures were examined, and seven viruses (6%) were isolated. These included two measles viruses, two foamy viruses, one reovirus, one adenovirus, and one myxovirus. One measles virus isolation was made from a lot of human kidney cell cultures from a 3-year-old child who had been exposed to measles and had been given 0.6 ml of γ-globulin at the time of exposure. The child died 2 weeks later. Attempts at

virus isolation from the tissue suspension and antibody studies provided no evidence that the child had been infected with measles virus. The presence of the measles virus infection in the kidney of this child would not have been recognized if the kidney cell cultures had not been kept for 33 days after planting. The second isolation of measles virus was made from a lot of HEK cell cultures purchased commercially. There was no history available for this case. In fact, the HEK cells had been used for the propagation of a slow-growing adenovirus. The presence of the measles virus was noted only when stained preparations were examined to ascertain the presence of adenovirus inclusions. To our surprise, measles virus intranuclear and intracytoplasmic eosinophilic inclusions occurred in both inoculated and uninoculated control HEK cultures. Thus, the adenovirus stock derived from the commercially made HEK cultures was inadvertently contaminated with a measles virus.

The isolation of a strain of myxovirus from one lot of HEK cells prepared from the kidney tissue of a premature baby deserves some attention. The infant died 12 hr after birth and had a respiratory disease syndrome. There was no evidence of hemadsorption when this lot of cultures was tested at 21, 24, and 30 days after planting, but 70% of the culture tubes showed hemadsorption when tested on day 50. This isolate was serologically identical to SV5. Since monkey cell cultures were used in the laboratory for various purposes, one cannot eliminate the possibility of SV5 laboratory contamination. However, of over 100 lots of HEK cell cultures tested, only one showed distinct hemadsorption, and even then the virus did not appear until after prolonged cultivation.

Benyesh-Melnick et al. (8), have reported the isolation of nine viruses from kidney cell cultures from 84 children coming to autopsy during a 2-year period, October 1961 to September 1963. Their isolates included three cytomegaloviruses, three adenoviruses, (types 1, 2 and 7), and one each of measles, varicella, and coxsackie B1 viruses. Thus, latent virus infections in human kidney tissues, although not as frequent as in monkeys, do occur fairly often.

CONCLUDING REMARKS

This survey will have served its purpose if it stimulates more investigators to delve into the pathogenesis, persistence, and epidemiology of latent virus infections in primates. Data on the mechanism of latent virus infections are limited, and much information remains to be gathered before we can understand the origin and persistence of virus infections in primate tissues.

The incidence of latent virus infections in the kidney tissues of so-called "normal" healthy monkeys is unusually high. The virus types recovered varied from year to year and from laboratory to laboratory. These variations may reflect the source from which the monkeys were obtained, the nature and degree of contact of monkeys with man and other animals, and the conditions under which the monkeys were quarantined. In general practice, latent virus infections in primary cell cultures are often not recognized. Virus stocks derived from such latently infected cultures or virus vaccines produced from these infected cells would undoubtedly be contaminated with indigenous viral agents. It is, therefore, urged that precautions be taken whenever primary cell cultures are used, in order to avoid misleading experimental data which might result from the presence of latent viruses.

The sources of latent virus infections in monkeys are obscure. Some of these agents, for example SV40, are undoubtedly viruses of primates, but others may be infectious agents acquired after contact with man. This concept is supported by the data on measles virus infection in monkeys and by the antibody conversions to parainfluenza 3 and SV5 in paired sera obtained from monkeys during quarantine. Thus, SV5, although originally isolated from monkeys, may well be a human myxovirus, since it is prevalent only in monkeys having human contacts. It has been suggested that monkeys be vaccinated with inactivated SV5 upon arrival (99) or be held in quarantine for 3 to 6 months (47) prior to their use in order to reduce SV5 or other latent viruses in their kidney tissues.

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LITERATURE CITED

1. Andrewes, C. H. 1964. Viruses of vertebrates. The Williams & Wilkins Co., Baltimore.
2. Archetti, I., and D. Steve-Bocciarelli. 1963. Structure of some simian adenoviruses. Virology **20**:399–404.
3. Archetti, I., D. Steve-Locciarelli, and G. Toschi. 1961. Structure of an enteric simian virus. Virology **13**:149–152.
4. Ashe, W. K., H. W. Scherp, and R. J. Fitzgerald. 1965. Previously unrecognized virus

from submaxillary glands of gnotobiotic and conventional rats. J. Bacteriol. **90**:1719–1729.
5. Ashkenazi, A., and J. L. Melnick. 1962. Induced latent infection of monkeys with vacuolating SV_{40} papova virus. Virus in kidneys and urine. Proc. Soc. Exptl. Biol. Med. **111**:367–372.
6. Atoynatan, T., and G. D. Hsiung. 1964. Ultrafiltration of simian viruses. Proc. Soc. Exptl. Biol. Med. **116**:852–856.
7. Benyesh-Melnick, M., and H. S. Rosenberg. 1964. The isolation of adenovirus type 7 from a fatal case of pneumonia and disseminated disease. J. Pediat. **64**:83–87.
8. Benyesh-Melnick, M., H. S. Rosenberg, and B. Watson. 1964. Viruses in cell cultures of kidney of children with congenital heart malformations and other diseases. Proc. Soc. Exptl. Biol. Med. **117**:452–459.
9. Bhatt, P. N., M. K. Goverdhan, M. F. Shaffer, C. D. Brandt, and J. P. Fox. 1966. Viral infections of monkeys in their natural habitat in Southern India. I. Some properties of cytopathic agents isolated from bonnet and langur monkeys. Am. J. Trop. Med. Hyg. **15**:551–560.
10. Bhatt, P. N., C. D. Brandt, R. A. Weiss, J. P. Fox, and M. F. Shaffer. 1966. Viral infections of monkeys in their natural habitat in Southern India. II. Serological evidence of viral infection. Am. J. Trop. Med. Hyg. **15**:561–566.
11. Bibrack, B. 1965. Isolierung und Charakterisierung eines Adenovirus aus Nieren von Schwein. Dissertation, Universität Munchen, Munich.
12. Black, P. H., J. W. Hartley, and W. P. Rowe. 1963. Isolation of a cytomegalovirus from African green monkey. Proc. Soc. Exptl. Biol. Med. **112**:601–605.
13. Black, P. H., and W. P. Rowe. 1963. Transformation in hamster kidney monolayers by vacuolating virus, (SV-40). Virology **19**: 107–109.
14. Blinnikov, K. S. 1960. Latent viruses in macacus rhesus monkeys. Probl. Virol. (USSR; English Transl.) **6**:701–704.
15. Brown, L. V. 1957. Pathogenicity for rabbit kidney cell cultures of certain agents derived from "normal" monkey kidney tissue. I. Isolation and propagation. Am. J. Hyg. **65**:189–209.
16. Burke, C. N., R. E. Luginbuhl, and C. F. Helmboldt. 1965. The isolation of a latent adenolike virus from chicken kidney cell cultures. Avian Diseases **9**:31–43.
17. Chang, P. W., and G. D. Hsiung. 1965. Experimental infection of parainfluenza virus type 5 in mice, hamsters, and monkeys. J. Immunol. **95**:591–601.
18. Chanock, R. M., K. M. Johnson, M. K. Cook, D. C. Wong, and A. Vargosko. 1961. The hemadsorption technique, with special reference to the problem of naturally occurring simian parainfluenza virus. Am. Rev. Respirat. Diseases **83**:125–129.
19. Choppin, P. W., and W. Stoeckenius. 1964. The morphology of SV_5 virus. Virology **23**:195–202.
20. Churchill, A. E. 1963. The isolation of parainfluenza 3 virus from fatal cases of pneumonia in *Erythrocebus patas* monkeys. Brit. J. Exptl. Pathol. **44**:529–537.
21. De Harven, E., and D. S. Yohn. 1966. The fine structure of the Yaba monkey tumor poxvirus. Cancer Res. **26**:995–1008.
22. Deinhardt, F., and A. W. Holmes. 1963. Isolation of a latent virus from the kidney of a newborn human. J. Lab. Clin. Med. **62**:872.
23. Eddy, B. E., G. S. Borman, G. E. Grubbs, and R. D. Young. 1962. Identification of the oncogenic substance in rhesus monkey kidney cell cultures as simian virus 40. Virology **17**:65–75.
24. Emery, J. B., and C. J. York. 1960. Occurrence of hemadsorption virus in normal monkey tissue culture. Virology **11**:313–315.
25. Endo, M., Y. Aoyama, T. Kaminema, T. Hayashida, and T. Kingo. 1959. A virus considered as spumous virus isolated from a culture of renal cells of a Japanese monkey. Japan. J. Exptl. Med. **29**:355–357.
26. Evans, A. S. 1958. Latent adenovirus infections of the human respiratory tract. Am. J. Hyg. **67**:256–266.
27. Flanagan, C. L., and I. Schultz. 1963. A mechanism of viruria. J. Clin. Invest. **42**:931–932.
28. Fong, C. K. Y., K. G. Bensch, and G. D. Hsiung. 1968. Productive and abortive infections of simian and nonsimian cells with a simian adenovirus SV_{15}. I. Microscopic observations. Virology **35**:297–310.
29. Fuentes-Marins, R., A. R. Rodriguez, S. S. Kalter, A. Hellman, and R. A. Crandall. 1963. Isolation of enteroviruses from the "normal" baboon (*Papio doguera*). J. Bacteriol. **85**:1045–1050.
30. Gajdusek, D. C., N. G. Rogers, M. Basnight, and C. J. Gibbs, Jr. Latent viruses from tissues of chimpanzees affected with experimental Kuru. Ann. N.Y. Acad. Sci., *in press*.
31. Gaylord, W. H., and G. D. Hsiung. 1961. The vacuolating virus of monkeys. II. Virus morphology and intranuclear distribution with some histochemical observations. J. Exptl. Med. **114**:987–996.
32. Gerber, P. 1962. An infectious deoxyribonucleic acid derived from vacuolating virus (SV 40). Virology **16**:96–97.
33. Giles, J. P., H. Liebhaber, S. Krugman, and C. Lattimer. 1964. Early viremia and viruria in infectious hepatitis. Virology **24**:107–108.
34. Granboulan, N., P. Tournier, R. Wicker, and W. Bernhard. 1963. An electron microscope study of the development of SV40 virus. J. Cell Biol. **17**:423–441.
35. Gresser, I., and S. L. Katz. 1960. Isolation of measles virus from urine. New Engl. J. Med. **263**:452–454.
36. Gresser, I., and S. Kibrick. 1961. Isolation of vaccinia virus and type 1 adenovirus from urine. New Engl. J. Med. **265**:743–744.
37. Gutekunst, R. R., and A. D. Heggie. 1961. Viremia and viruria in adenovirus infections.

Detection in patients with rubella or rubelliform illness. New Engl. J. Med. **264**:374–378.
38. Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1957. Serial propagation of the guinea pig salivary gland virus in tissue culture. Proc. Soc. Exptl. Biol. Med. **96**:281–285.
39. Heberling, R. L., and F. S. Cheever. 1965. Some characteristics of the simian enteroviruses. Am. J. Epidemiol. **81**:106–123.
40. Hoffert, W. R., M. E. Bates, and F. S. Cheever. 1958. Study of enteric viruses of simian origin. Am. J. Hyg. **68**:15–30.
41. Holmes, A. W., R. G. Caldwell, R. E. Dedmon, and F. Deinhardt. 1964. Isolation and characterization of a new herpes virus. J. Immunol. **92**:602–610.
42. Holmes, A. W., J. A. Devine, E. Nowakowski, and F. Deinhardt. 1966. The epidemiology of a herpes virus infection of New World monkeys. J. Immunol. **96**:668–671.
43. Hsiung, G. D. 1959. Use of human kidney cultures in the study of enteroviruses. Proc. Soc. Exptl. Biol. Med. **102**:612–616.
44. Hsiung, G. D. 1965. Use of ultrafiltration for animal virus grouping. Bacteriol. Rev. **29**: 477–486.
45. Hsiung, G. D. 1967. Recent advances in the study of simian viruses. Conf. Experimental Medicine and Surgery in Primates. Ann. N.Y. Acad. Sci., *in press*.
46. Hsiung, G. D. 1968. Detection of latent viruses in kidney tissues. J. Natl. Cancer Inst. *in press*.
47. Hsiung, G. D., and T. Atoynatan. 1966. Incidence of naturally acquired virus infections of captive monkeys. Am. J. Epidemiol. **83**:38–47.
48. Hsiung, G. D., T. Atoynatan, and L. Gluck. 1966. Multiple virus infection of monkey kidney cells in culture. Proc. Soc. Exptl. Biol. Med. **121**:562–566.
49. Hsiung, G. D., K. G. Bensch, and P. H. Prose. 1967. Electron microscopy of monkey kidney cells infected with both SV_{40} and measles virus. Nature **215**:178–179.
50. Hsiung, G. D., J. M. Coyle, P. W. Chang, and T. Atoynatan. 1967. Studies of the possible origin of virus infections in captive baboons and other non-human primates. Baboon Med. Res. **2**:797–812.
51. Hsiung, G. D., H. Fischman, C. K. Y. Fong, and R. H. Green. 1968. Characterization of a cytomegalovirus isolated from the equine kidney cell culture, *to be published*.
52. Hsiung, G. D., and W. Gaylord. 1961. The vacuolating virus of monkeys. I. Isolation, growth characteristics, and inclusion body formation. J. Exptl. Med. **114**:975–986.
53. Hsiung, G. D., P. Isacson, and R. W. McCollum. 1962. Studies of a myxovirus isolated from human blood. I. Isolation and properties. J. Immunol. **88**:284–290.
54. Hsiung, G. D., and J. L. Melnick. 1958. Orphan viruses of man and animals. Ann. N.Y. Acad. Sci. **70**:342–360.
55. Hull, R. N., I. S. Johnson, C. G. Culbertson, C. B. Reimer, and H. F. Wright. 1965. Oncogenicity of the simian adenoviruses. Science **150**:1044–1046.
56. Hull, R. N., J. R. Minner, and J. W. Smith. 1956. New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathic agents, SV_1, SV_2, SV_4, SV_5, SV_6, SV_{11}, SV_{12}, and SV_{15}. Am. J Hyg. **63**:204–215.
57. Hull, R. N., and J. R. Minner. 1957. New viral agents recovered from tissue cultures of monkey kidney cells. II. Problems of isolation and identification. Ann. N.Y. Acad. Sci. **67**:413–423.
58. Hull, R. N., J. R. Minner, and C. C. Mascoli. 1958. New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery of additional agents both from cultures of monkey tissues and directly from tissues and excreta. Am. J. Hyg. **68**:31–44.
59. Hull, R. N., and J. C. Nash. 1960. Immunization against B virus infection. I. Preparation of an experimental vaccine. Am. J. Hyg. **71**:15–28.
60. Johnston, P. B. 1961. A second immunologic type of simian foamy virus: monkey throat infections and unmasking by both types. J. Infect. Diseases **109**:1–9.
61. Jordan, L. E., G. Plummer, and H. D. Mayor. 1965. The fine structure of foamy virus. Virology **25**:156–159.
62. Kalter, S. S., J. Ratner, G. V. Kalter, A. R Rodriguez, and C. S. Kim. 1967. A survey of primate sera for antibodies to viruses of human and simian origin. Am. J. Epidemiol **86**:552–568.
63. Klein, S. W., and W. C. Huang. 1963. Adenovirus recovered from tissue cultures of human kidney cells. J. Pediat. **63**:774.
64. Koprowski, H., J. A. Ponten, F. Jensen, R. G. Ravdin, P. Moorhead and E. Saksela. 1962. Transformation of cultures of human tissue infected with simian virus SV40. J. Cellular Comp. Physiol. **59**:281–292.
65. Malherbe, H., and R. Harwin. 1957. Seven viruses isolated from the vervet monkey. Brit. J. Exptl. Pathol. **38**:539–541.
66. Malherbe, H., and R. Harwin. 1963. Cytopathic effects of vervet monkey viruses. S. African Med. J. **37**:407–411.
67. McNair Scott, T. F., and T. Tokumaru. 1965. The herpesvirus group in F. L. Horsfall, Jr. and I. Tamm (ed) Viral and rickettsial infections of man, 4th ed., p. 906–909. J. B. Lippincott Co., Philadelphia.
68. Melnick, J. L. 1968. Latent viral infections in donor tissues and in recipients of vaccines. J. Natl. Cancer Inst., *in press*.
69. Melnick, J. L., and D. D. Banker. 1954. Isolation of B. virus (Herpes group) from the central nervous system of a rhesus monkey. J. Exptl. Med. **100**:181–194.
70. Melnick, J. L., M. Benyesh-Melnick, K. O. Smith, and F. Rapp. 1965. Recent developments in detection of hidden virus infections. Perspectives Virol. **4**:72–112.

71. Melnick, J. L., and R. M. McCombs. 1966. Classification and nomenclature of animal viruses. Progr. Med. Virol. **8**:400–409.
72. Melnick, J. L., M. Midulla, I. Wimberly, J. G. Barrera-Oro, and B. M. Levy. 1964. A new member of the herpesvirus group isolated from South American marmosets. J. Immunol. **92**:596–601.
73. Meyer, H. M., Jr., B. E. Brooks, R. D. Douglas, and N. G. Rogers. 1962. Ecology of measles in monkeys. Am. J. Diseases Children **103**: 307–313.
74. Meyer, H. M., Jr., H. E. Hopps, N. G. Rogers, B. E. Brooks, B. C. Bernheim, W. P. Jones, A. Nisalak, and R. D. Douglas. 1962. Studies on Simian virus 40. J. Immunol. **88**:796–806.
75. Miller, R. H., A. R. Pursell, F. E. Mitchell, and K. M. Johnson. 1964. A newly discovered myxovirus (SV41) isolated from cell cultures of cynomolgus monkey kidney. Am. J. Hyg. **80**:365–376.
76. Niven, J. S. F., J. A. Armstrong, C. H. Andrews, H. G. Pereira, and R. C. Valentine. 1961. Subcutaneous "growths" in monkeys produced by a poxvirus. J. Pathol. Bacteriol. **81**:1–14.
77. O'Conor, G. T., A. S. Rabson, I. K. Berezesky, and F. J. Paul. 1963. Mixed infection with simian virus 40 and adenovirus 12. J. Natl. Cancer Inst. **31**:903–917.
78. Orsi, E. V., L. Rodriguez, M. Franko, and P. Adamkiewicz. 1968. Demonstration of latent SV_{40} infection and membrane changes in cell cultures of presumed healthy African green monkeys. Bacteriol. Proc., p. 177.
79. Pollard, M. 1965. The use of germfree animals in virus research. Progr. Med. Virol. **7**:362–376.
80. Rabson, A. S., G. T. O'Conor, F. J. Paul, and I. E. Berezesky. 1966. Virus: Mixed infection with herpes simplex and simian virus 40. Science **151**:1535–1536.
81. Rapoza, N. P. 1967. A classification of simian adenoviruses based on hemagglutination. Am. J. Epidemiol. **86**:736–745.
82. Rapp, F., J. L. Melnick, J. S. Butel, and T. Kitahara. 1964. The incorporation of SV_{40} genetic material into adenovirus 7 as measured by intranuclear synthesis of SV_{40} tumor antigen. Proc. Natl. Acad. Sci. U.S. **52**:1348–1352.
83. Rogers, N. G., M. Basnight, C. J. Gibbs, Jr., and D. C. Gajdusek. 1967. Latent viruses in chimpanzees with experimental Kuru. Nature **216**:446–449.
84. Rowe, W. P., and S. G. Baum. 1964. Evidence for a possible genetic hybrid between adenovirus type 7 and SV_{40} viruses. Proc. Natl. Acad. Sci. U.S. **52**:1340–1347.
85. Rowe, W. P., J. W. Hartley, H. G. Cramblett, and F. M. Mastrota. 1958. Detection of human salivary gland virus in the mouth and urine of children. Am. J. Hyg. **67**:57–65.
86. Rowe, W. P., J. W. Hartley, S. Waterman, H. C. Turner, and R. J. Huebner. 1956. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc. Soc. Exptl. Biol. Med. **92**:418–424.
87. Rowe, W. P., R. J. Huebner, L. K. Gilmore, R. H. Parrott, and T. G. Ward. 1953. Isolating of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exptl. Biol. Med. **84**:570–573.
88. Ruckle, G. 1958. Studies with the monkey-intra-nuclear inclusion agent (minia) and foamy agent derived from spontaneously degenerating monkey kidney cultures. I. Isolation and tissue culture behavior of the agents and identification of minia as closely related to measles virus. Arch. Ges. Virusforsch. **8**:139–166.
89. Rustigian, R., P. Johnston, and H. Reihart. 1955. Infection of monkey kidney tissue cultures with virus-like agents. Proc. Soc. Exptl. Biol. Med. **88**:8–16.
90. Schultz, E. W., and K. Habel. 1959. SA Virus. A new member of the myxovirus group. J. Immunol. **82**:274–278.
91. Schultz, I., and F. A. Neva. 1965. Relationship between blood clearance and viruria after intravenous injection of mice and rats with bacteriophage and poliovirus. J. Immunol. **94**:833–841.
92. Shah, K. V., and C. H. Southwick. 1965. Prevalence of antibodies to certain viruses in sera of free-living rhesus and of captive monkeys. Indian J. Med. Res. **53**:488–500.
93. Shein, H. M., and J. F. Enders. 1962. Transformation induced by simian virus 40 in human renal cell cultures. I. Morphology and growth characteristics. Proc. Natl. Acad. Sci. U.S. **48**:1164–1172.
94. Smith, M. G. 1959. The salivary gland viruses of man and animals. (cytomegalic inclusion disease). Progr. Med. Virol. **2**:171–202.
95. Spertzel, R. O., D. L. Huxsoll, S. J. McConnell, L. N. Binn, and R. H. Yager. 1965. Recovery and characterization of a herpes-like virus from dog kidney cell culture. Proc. Soc. Exptl. Biol. Med. **120**:651–655.
96. Stiles, G. E. 1968. Serologic screening of rhesus and grivet monkeys for SV_{40} and the foamy viruses. Proc. Soc. Exptl. Biol. Med. **127**:225–230.
97. Stiles, G. E., J. L. Bittle, and V. J. Cabasso. 1964. Comparison of simian foamy virus strains including a new serotype. Nature **201**:1350–1351.
98. Sweet, B. H., and M. R. Hilleman. 1960. The vacuolating virus, SV_{40}. Proc. Soc. Exptl. Biol. Med. **105**:420–427.
99. Tribe, G. W. 1966. An investigation of the incidence, epidemiology, and control of simian virus 5. Brit. J. Exptl. Pathol. **47**:472–479.
100. Trombetta, N. 1964. Observations on latent viruses in kidney cultures from rhesus monkeys. Ateneo Parmense **35**:335–348.

101. Tyrrell, D. A., F. E. Buckland, M. C. Lancaster, and R. C. Valentine. 1960. Some properties of a strain of SV_{17} virus isolated from an epidemic of conjunctivitis and rhinorrhoea in monkeys (Erythrocebus patas). Brit. J. Exptl. Pathol. **41**:610–616.
102. Utz, J. P. 1964. Viruria in man. Progr. Med. Virol. **6**:71–81.
103. Utz, J. P., and A. I. Shelokov. 1958. Coxsackie B virus infection. Presence of virus in blood, urine, and cerebrospinal fluid. J. Am. Med. Assoc. **168**:264–267.
104. Utz, J. P., C. F. Szwed, and J. A. Kasel. 1958. Clinical and laboratory studies of mumps. II. Detection and duration of excretion of virus in urine. Proc. Soc. Exptl. Biol. Med. **99**:259–261.
105. Vogel, J., and A. Shelokov. 1957. Adsorption-hemagglutination test for influenza virus in monkey kidney tissue culture. Science **126**: 358–359.
106. von Magnus, P., E. K. Andeenrs, K. P. Petersen, and A. Birch-Andersen. 1959. A pox-like disease in cynomolgus monkeys. Acta. Pathol. Microbiol. Scand. **46**:156–176.
107. Weller, T. H., and F. A. Neva. 1962. Propagation in tissue culture of cytopathic agents from patients with rubella-like illness. Proc. Soc. Exptl. Biol. Med. **111**:215–225.
108. Wood, W., and F. T. Shimada. 1954. Isolation of strains of virus B from tissue cultures of cynomolgus and rhesus kidney. Can. J. Public Health **45**:509–518.
109. Yohn, D. S., V. A. Haendiges, and J. T. Grace, Jr. 1966. Yaba tumor poxvirus synthesis in vitro. I. Cytopathological, histochemical, and immunofluorescent studies. J. Bacteriol. **91**:1977–1985.
110. Yoshida, E., H. Yamamoto, and H. Shimojo. 1965. Contamination of cynomolgus monkey kidney cell cultures by hemagglutinating simian virus (SV_5). Japan. J. Med. Sci. Biol. **18**:151–156.