Both agents were unstable in acid pH; thus they were designated as rhinoviruses. The viruses were immunologically distinct from each other and from all previously characterized rhinoviruses. Nineteen other virus strains serologically related to the prototype viruses were recovered from recruits at a military training camp. Statistical analysis suggested that viruses similar to prototype strain 1200 were associated with occurrence of mild "cold-like" illnesses. These studies also indicated that homologous neutralizing antibodies protected against infection by these viruses.

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Ultrafiltration of Simian Viruses.* (29390)

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Most simian viruses (SV) have been isolated from monkey tissues taken from apparently healthy asymptomatic animals. The extensive use of primary monkey kidney cell cultures in research and for the commercial production of vaccines has resulted in recovery of large numbers of viruses from these animals, but classification of the simian viruses is a problem. Hull and coworkers(1-3) grouped these viruses into 8 categories based on cytopathic changes in infected tissue culture and on serological differences. Hsiung and Melnick(4) suggested that studies on host cell infectivity spectra and plaque morphology be used for grouping these viruses, and recently, Malherbe and Harwin(5) grouped viruses obtained from monkeys according to cytopathology as observed in stained cultures. Although there is some overlapping in the grouping of these viruses

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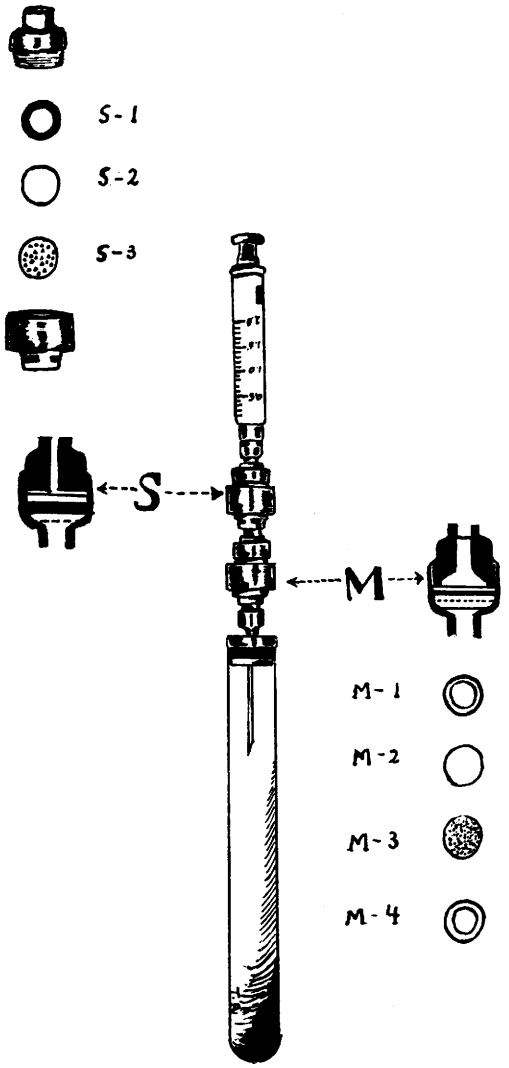

FIG. 1. Filter set. S = Seitz filter adapter containing S-1, rubber ring; S-2, Seitz filter discs; S-3, screen. M = Millipore membrane filter adapter containing M-1, Teflon O-ring gasket; M-2, Millipore membrane; M-3, support screen; M-4, Teflon flat gasket.

by their biological and serological properties, a more fundamental approach based on their physical properties is under consideration. The present study is concerned with size determination of some simian viruses as measured by ultrafiltration, and the application of the technique to virus taxonomy and identification(6).

Materials and methods. Virus strains. The viruses used are listed in Tables I and II. SV_5 was obtained through the courtesy of Dr. K. E. Jensen, Charles Pfizer Co., Terre Haute, Ind.; SV_{30}, SV_{31}, SV_{32}, SV 33, SV_{36}, originally designated as P_5, P_6, P_7, P_{10}, and P_9 respectively, were supplied by Dr. F. S. Cheever(7). All other SV series were made available by Dr. R. N. Hull(1,2,3), except SV_{40}, which was isolated from patas monkey kidney cell culture in our laboratory(8).

Tissue cultures and virus infectivity titrations. Primary rhesus monkey kidney cultures either in tubes or in bottles were used for virus infectivity titrations, except in the case of SV_{40}, for which African green or patas monkey kidney cultures were used. All viruses except SV_5 were assayed by the cytopathic effect (CPE) and agar overlay plaque techniques. The hemadsorption and plaque methods were used for SV_5.

Filtration.† A 2 ml syringe fitted with 2 Swinny filter adapters, one attached to another was assembled as shown in Fig. 1. The S adapter, containing a one-half inch Seitz filter disc or a GS Millipore membrane (220 ± 20 mμ), was used for clarification of the infected tissue culture fluids. The M adapter, containing either a Millipore membrane or a gradacol membrane of various porosities, was attached to the S adapter at one end and at the other end to a hypodermic needle. The entire assembled filter set (except the membranes) was autoclaved at 15 lb for 30 minutes. After cooling, the appropriate membrane was inserted into the adapter, and the virus fluid was introduced into the syringe. A vacuum tube (Vacutainers, 20 ml capacity 165 × 16 mm) was used to collect the final filtrate (Fig. 1).

Millipore membranes used were as follows: VC type of 100 mμ ± 8 mμ, VM type of 50 mμ ± 3 mμ, and VF type of 10 mμ ± 2 mμ. These membranes were sterilized by exposure to a UV lamp at 12 inches distance for 10 minutes for each side. The various grada-

† Gradacol membranes were supplied by the Wright-Fleming Institute, St. Mary's Hospital, London and were kindly made available by Dr. Marvin Harris, Nat. Inst. Health, Bethesda, Md. Millipore filters were purchased from the Millipore Filter Corp., Bedford, Mass. Swinny filter adapters and Vacutainers were purchased from Becton Dickinson & Co., Rutherford, N. J.

TABLE I. Use of Millipore for Ultrafiltration of Simian Viruses.

Virus strain	Avg virus infectivity titers log TCD_{50}/0.1 ml				
	Unfiltered	Seitz or millipore membrane (220 mμ)	Millipore membranes Avg pore diameter (mμ) 100	50	10
SV_5*	5.8	4.0†	0	0	0
SV_1	5.4	4.7	3.7	0	0
SV_{11}	4.3	4.1	1.7	0	0
SV_{15}	5.5	5.2	2.7	0	0
SV_{17}	5.7	5.1	.7	0	0
SV_{23}	5.7	5.1	2.5	0	0
SV_{25}	4.1	3.5	1.7	0	0
SV_{27}	5.7	4.7	1.7	0	0
SV_{30}	4.5	3.5	1.7	0	0
SV_{31}	5.5	4.7	1.9	0	0
SV_{32}	5.7	5.5	1.1	0	0
SV_{33}	3.1	2.5	1.4	0	0
7852‡	4.5	3.7	1.7	0	0
7853‡	5.1	4.5	1.0	0	0
SV_{12}	4.5	4.1	1.7	0	0
SV_{16}	6.1	5.5	4.1	2.1	0
SV_{18}	5.8	5.5	4.1	3.5	0
SV_{26}	5.1	4.7	4.3	3.1	0
SV_{29}	4.3	4.0†	3.8	2.8	0
SV_{35}	4.7	4.5†	4.1	3.1	0

* Results were obtained by hemadsorption.
† Seitz filter discs were used for clarification.
‡ Virus strains were isolated in our laboratory by J. T. Riordan.

col membranes were stored in sterile water and subsequently were cut with a sterile cork borer to fit the Swinny adapters.

Results. Simple Millipore filtration. Simian virus-infected culture fluids (coded) were filtered, using Millipore membranes of 100, 50 and 10 mμ porosities. Each filtrate was assayed at serial 10-fold dilutions using CPE in tube cultures, 2 to 6 tubes per dilution, and/or plaques in bottle cultures, 2 bottles per dilution. The results of the screening test are shown in Table I. In most experiments 2 or more filtrations were carried out with similar results. In certain cases Millipore membranes of different lots were used and results were essentially the same.

As shown in Table I, no infectious virus could be detected in filtrates of SV_5 after the virus fluid was passed through Millipore filters of 100, 50 and 10 mμ pore sizes. A significant amount of virus was recovered in the filtrates of SV_{16}, SV_{18}, SV_{26}, SV_{29}, and SV_{35} when passed through the 100 mμ and 50 mμ pore size filters. On the other hand, SV_1, SV_{11}, SV_{15}, SV_{17}, SV_{23}, SV_{25}, SV_{27}, SV_{30}, SV_{31}, SV_{32}, SV_{33}, 7852, 7853, and SV_{12} were filterable through porosities of 100 mμ but not of 50 mμ.

Comparison of Millipore and gradacol membrane filtration. For confirmation several virus strains, representing each known virus group, were used. Gradacol membranes of the appropriate average pore diameter were selected according to each virus size range as determined by the simple Millipore filtration. For example, gradacol membranes with an average pore diameter (APD) greater than 100 mμ were used for filtering SV_5. Membranes of APD smaller than 50 mμ but larger than 10 mμ were used for SV_{16}, SV_{18}, SV_{26}, SV_{29}, and SV_{35}. For SV_1, SV_{11}, SV_{23}, SV_{36} and SV_{12}, gradacol membranes APD between 50-130 mμ were used. The results of several experiments are summarized in Table II.

It can be seen that a fall in virus titer of 0.3-1.5 logs was noted in filtrates obtained after Seitz filtration or after clarification with a Millipore membrane of 220 mμ. Results obtained from filtration of viruses through gradacol and Millipore membranes were essentially similar. Thus, SV_5 was arbitrarily classified as a large virus of size 220-130 mμ, which is within the range of other parainfluenza viruses of the myxovirus group (9). On the other hand, SV_{16}, SV_{18}, SV_{26}, SV_{29} and SV_{35} were classified as small (*i.e.* 30-40 mμ), which is within the range of human enteroviruses(10). SV_1, SV_{11}, SV_{23} and SV_{36} (adenoviruses) and SV_{12} (reovirus) were classified in the medium size virus group of 130-50 mμ range. According to other reports adenovirus type 4 strain RI-67 passed through gradacol membrane 160 but not 140 mμ(11). Reovirus type 1, the Long strain, formerly known as ECHO-10, passed through a 130 mμ but not a 120mμ membrane(12). In the present study virus strains belonging to the adenovirus group (SV_1, SV_{11}, SV_{23}) and the reovirus group (SV_{12}) passed through a 100 mμ Millipore membrane with significant reduction in virus titers. Inconsistent results have been observed with the latter groups of viruses, for example, SV_{36},

TABLE II. Comparison of Millipore Membrane and Gradacol Membrane Filtration.

Virus group†	Virus strain	Virus titers log PFU/0.1 ml												Virus size arbitrarily
		Unfiltered	Seitz	Avg pore diameter (mμ) 310	240	(220)*	130	110	(100)*	87	75	54	(50)*	40	30	(10)*	
Myxo-	SV_5	5.6	5.1	4.8	3.4	(2.8)	0	0	(0)				(0)			(0)	Large
Adeno-	SV_1	5.4	3.3			(4.7)	3.1	3.1	(3.7)	1.5	0	0	(0)			(0)	Medium
	SV_{11}	4.3				(4.1)	1.3	1.3	(1.3)	0	0	0	(0)			(0)	"
	SV_{28}	5.7				(5.1)	2.1	2.1	(2.5)	0	0	0	(0)			(0)	"
	SV_{36}‡	>7.1				(6.0)	1.1	0	(0)	0	0	0	(0)			(0)	"
Reo-	SV_{12}‡	4.2				(3.5)	1.4	1.2	(1.4)	.3	0	0	(0)			(0)	"
Entero-	SV_{16}	6.6	6.3						(5.9)			4.1	(3.8)	2.8	0	(0)	Small
	SV_{18}	5.8	5.4						(5.1)			4.3	(4.4)	2.4	0	(0)	"
	SV_{26}	5.1	4.7						(4.3)			2.9	(3.1)	1.5	0	(0)	"
	SV_{29}	5.6	5.2						(4.8)			3.8	(4.3)	3.5	0	(0)	"
	SV_{35}‡	4.7	4.5						(4.1)			3.1	(3.1)	2.7	0	(0)	"
Papova-	SV_{40}‡	5.3	5.0						(4.5)			3.7	(3.7)	2.8	0	(0)	"

* Numbers in parentheses were determined following Millipore filtration; others were determined after gradacol membrane filtration.
† Virus groups are listed according to R. N. Hull.
‡ By CPE method, SV_{12}, SV_{35}, and SV_{36} in rhesus monkey cell cultures and SV_{40} in green monkey cell cultures.

an adenovirus, was retained by the filter of 100 mμ porosity.

Separation of mixed infection. Mixtures of simian viruses containing equal numbers of plaque forming units (PFU) of SV_1 and SV_{18} were filtered using 50 mμ Millipore membranes. The final filtrates were assayed in bottle cultures for identity of each virus by the plaque method. In 3 repeated experiments SV_{18} but not SV_1 was recovered in the filtrate. Thus, the separation of SV_1 (adenovirus) and SV_{18} (enterovirus) was easily accomplished by this procedure.

Discussion. Experiments on ultrafiltration of animal viruses have been limited in recent years because of the short supply of gradacol membranes. Filtration procedure was laborious and time-consuming and could not be conveniently used in a routine laboratory. In the present study, using Millipore membranes in combination with the Seitz filters, unknown viruses could be readily grouped into 3 size ranges as follows: (Also see last column of Table II.)

Virus size (arbitrarily)	Seitz filter	Millipore filters (mμ) 100	50	10
Large	+	0	0	0
Medium	+	+	0	0
Small	+	+	+	0

+ = Virus passed through filter. 0 = Virus retained by filter.

For obtaining more specific values further size determinations can be made within the range indicated by the preliminary filtration. Obviously this grouping is only approximate, and final decisions on specific size can be made by other methods.

According to the above category and the results in Table II, SV_5 would be regarded as a "large" virus; SV_1, SV_{11}, SV_{12}, SV_{23}, and SV_{36} would be "medium" size viruses; SV_{16}, SV_{18}, SV_{26}, SV_{29}, SV_{35}, and SV_{40} would be considered "small." The data obtained from the present study for different virus groups and those reported for myxo-, entero-, and papovaviruses, in general are in fairly good agreement. SV_{26}, SV_{29}, and SV_{35} were isolates from the central nervous system of monkeys and it was suggested by Hull(13) that these viruses may belong to the entero-

virus group. Our results obtained from ultrafiltration indicated that the sizes of SV_{26}, SV_{29} and SV_{35} are indeed within the range of the enteroviruses.

Some variation has been observed within the simian adenovirus group. Archetti and Steve-Bocciarelli(14) report that simian adenoviruses SV_1, SV_{11}, and SV_{23}, were similar morphologically 80 mμ in diameter, with an internal core of 45 mμ. Inconsistent results have been obtained with SV_{11}, SV_{12} and SV_{36}. In most experiments virus titers were significantly reduced after passing through a 100 mμ filter, but in other experiments infectious virus was not detected in the filtrate. It becomes clear that in some instances confirmation of size should be made using gradacol membranes.

Simian virus mixed infections have caused difficulties in final identification. The separation of SV_1 and SV_{18} by filtration using a Millipore membrane of 50 mμ porosity indicates that the use of Millipore membrane of appropriate pore diameters can facilitate the separation of 2 viruses of different size ranges.

In general, the results of the filtration measurements on virus of large and small sizes were consistent and reproducible. This was at least partially due to the amount of the pressure applied in each filtration test (a 20 ml Vacutainer tube) and the simplicity of the filter set. The method can, therefore, be recommended for a routine diagnostic virus laboratory where unknown agents are encountered.

Summary. Millipore filters of 100, 50 and 10 mμ pore sizes were found to be satisfactory for estimating the size of simian viruses, and classifying them into 3 size categories of large, medium and small. SV_5 was of large size; SV_1, SV_{11}, SV_{12}, SV_{15}, SV_{17}, SV_{23}, SV_{25}, SV_{27}, SV_{30}, SV_{31}, SV_{32}, SV_{33}, and SV_{36} were in the medium size range; SV_{16}, SV_{18}, SV_{26}, SV_{29}, SV_{35} and SV_{40} belong to the small virus group. Simplicity of the filter set would permit its use in a routine virus laboratory.

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