Characterization of Two Newly Recognized Rhinovirus Serotypes of Human Origin. (31350)

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(Introduced by R. J. Huebner)
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Rhinoviruses have emerged as the major known etiologic agents of adult upper respiratory illnesses(1-4). Their importance in respiratory illnesses of infancy and childhood has not been adequately determined. Rhinoviruses are classified as a subgroup of the picornaviruses by virtue of certain biophysical properties which include (1) small size (15-30 mμ); (2) ribonucleic acid (RNA) core; (3) ether resistance; and (4) complete or almost complete inactivation at pH 3.0(5). This last property serves to distinguish the rhinovirus from the enterovirus subgroup of picornaviruses.

This report describes 2 new rhinoviruses, 1505 and 8213, which were isolated during epidemiologic studies of respiratory disease in 2 different populations. These 2 viruses were found to be distinct by neutralization tests from all 85 previously described rhinoviruses (6-18). In addition, they were also found to be different from 11 rhinoviruses presented at the 1965 Rhinovirus Workshop in Bethesda, Md., by various laboratories as possible candidate rhinovirus strains which were still in the process of being studied(19).

Materials and methods. Virus isolation. Specimen #1505 represented an oropharyngeal swab obtained from a child in a study at Camp Lejeune, N. C.(20). A dry cotton swab was wiped across the posterior pharynx and tonsillar pillars several times and then immersed in a vial containing veal infusion broth with 0.5% bovine albumin. The swab was stirred vigorously in the fluid and the fluid milked out by rubbing the swab along the inner surface of the vial. For virus isolation, 0.2 ml of the unfrozen specimen was inoculated into 2 fetal human diploid cell strain (HDCS) cultures, 2 rhesus monkey kidney cell cultures, and 2 Hep-2 cultures. These tube cultures were examined periodically for the appearance of cytopathic effect. Cells and fluid were harvested and frozen at —60°C when the entire cell monolayer had degenerated.

Specimen #8213 represented an oropharyngeal swab obtained from a child in a study at Children's Hospital in the District of Columbia(21). Procedures were carried out as above except that human embryonic kidney (HEK) cell cultures were used for primary isolation instead of HDCS cultures.

Tissue culture. Tube cultures of HDCS, WI-26 or WI-38, rhesus or vervet monkey kidney (MK) cells, Hep-2 cells, and KB cells were purchased from commercial sources. The maintenance media for HDCS cultures, unless otherwise specified, were composed of equal parts of Eagle's Minimum Essential Medium (MEM) and medium 199 supplemented with 2% inactivated (56°C for 30 minutes) calf serum, 0.02 M glutamine, 100 units of penicillin per ml and 100 μg of streptomycin per ml. The MK and HEK cell cultures were maintained in the same medium as above but without calf serum. Both KB and Hep-2 cell cultures were maintained in the same medium as the HDCS cultures except that 2% inactivated chick serum was used in place of the calf serum. All cultures were incubated on rotating drums at 12 r.p.h. at 33°C.

Infectivity titrations. HDCS cultures were used in infectivity titrations. Ten-fold dilutions of virus were made in Hanks' Balanced Salt Solution (HBSS) containing 0.5% gelatin, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Two-tenths ml of the appropriate virus dilutions was inoculated

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into each of 2 to 4 tube cultures; the cultures were examined for the appearance of cytopathic effect (CPE) 3 times weekly for approximately 10 days. Infectivity titers were based on CPE and calculated by the Reed and Muench method(22).

Terminal dilution purification. Each rhinovirus was "purified" by 3 terminal dilution passages in HDCS cultures. Two-tenths ml of 3.2-fold dilutions of virus was inoculated into each of 10 culture tubes, and the cultures were maintained for approximately one week. Successive terminal dilution passages were made with the single positive culture or with one of 2 positive cultures at the limiting dilution.

Ether sensitivity test. Diethyl ether was mixed with viruses 1505 and 8213 in a proportion of 1 to 4 and each mixture held at 4°C for approximately 18 hours. The ether was eliminated by bubbling nitrogen gas through the mixture for 5 minutes, and the infectivity titer was determined in HDCS cultures using 4 tubes per 10-fold dilution. Viruses 1505 and 8213 without diethyl ether were handled in a similar manner. A known ether sensitive virus, herpes simplex, and a known ether resistant virus, Echo 28, were handled in a similar manner as the test viruses above.

Sensitivity to 5-iodo-2-deoxyuridine (5-IUDR). The maintenance media in tests with 5-IUDR consisted of equal parts of Eagle's MEM and 199 with 2% inactivated horse serum, 0.02 M glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Two-tenths ml of 10-fold dilutions of virus was inoculated into 2 HDCS tube cultures containing a final concentration of $10^{-4.3}$ M 5-IUDR, into 2 HDCS tube cultures lacking 5-IUDR, and into 2 HDCS tube cultures containing a final concentration of $10^{-4.3}$ M 5-IUDR and $10^{-4.0}$ M thymidine. The cultures were examined for the appearance of CPE at 3 and 6 days after inoculation. Included in each test was vaccinia virus, a known deoxyribonucleic acid (DNA) virus, and poliovirus type 1 (LSC-1 strain), a known ribonucleic acid (RNA) virus. The test was satisfactory if poliovirus was not inhibited by the 5-IUDR and if the vaccinia virus was completely inhibited by 5-IUDR but not inhibited by 5-IUDR plus thymidine.

Acid lability. The method of Ketler, Hilleman and Hamparian was employed(23). Virus pools of 1505 and 8213 were diluted 1:10 in Eagle's MEM prepared without bicarbonate, pH 2.7, and in Eagle's MEM without bicarbonate but buffered with 0.01 M tris(hydroxymethyl)-aminomethane (Tris), pH 7.0, and both mixtures were incubated three hours at room temperature. A known acid labile virus, Echo 28, and a known acid stable virus, poliovirus type 1 (LSC-1 strain) were handled in a similar manner as the test viruses above. Infectivity titers were determined in HDCS cultures using 4 tubes per 10-fold dilution.

Size determination. Gradacol filtration was employed for virus size estimation. Procedures described previously were followed throughout(11).

Magnesium stabilization. The method of Wallis and Melnick was employed(24). One part of 4 M magnesium chloride was mixed with 3 parts of virus and incubated at 50°C for one hour. A control mixture consisting of one part HBSS and 3 parts virus was also incubated at 50°C for one hour. Ten-fold dilutions of each mixture were inoculated into 4 HDCS tube cultures.

Preparation of antisera. Antisera were prepared in goats and guinea pigs. For goat immunization, triply terminally diluted 1505 and 8213 viruses were grown in KB cultures and harvested when CPE was complete. The cultures were frozen and thawed 3 times and cellular debris removed by centrifugation at 2000 r.p.m. for 20 minutes in an International PR-2 centrifuge at 4°C. The goat receiving 8213 virus was given 2 injections a week for 2 weeks of 10 ml of unconcentrated fluorocarbon 113 treated virus which was emulsified with 10 ml of complete Freund's adjuvant; the animal was bled approximately 3 weeks after the last injection. The fluorocarbon treatment was the same as described previously(11). The goat receiving 1505 virus was given 2 injections a week for 2 weeks of 10 ml of unconcentrated fluorocarbon 113 treated virus which was emulsified with 10 ml of complete Freund's adjuvant; an additional injection of material as de-

scribed above was given in approximately 2 weeks, followed by an additional injection in approximately 3 weeks. The goat was bled one week later.

Hyperimmune antisera to viruses 1505 and 8213 were prepared in guinea pigs according to a previously described procedure except that the virus (which had not been triply terminally diluted) was concentrated 10 times by centrifugation(11,16).

Antisera for 84 of the 85 previously described rhinoviruses and 9 of the 11 rhinoviruses presented at the Rhinovirus Workshop were prepared either at NIH or by other laboratories. Sera not prepared at NIH were kindly supplied by the following investigators: Dr. J. C. Holper, Abbott Laboratories, supplied all bovine sera; Dr. V. V. Hamparian, Ohio State University, supplied guinea pig sera for 15 coryzaviruses; Dr. W. S. Jordan, Jr. and Dr. J. M. Gwaltney, Jr., University of Virginia, supplied guinea pig sera for 16 coryzaviruses and sera for CH 79, 82 and 310 (sera to CH 47 and CH 202 were not available); Dr. D. Hamre, University of Chicago, supplied canine serum for virus 037211 and guinea pig sera for other viruses described by her laboratory for which calf or goat sera were not available; Dr. J. L. Melnick and Dr. C. A. Phillips, Baylor University, supplied baboon sera for the Baylor Viruses; Dr. E. H. Lennette and Dr. J. H. Schieble, State of California Department of Public Health, supplied guinea pig sera for viruses FO1-3610, FO1-3772, FO1-3774, FO1-3928, and FO1-4081; and Dr. W. J. Mogabgab, Tulane University, supplied rabbit serum for virus K779. Serum to virus 63-0499 was not available; (however, this virus was kindly supplied by Dr. R. Deibel of the New York State Department of Health).

Serum neutralization tests. Equal volumes of virus and initial serum dilutions of 1:10 and/or 1:20 (or 4-fold serum dilutions depending on the test) were incubated at room temperature for 2 hours. All sera were inactivated at 56°C for 30 minutes. Two-tenths ml of the mixture was inoculated into each of 2 HDCS cultures. These were examined for CPE at a time when a simultaneous titration indicated that 3 to 100 TCD_{50} of virus was present. The cultures were examined at 2- to 3-day intervals for approximately one week. Serum neutralization end points were calculated according to the Reed & Muench method(22).

Inoculation of suckling mice. NIH random bred general purpose Swiss mice less than 24 hours old were used. One litter of mice was inoculated with each rhinovirus intracerebrally (0.03 ml) and intraperitoneally (0.03 ml) and observed 8 to 12 days. Two mice each from the litter inoculated with 8213 virus were sacrificed on days 2, 4, and 6, while 2 mice each from the litter inoculated with 1505 virus were sacrificed on days 4, 8, and 12. Twenty per cent carcass and brain suspensions were prepared from the mice sacrificed 2 and 4 days after inoculation with strain 8213, and from the mice sacrificed 4 days after inoculation with strain 1505. These suspensions were tested for virus in HDCS cultures.

Results. Virus 8213 was isolated from a throat swab obtained on June 10, 1959, the initial day of hospitalization at Children's Hospital (D.C.) of a 21-month-old female infant (study #8213), admitted with a diagnosis of bronchiolitis. The isolation was made in HEK tissue cultures. The virus was not isolated in MK or Hep-2 tissue cultures. However, after passage into cultures of HDCS WI-38, it could be adapted to grow in both MK (rhesus and vervet) and KB cells. Reisolation of the virus from the original specimen was made successfully in cultures of HDCS WI-38. Blood specimens obtained from the patient on the day of admission and 3 weeks later demonstrated a significant rise in neutralizing antibody for strain 8213 (a rise from $<$1:4 to 1:128). Two additional strains resembling strain 8213 were recovered—one from a throat swab obtained on May 20, 1959, from a 26-month-old female in-patient (study #7961) at Children's Hospital (D.C.) with a diagnosis of bronchiolitis; the other from a throat swab obtained on June 16, 1959, from a 23-month-old female in-patient (study #8269) with a non-respiratory disease at the same hospital.

Virus 1505 was isolated from a throat swab

TABLE I. Effect of 5-Iodo-2-Deoxyuridine (IUDR) and 5-IUDR plus Thymidine on Virus Multiplication.

Virus	Infectivity titer* in indicated medium: Maintenance medium†	Maintenance medium & $10^{-4.3}$M 5-IUDR	Maintenance medium & $10^{-4.3}$M 5-IUDR & $10^{-4.0}$M thymidine
1505	4.0	3.5	3.5
8213	3.5	4.0	3.5
Vaccinia	4.5	1.5	4.0
Polio type 1	5.0	5.0	5.5

* Infectivity titer expressed as Log_{10} TCD_{50}/0.2 ml.

† Maintenance medium as described in *Materials and methods.*

obtained on September 8, 1961, from a 5-year-old male (study #1505) who was not ill and who was seen in the immunization clinic at Camp Lejeune, N. C. The isolation was made in cultures of HDCS WI-26. The virus was not isolated in MK or Hep-2 cell cultures. After passage in HDCS cultures, 1505 virus could not be propagated in MK tissue cultures but could be adapted to grow in KB cell cultures. Several reisolation attempts from the original specimen in cultures of HDCS WI-38 were not successful. No blood specimens were obtained from the patient. An additional isolate resembling the 1505 strain was recovered from a throat swab obtained on February 11, 1960, from a 15-month-old female (study #11121) attending Well Baby Clinic at the Children's Hospital (D.C.). This strain was reisolated from the original specimen in cultures of HDCS WI-26. Another isolate resembling strain 1505 was recovered from a throat swab obtained on July 10, 1962, from a 3½-month-old female (study #81264) seen in the out-patient clinic at Camp Lejeune with a diagnosis of bronchiolitis. This infant developed a rise in neutralizing antibody to the homologous virus (acute phase serum titer $<1:4$ and convalescent phase serum titer 1:64) whereas an antibody rise to strain 1505 was not detected, indicating the possibility of antigenic heterogeneity in this virus serotype.

Size estimation. Virus 8213 passed through the 85 mμ, 55 mμ, and 20 mμ average pore diameter (APD) gradacol membranes while virus 1505 passed the 83 mμ and 59 mμ APD gradacol membranes but was retained by the 21 mμ APD membrane. According to Black's formula, virus 8213 would have an estimated size of approximately 13 mμ, while virus 1505 would have an estimated size of approximately 25 mμ(25).

Determination of nucleic acid type. Multiplication of both 1505 and 8213 viruses was not inhibited by $10^{-4.3}$ M 5-IUDR, a concentration inhibitory for DNA viruses. As seen in Table I, vaccinia virus (a known DNA virus) was markedly inhibited by 5-IUDR while poliovirus type 1 (a known RNA virus) was unaffected. Vaccinia virus multiplication was not inhibited when thymidine was added to the 5-IUDR in the maintenance medium. These findings indicate, in an indirect way, that the nucleic acid core of viruses 1505 and 8213 is composed of RNA.

Ether sensitivity. Table II shows that both 1505 and 8213 viruses were resistant to inactivation by diethyl ether. The table also shows that a control virus, Echo 28 (a known ether resistant virus), was resistant to inactivation by diethyl ether while another con-

TABLE II. Biochemical Properties of Rhinoviruses 1505 and 8213.

Virus	Ether resistance: Infectivity titer* after Ether exposure	Ether resistance: Infectivity titer* after No ether exposure	Acid lability: Infectivity titer* after exposure to pH 2.7	Acid lability: Infectivity titer* after exposure to pH 7.0	Magnesium stabilization: Infectivity titer* after exposure to 50° for 1 hr With $MgCl_2$	Magnesium stabilization: Infectivity titer* after exposure to 50° for 1 hr Without $MgCl_2$
1505	4.8	4.3	$<.8$	4.5	3.5	$<.8$
8213	5.3	5.0	$<.8$	5.3	3.5	$<.8$
Echo 28	4.3	4.0	$<.8$	4.0	N.T.	N.T.
Herpes simplex	$<.8$	5.3	N.T.	N.T.	N.T.	N.T.
Polio type 1	N.T.	N.T.	≤ 6.5	≤ 6.5	N.T.	N.T.

* Infectivity titer expressed as log_{10} TCD_{50}/0.2 ml. N.T. = Not tested.

trol virus, herpes simplex (a known ether sensitive virus), was inactivated by the diethyl ether.

Acid lability. Both 1505 and 8213 viruses were completely inactivated by exposure to pH 2.7 for 3 hours at 25°C (Table II). Table II also shows that a control virus, Echo 28 (a known acid labile virus), was completely inactivated while another control virus, poliovirus type 1 (a known acid stable virus), was not inactivated by exposure to pH 2.7 for 3 hours.

Magnesium stabilization. Both 1505 and 8213 viruses were stabilized by magnesium on exposure to 50°C for one hour. Both viruses were inactivated at 50°C in the absence of magnesium (Table II).

Pathogenicity in mice. Both 1505 and 8213 viruses produced no evidence of illness in suckling mice inoculated intraperitoneally and intracerebrally and observed for 8 to 12 days. A 20% suspension of muscle and brain from mice sacrificed at 2 and 4 days for virus 8213 and at 4 days for virus 1505 produced no CPE when inoculated into cultures of HDCS WI-26.

Serologic studies. 1505 and 8213 viruses were shown to be distinct from each other by reciprocal neutralization tests. A 1 in 10 or 1 in 20 dilution of 1505 goat antiserum (with a neutralizing antibody titer of 1:2560 *vs* 32 TCD_{50} of homologous virus) failed to neutralize any of the 85 previously described rhinoviruses with the exception of the 1660 and 342-H viruses; a 1 in 10 or 1 in 20 dilution of 8213 goat antiserum (with a neutralizing antibody titer of 1:2560 *vs* 32 TCD_{50} of the homologous virus) failed to neutralize any of the 85 previously described rhinoviruses with the exception of the 1660 virus (Table III). The 11 viruses presented at the Rhinovirus Workshop were also not neutralized by the 1505 and 8213 antisera (Table III).

Table IV shows the results of a reciprocal neutralization test with 1660 and 1505 viruses using a 1505 guinea pig serum in addition to the 1505 goat serum. It is seen that the two viruses are distinct not only because the one way cross reaction was of such a low level (less than 20-fold of the homologous titer)

TABLE III. Rhinoviruses and Rhinovirus Antisera Used in Reciprocal Neutralization Tests with 1505 and 8213 Viruses.

		Antiserum to indicated virus		
Reference	Virus†	Homologous titer	TCD_{50} in test	Animal in which sera made
6,7	*Echo 28	640	100	Goat
8	*HGP	640	100	Calf
	*FEB	1280	32	Goat
	*B632	2560	32	Calf
	*Thompson	320	10	Goat
	*Norman	2560	32	"
	*1660	2560	32	Calf
9,23	‖*68-T11	640	320	‡Goat
	*MRH-T12	5120	100	‡ "
	*211-T13	5120	100	‡ "
	*204-T14	2560	32	‡ "
	*1-T15	640	32	‡ "
	*181-T16	640	100	‡ "
	*5986-T17	5120	10	‡ "
	*6072-T18	>5120	32	‡ "
	*15-T19	≧ 160	30–300	Guinea pig
	21-T20	"	"	" "
	*47-T21	"	"	" "
	*127-T22	"	"	" "
	*5007-T23	"	"	" "
	*5124-T24	"	"	" "
	*5146-T25	"	"	" "
	*5426-T26	"	"	" "
	5660-T27	"	"	" "
	*5870-T28	5120	32	‡Goat
	*6101-T29	≧160	30–300	Guinea pig
	*5582-T30	"	"	" "
10	*K779	5120	3	‡Rabbit
11	*353	>2560	100	‡Goat
	*1059	640	200	‡ "
	*1734	640	320	‡Calf
	*11757	160	32	‡Goat
	*33342	640	320	‡Calf
12	*106F	1280	100	‡Calf
	*140F	640	32	‡ "
	*179E	640	100	‡ "
13	*515-T34	1024	100	Guinea pig
	611-T35	128	100	" "
	1770-T36	1024	100	" "
	*2268-T37	2048	100	" "
	6660-T38	1024	100	" "
	6669-T39	256	300	" "
	1963-T40	256	100	" "
	*6360-T41	256	33	" "
	*6692-T42	≧160	30–300	" "
	*1936-T43	256	33	" "
	6258-T44	64	33	" "
	605-T45	128	33	" "
	*1979-T46	512	100	" "
	425-T47	≧160	30–300	" "
	1983-T48	"	"	" "
	2253-T49	128	100	" "
	477-T50	≧160	30–300	" "
	1857-T51	256	33	" "
	1671-T52	512	100	" "
	464-T53	128	100	" "
14	*363	640	32	‡Goat
	*1200	5120	32	‡ "

(Continued next page)

TABLE III (continued).

Reference	Virus†	Antiserum to indicated virus: Homologous titer	TCD_{50} in test	Animal in which sera made
15	*101-1	1280	10	‡Calf
	*107E	5120	3	Guinea pig
	*113E	320	32	‡Goat
	*127-1	320	100	Calf
	*137-3	320	≧320	‡ "
	*137F	1280	100	‡Goat
	*143-3	≧5120	10	‡Calf
	*147H	1280	50	Guinea pig
	*151-1	640	3	‡Calf
	*164A	2560	32	‡ "
	*182E	640	10	‡Guinea pig
	*184E	800	30	" "
	*191-1	1280	32	‡ " "
	*201-3C	2400	50	‡ " "
	*203F	60	30	" "
	*313G	640	32	" "
	*342H	2560	100	‡Calf
16	*209	2560	32	‡Goat
	*1794	1280	32	‡ "
	*56110	1280	100	‡Calf
	*56822	5120	320	‡Goat
	*58750	5120	32	‡ "
	*71560	640	100	‡ "
17	63-0499	Serum not available		
18	*Baylor 1	160	100	‡Baboon
	*Baylor 2	1280	32	‡ "
	*Baylor 3	160	32	‡ "
19,26	*CH 47§	Serum not available		
	*CH 79§	512	100	‡Goat
	*CH 82§	2560	100	‡ "
	*CH 202§	Serum not available		
	*CH 310§	512	100	‡Goat
19,27	*FO1-3610	2560	100	‡Guinea pig
	*FO1-3772	1024	300	‡ " "
	*FO1-3774	160	32	‡ " "
	*FO1-3928	1280	32	‡ " "
	*FO1-4081	5120	10	‡ " "
19,28	*037211	2560	100	‡Dog

* Triply terminally diluted virus.

† The following viruses appear to be similar, if not identical: B632 and K779 (12); 127-T22 and 203F (29); 5007-T23 and 353 (13); 5146-T25 and 147H (29); 5660-T27 and 127-1 (29); 6101-T29 and 113E (29); 5582-T30 and 179E (13); 6660-T38 and CH 82 (30); 6669-T39 and 182E (29); 605-T45 and 313G (29); 1979-T46 and Baylor 3 and CH 310 (30); 477-T50 and Baylor 2 and CH 202 (30); 137F and 56110 (29); 184E and 1794 (29); Baylor 1 and 037211 (29).

‡ Serum prepared from triply terminally diluted virus.

§ These viruses were formerly designated as follows: CH 47 as Chv/1/59; CH 79 as Chv/2/59; CH 82 as Chv/3/59; CH 202 as Chv/7/59; CH 310 as Chv/1/60 (4); [Chv/5/60 cited in reference 4 is similar, if not identical, to coryzavirus T23 (30)].

‖ T = Type.

but also because no relationship was detectable with the 1505 guinea pig serum.

Table IV also shows the results of a reciprocal neutralization test with 342-H and 1505 viruses using the 1505 guinea pig serum in addition to the 1505 goat serum. The table shows that these two viruses are also distinct since the one way cross reaction was of such a low level (less than 20-fold of the homologous titer) and also because no relationship was detectable with 1505 guinea pig serum.

In addition, Table IV shows the results of a reciprocal neutralization test with 1660 and 8213 viruses using an 8213 guinea pig serum in addition to the 8213 goat serum. The Table shows that the 2 viruses are distinct since the one way cross reaction was of such a low level (less than 20-fold of the homologous titer) and since no relationship was detectable with the 8213 guinea pig serum.

Antisera for 84 of the 85 previously described rhinoviruses were available for neutralization tests. These 84 antisera which were used in the neutralization tests at dilutions of 1 in 10 or 1 in 20 (which in most cases contained at least 20 antibody units), failed to inhibit 3-100 TCD_{50} of 8213 virus (Table III). Antisera for 82 of these 84 previously described rhinoviruses used at dilutions as described above also failed to inhibit 3-100 TCD_{50} of 1505 virus. A 1:40 dilution of both K779 and 164-A antisera was found to neutralize virus 1505; however, this low level one way cross reaction was found to be less than 20-fold of the homologous titer of both K779 and 164-A antisera. Antisera for only 9 of the 11 viruses presented at the Rhinovirus Workshop were available and were also used at dilutions of 1 in 10 or 1 in 20; all 9 failed to inhibit 3-100 TCD_{50} of 1505 and 8213 viruses (Table III).

Table III also shows those rhinoviruses which appear to be similar, if not identical, as determined by neutralization tests performed in various laboratories.

Discussion. Viruses 1505 and 8213 possess properties characteristic of agents in the rhinovirus subgroup of picornaviruses(5). Reciprocal neutralization tests with enteroviruses, the other large subgroup of picorna-

TABLE IV. Cross Neutralization Tests of Viruses 1660 and 342-H with Virus 1505 and Cross Neutralization Test of Virus 1660 with Virus 8213.

	Reciprocal of neutralizing antibody titer of indicated antiserum to indicated virus					
Virus	1660 Bovine serum	342-H Bovine serum	1505 Guinea pig serum	1505 Goat serum	8213 Guinea pig serum	8213 Goat serum
1660	1280 (32)*	NT†	<20 (32)	20 (32)	<20 (32)	40 (32)
342-H	NT†	≥5120 (32)	<10 (32)	40 (32)	NT†	NT†
1505	<20 (32)	<20 (100)	640 (32–100)	2560 (32–100)	NT†	NT†
8213	<20 (32)	NT†	NT†	NT†	≥5120 (32)	2560 (32)

* Figures in parentheses represent TCD_{50} of each virus used in test. † NT = Not tested.

viruses, were not deemed necessary since all prototypes and all of a sample of field strains of enteroviruses have been found to be acid stable while rhinoviruses are acid labile(23, 31,32).

Neutralization tests were performed with 85 previously described rhinovirus strains and with 11 additional rhinoviruses presented at the Rhinovirus Workshop in Bethesda, Md., as possible candidate rhinoviruses which were still being studied by their respective laboratories. It was found that viruses 1505 and 8213 were distinct from all these rhinoviruses. It was of interest that the 8213 hyperimmune goat antiserum demonstrated a low level of neutralizing activity against 1660 virus and the 1505 hyperimmune goat antiserum possessed a low level of neutralizing activity against both 1660 and 342-H viruses but such activity was not detectable with either 1505 or 8213 hyperimmune guinea pig antiserum. In general, rhinovirus antisera prepared in large animals such as goats and cattle (in order to provide large quantities of sera) have been quite satisfactory in neutralization tests. However, occasional low level neutralizing activity to heterologous rhinoviruses such as that described in this study has been observed with both homotypic goat and bovine antisera, but not with the homotypic guinea pig serum. In most cases where such low level neutralizing activity has been observed by us, the preimmunization serum of the goat did not contain detectable heterotypic antibody. It is possible that goats and cows either have a greater rhinovirus infection experience than guinea pigs and upon immunization with a human rhinovirus react with a broadened antibody response due to shared antigens, or that guinea pigs have a more specific antibody response than cows and goats.

It is apparent that the number of prototype rhinoviruses will be much fewer than those listed in Table III since many of the rhinoviruses described have not been tested by neutralization tests against all the other described rhinoviruses on the list. One can see from Table III that 13 pairs of viruses and 2 groups of 3 viruses are similar, if not identical, reducing the number of rhinoviruses by 17. In our own laboratory 4 other viruses, 100319, 55216, 16413, 19143, were studied as completely as viruses 1505 and 8213, but towards the conclusion of the cross neutralization tests it was found that these viruses were identical to coryzavirus types 24, 26, 34, and 45 respectively and therefore were not presented here as new viruses. When the necessary cross neutralization tests with each rhinovirus are completed it is probable that other viruses will prove to be identical and the number of rhinovirus candidates will further diminish. Elaborate cross testing is in progress in a collaborative program involving rhinovirus laboratories under the auspices of the NIAID Committee for Vaccine Development and the World Health Organization, in order to determine the total number of distinct prototype rhinoviruses and to determine which viruses are identical to others in an attempt to arrive at a suitable system of nomenclature which would facilitate epidemiologic studies. Both 1505 and 8213 viruses have been submitted to this collaborative program as candidate rhinoviruses.

Summary. Two new viruses, 1505 and 8213, were recovered from the human oropharynx in two separate epidemiological studies. The viruses were found to possess

characteristics of agents in the rhinovirus subgroup of picornaviruses: small size (15-30 mμ), RNA core, ether stability, inactivation at pH 3.0. Both 1505 and 8213 viruses were tested by neutralization tests against 85 previously described rhinovirus serotypes and 11 possible candidate rhinoviruses, and both were found to be distinct antigenically from all ninety-six.

ADDENDUM: Following the completion of this study, the two rhinovirus strains were assigned serotype numbers by the participants in the WHO-NIAID Rhinovirus Collaborative Nomenclature Program. Rhinovirus strain 1505 was designated rhinovirus 48 and rhinovirus strain 8213 was designated rhinovirus 49.

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Comparison of Parenterally and Orally Administered Diethylstilbestrol In Production of Aortic Ruptures in Turkeys.* (31351)

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Aortic ruptures have been produced in turkeys by diethylstilbestrol (DES) injections. Fatal dissecting aneurysms occurred

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