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A New Virus Isolated from the Human Respiratory Tract.* (30734)

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In the winter of 1962, five agents were isolated in secondary human kidney tissue cultures which presented a cytopathic effect (CPE) quite distinct from that customarily produced by known viruses associated with respiratory illnesses. This report presents the evidence for considering these agents as strains of a new respiratory virus, possibly associated with mild upper respiratory illnesses of man.

Materials and methods. The sampling procedure employed in the study of respiratory illnesses among medical students enrolled in the program at the University of Chicago as well as methods for virus isolation in tissue cultures have been described(1,2).

Serologic methods. Antiserum to the prototype strain of the new virus was prepared in guinea pigs following the procedure used for rhinovirus antisera(2). Neutralization tests were carried out by the same method employed for rhinoviruses. The method for complement fixation (CF) tests with respiratory syncytial virus was used(3). CF antigen was prepared from WI-38 cells inoculated with high multiplicity of virus and harvested at the time of maximum CPE. The crude pool was frozen and thawed 3 times and centrifuged to remove the cells and debris.

Results. Isolation and growth in tissue culture. This virus was recovered from 5 specimens, 4 obtained from individuals with minor upper respiratory illnesses and one from a well individual during the winter of 1962 (Table I). All of these specimens yielded virus only after a second blind passage in human kidney cells. No virus was recovered in the secondary monkey kidney cultures or H.Ep. 2 cell cultures inoculated with these specimens. These viruses produced CPE in human diploid cell strains and these cultures, HEL(1) or WI-38(4), were used for all further experiments. The CPE in HEL or WI-38 was slow, with first changes noted after 6 days' incubation at 33°C on roller drums. The cell monolayer became "stringy" in appearance but this developed generally rather than focally. Inclusion bodies were not found in cells stained by H and E. Many small vacuoles in the cytoplasm of cells were the first changes noted in stained cells.

The virus isolated from student specimen 229E was chosen as the prototype strain and purified by 3 serial selections at limiting dilutions in WI-38 cells. Guinea pig antiserum prepared with purified virus had a homologous titer of 1:1200 in neutralization tests with 20 $TCID_{50}$ of 229E virus and also neutralized the other 4 strains shown in Table I.

Attempts to reisolate these viruses from the frozen original specimens were successful in 3 out of the 5 cases. The reisolations were accomplished by the use of the WD human diploid cell strain, obtained from Dr. L. Hayflick of the Wistar Institute. None of the WI-38 cultures showed any evidence of CPE even though as many as 4 blind passages were carried out. Obvious CPE developed in WD cultures by the second passage.

Characterization of the new virus, 229E. Hemagglutination tests. High titered pools of 229E grown in WI-38 cell cultures were inoculated into secondary monkey kidney cultures and H.Ep. 2 cell cultures and 2 blind passages carried out. There was no evidence of CPE in either cell culture nor was there hemadsorption of guinea pig red blood cells on the monkey kidney cultures. WI-38 cell cultures infected with this virus also did not hemadsorb guinea pig cells. High titer pools of 229E virus were tested for hemagglutinin with both guinea pig and chicken red blood cells at 4°C room temperature and 37°C. No hemagglutinin was demonstrated. Plaques were produced by 229E virus on WI-38 cultures under a methyl cellulose overlay after 7 days' incubation at 33°C.

Ether sensitivity. Two strains of the new

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TABLE I. Isolations of a New Respiratory Virus.

Specimen No.	Original isolation: Date of specimen	Specimen type	TC* used	Pass +	Time to CPE, days	Reisolation: Date of reisolation attempt	Result	TC* used
229E	1/19/62	Acute URI	HK L42	2nd	7	6/12/62	neg	HK, HEL
241G	3/ 6/62	"	HK L43	"	5	12/16/64	+	WI-38, WD
243D	2/28/62	"	HK L42	"	7	12/16/64	+	WI-38, WD
276D	2/27/62	Well	HK L42	"	12†	12/16/64	+	WI-38, WD
299G	2/ 1/62	Acute URI	HK L42	"	7	7/29/64	neg	WI-38

* HK = human kidney, L refers to lot numbers; HEL, WD and WI-38 are human diploid cells.
† One tube.

respiratory virus (229E and 299G) have been tested and shown to be inactivated by treatment with 20% ether at 4°C overnight.

Nucleic acid determination. The 229E virus has been tested with both 5-fluorodeoxyuridine (FUDR) and 5-iododeoxyuridine (IUDR) (Table II). Neither of these compounds inhibited the multiplication of 229E in WI-38 cultures. Another strain, 299G, was also tested and found not to be inhibited by IUDR. These results indicate that the nucleic acid of these viruses is RNA. Controls for these experiments included vaccinia virus which was inhibited by both these compounds and poliovirus type 1 which was not inhibited by either compound. The inhibition of vaccinia virus by IUDR could be reversed by addition of thymidine.

Stability. Preliminary tests indicated that 229E is a relatively stable virus. There was no loss in titer after storage for 10 days at 4°C nor after 2 hours at 37°C. Infectivity was destroyed after incubation at 56°C for 10 minutes.

Size. The size of 229E was determined by filtration through gradocol membranes. It passed through a 170 mμ membrane but not through a 110 mμ. Employing Black's factor (5), the size of the virus is 89 mμ.

Egg inoculation. A pool of 229E having a titer of $10^{-3.2}$ was inoculated intra-amniotically, 0.1 ml, to each of eight 10-day-old chick embryos. After 5 days' incubation at 37°C the amniotic and allantoic fluids from these embryos were tested individually for hemagglutinin with chicken red blood cells at room temperature and at 4°C. All were negative. There were no deaths among the embryos inoculated and no gross lesions in embryos or membranes at time of harvest.

Cultures for Mycoplasma. Cell cultures employed in our laboratories for isolation of viruses frequently contain Mycoplasma. After treatment of WI-38 cultures with 50 μg/ml of aureomycin, no Mycoplasma could be detected by culture either anaerobically or aerobically on PPLO agar plates, nor were they isolated from pools of 229E virus grown in such WI-38 cultures.

Serologic tests. The 229E virus was tested for neutralization by antisera for some of the known myxoviruses shown in Table III. None of the antisera neutralized 229E virus. Recently 229E CF antigen prepared in our laboratories was tested by Dr. Robert Chanock of NIAID with the antisera prepared in his laboratory to the known myxoviruses. These were ferret antisera for respiratory syncytial

TABLE II. Determination of Nucleic Acid of a New Respiratory Virus, Strains 229E and 299G.

Virus	Titer ($TCID_{50}$): Control	FUDR 7.6×10^{-5} M	IUDR $10^{-4.3}$ M	IUDR $10^{-4.3}$ + 10^{-3} M thymidine
229E	3.5	3.5		
Vaccinia	3.5	1.0		
Polio 1	6.5	6.5		
229E			4.5	5.2
299G			4.2	4.5
Vaccinia	3.7		<1.0	3.7
Polio 1			5.5	6.5

TABLE III. Neutralization Test with 229E Virus and Known Myxovirus Antisera.

Antiserum	Animal species	Homologous titer		Dilution used in 229E test	Result
		Test used	Titer		
Respiratory syncytial Strain 1996	Ferret	Neut	256	1/16	Negative
Measles*	Monkey	CF	256	1/16	"
Mumps*	Guinea pig	CF	256	1/16	"
Parainfluenza 1	Rabbit	HI	512	1/16	"
" 2	"	HI	256	1/16	"
SV-5*	"	HI	640	1/16	"
Parainfluenza 3	"	HI	256	1/8	"
" 4	"	HAd H	64	1/16	"
Influenza C	Rooster	HI	512	1/16	"

* Purchased from Microbiological Associates, Inc.
† Hemadsorption-inhibition test.

virus and measles; guinea pig antisera for parainfluenza 1 (HA-2 and Sendai), parainfluenza 2 (CA and SV5), parainfluenza 3 (HA-1 and SF-4), parainfluenza 4, mumps, influenza A_2, influenza B, influenza C, and NDV. Homologous titers ranged from 1:40 to 1:1280. There were no cross reactions by complement fixation test with these sera and 229E antigen.

Antibody in human sera. The results of neutralization tests and complement fixation tests on the sera of students from whom 229E virus was isolated are summarized in Table IV. A 4-fold or greater rise in neutralizing antibody was detected in sera from all of the students from whom the virus had been isolated, and 4 out of 5 showed a rise by CF test. However, CF titers with one exception (243E,F) were low.

TABLE IV. Neutralization and Complement Fixing Antibody in Sera of Students from Whom 229E Was Isolated.

Student No.	Time serum drawn after onset of illness, days	Antibody titers	
		Neutralization test	CF test
229D	Pre	2	<4
229F	20	16	8
229G	38	16	4
229H	71	32	8
241F	Pre	4	<4
241H	37	32	<4
243D	Pre	8	<4
243E	29	>32	64
243F	46	>32	32
276D	Pre	4	<4
276E	42*	>32	8
276F	87	>32	4
299G	Pre	16	<4
299H	29	>32	8
299K	69	>32	4

* Not ill, time after isolation of virus.

Discussion. The ether sensitive RNA virus described above was isolated during the second year of a 5-year study of URIs among medical students at the University of Chicago. No further isolations were made in the succeeding years. However, human kidney cultures were replaced by WI-38 cultures for virus isolation the fourth and fifth years of the study. Although this virus could be adapted to grow in WI-38 cells, limited experience with reisolation indicated that WI-38 cultures might not be optimal for isolation. The WD diploid cell was apparently more sensitive. Unfortunately, this cell strain has not been recovered from frozen storage at the Wistar Institute.

Demonstration of an antibody rise to 229E virus by 2 testing methods in sera of students from whom the virus was isolated provides evidence for the human origin of this virus. Four of the five isolations were made from specimens from URIs suggesting that this virus may be etiologically associated with these illnesses. Further serologic testing is in progress to determine the frequency of infection with this agent.

Summary. A new ether sensitive RNA virus was isolated during surveillance of URI among medical students in the winter of 1962. This virus is antigenically unrelated to all known human myxoviruses.

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Non-Specific Hemadsorption by Rhesus Monkey Kidney Cells. (30735)

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The value of primary rhesus monkey kidney tissue culture for the isolation of myxoviruses of human origin has been well established. The hemadsorption and the hemadsorption-inhibition tests, with their many modifications, have been accepted as indispensable tools for myxovirus detection and identification(1,2). However, the problems associated with adventitious simian agents, and the suspected frequency of occurrence of these agents, have caused the usefulness of both the tissue system and the hemadsorption technique to be seriously questioned.

Many laboratories have reported that a lot of monkey kidney tissue completely free of at least some hemadsorption was rare. This has also been our finding. Of particular concern is the SV_5 virus of Hull(3) which has all the characteristics of a myxovirus: it hemadsorbs, hemagglutinates, elutes from red blood cells (RBC's) by enzymatic action, and demonstrates minimal cytopathic effect in early passage. Prior experience with SV_5 contamination, and the assumption by many laboratory workers that SV_5 may behave similarly to SV_{40}(4) or SV_{13}(3,5), have resulted in the general impression that SV_5 contamination is almost universal. Any adsorption of RBC's to uninoculated tissue has been thought to indicate the presence of SV_5 or some other myxovirus that either multiplies very slowly or is only prevented from exploding into a full blown infection by the use of specific antiserum in the tissue culture medium.

It has been our contention that hemadsorption resulting from the presence of adventitious myxoviruses may be easily recognized and identified. However, we have been unable to find in the literature any detailed explanation of the nature of hemadsorption unassociated with virus infection. This report, based on the use of rhesus monkey kidney tissue for a controlled period of over 2½ years, attempts to define the phenomenon more clearly.

Materials and methods. Monkey kidney cultures. Immature rhesus monkeys were obtained directly from India through a commercial import agency. Monkeys were received at the Communicable Disease Center approximately every 3 months in lots of 100 and held for a quarantine period of 3 to 4 weeks. No attempts were made to keep individual monkeys isolated.

Kidneys for tissue culture were obtained from a single monkey and blood was collected at the time of sacrifice for later serologic studies. Kidney cells were prepared by the Tissue Culture Unit at the Communicable Disease Center and grown in screw-capped tubes in Melnick's medium of 5% calf serum and 95% Hanks' solution containing 0.5% lactalbumin hydrolysate. Penicillin and streptomycin were added to this and all following