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Source: *The Journal of Infectious Diseases*, Vol. 119, No. 3 (Mar., 1969), pp. 282-290
Published by: Oxford University Press
Stable URL: http://www.jstor.org/stable/30102315
Accessed: 12-09-2016 04:12 UTC

Isolation from Man of "Avian Infectious Bronchitis Virus-like" Viruses (Coronaviruses*) similar to 229E Virus, with Some Epidemiological Observations

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With standard tissue culture techniques it has been possible to determine the etiologic agent of only 20%–35% of upper respiratory illnesses in adults [1–4]. However, in 1965 Tyrrell and Bynoe reported successful attempts to increase the efficiency of virus recovery from patients with upper respiratory tract illness using human embryonic tracheal and nasal organ cultures. They found that organ cultures were more sensitive than conventional tissue cultures for isolation of some rhinoviruses; in addition, they isolated an ether-labile virus, strain B814, which could be grown only in organ culture and which produced common cold-like illnesses in volunteers [5]. In 1966, Hamre and Procknow described the recovery of a new virus, using standard tissue culture techniques, from 5 students, 4 of whom had an upper respiratory tract illness [6]. The prototype strain, 229E, was ether-labile, possessed an RNA core, measured approximately 89 mμ, and yet was apparently unrelated to any of the myxoviruses of man. In 1967, we described the isolation of 6 new ether-labile viruses (hereafter referred to as National Institutes of Health organ culture [NIH O.C.] viruses) from patients with upper respiratory illnesses; these agents could be isolated only in human embryonic tracheal organ cultures [7]. The B814 virus, the 229E virus, and the NIH O.C. viruses were all shown to possess a similar morphology which resembled that of the avian infectious bronchitis virus (IBV) but was distinct from that of the myxo- or paramyxoviruses [7–9].[1] Subsequently, it was found that mouse hepatitis virus (MHV) shared the common morphologic features of these viruses [10–13].

As a group, the "IBV-like" viruses of man are fastidious in their host-cell requirements: neither the B814 nor the 6 NIH O.C. viruses could be adapted to grow in a monolayer tissue culture system; 229E virus was isolated in such tissue culture, but only with difficulty. Virus 229E was isolated originally after a second blind passage in human embryonic kidney (HEK) cells; attempts to isolate this virus in human diploid cell strain (HDCS) WI38 cultures after 4 blind passages were unsuccessful although it was adapted subsequently to grow in these cells [6]. Virus 229E was capable of producing respiratory illness in volunteers after 1 passage in human embryonic tracheal organ cultures [11].

In an attempt to study the incidence of 229E virus infection in a population of civilian adults, complement fixation (CF) tests were performed on paired sera obtained during a previously reported cross-sectional study of upper respiratory illness which spanned 2 years (1962–1964) [2]. Five of 256 patients developed serologic (CF) evidence of 229E virus infection. At the same time, various experimental cell cultures were

Received for publication October 21, 1968.

The authors are indebted to Mrs. Erminie B. Compton and Miss Carol L. Voss for assistance in compilation of data.

* Subsequent to the submission of this manuscript for publication the term "coronavirus" was proposed to include the avian infectious bronchitis virus (IBV) group, the mouse heaptitis virus (MHV) group, and the human "avian IBV-like" virus group (Coronaviruses, Nature 220:650, 1968).

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[1] D. A. J., Tyrrell, Presentation at Pan American Health Organization, First International Conference on Vaccines against Viral and Rickettsial Diseases of Man (November, 1966).

under evaluation for their sensitivity to rhinoviruses and strain 229E. We therefore took advantage of this opportunity by attempting to isolate virus strains resembling 229E from the original specimens of these 5 patients in such cell cultures. In addition, specimens were obtained from patients with upper respiratory tract illnesses who participated in a more recent (1965–1967) cross-sectional study.[2] With the use of semi-continuous human embryonic intestine (HEI) cell cultures, a virus similar to 229E virus was isolated from 3 of the 5 patients with serologic evidence of infection and from 6 patients in the more recent study whose serologic response to 229E virus was not known at the time of the attempts at isolation. The recovery of these 9 virus strains represents the only reported isolation of viruses similar to 229E virus from natural infections since the original description of this agent. A description of these virus strains and certain observations concerning their epidemiology form the basis of this report.

Materials and Methods

Human embryonic intestine (HEI) tissue culture. HEI tissue culture tubes (MA 177) were purchased from Microbiological Associates. The intestine was obtained from a $3^1/_2$-lb stillborn male infant with an estimated gestational age of 34 weeks. This fibroblast cell strain could not be grown consistently beyond the twentieth passage; passages 13–17 were used for isolation of the 229E related viruses. Growth medium consisted of Eagle's minimum essential medium (MEM) in Earle's balanced salt solution (BSS), supplemented with 0.1 mmole each of 7 "nonessential" amino acids, 1 mmole sodium pyruvate, 10% inactivated (56 C for 30 min) fetal calf serum, 100 units of penicillin, and 100 μg of streptomycin per milliliter; maintenance medium consisted of equal parts of Eagle's MEM in Earle's BSS and Medium 199 in Hanks' BSS supplemented with 2% inactivated calf serum and antibiotics as above [14].

Source of specimens and virus isolation procedures. Specimens were obtained on or before the fourth day of illness from employees of the NIH who had acute upper respiratory tract illnesses. In the 1962–1964 study, 0.2 ml of freshly collected nasopharyngeal wash fluid (veal infusion broth with 0.5% bovine albumin) was inoculated into 2 roller tubes each of Hep-2, rhesus monkey kidney (MK), and HDCS WI26 or WI38 [2]. In the 1965–1967 study of NIH employees, 0.85% NaCl was used as nasal wash fluid, and this fluid was immediately diluted approximately $^1/_2$ in veal infusion broth with 0.5% bovine albumin. These specimens were inoculated within 1 hr of collection into 2 roller tubes each of Hep-2, MK, WI26 and/or WI38, human aorta (AT-39), and HEK cultures [15]. The remaining nasal fluid was stored at −60 C. Tissue cultures were obtained from commercial sources, maintained as previously described, incubated at 33–34 C on drums rotating at 12 revolutions per hour, and observed for cytopathic effect (CPE) twice weekly [16]. MK cultures were tested for hemadsorption at 5–7-day intervals, and a single blind subpassage of the HEK culture harvests was made at 21 days. Acute phase sera were obtained at the time the washings were collected and convalescent sera about 3 weeks later. Five specimens from the 1962–1964 study and all specimens from the more recent study (1965–1967) were also inoculated as above into HEI tissue culture; although some specimens were inoculated into HEI cultures within 1 hr of collection, most had been stored at −60 C prior to inoculation.

Organ cultures. Human embryonic tracheal organ cultures were prepared and maintained by a modification of the method of Hoorn and Tyrrell as described previously [7, 17, 18].

Electron microscopy. One to 5 ml of tissue culture or O.C. fluid was clarified by low-speed centrifugation at 2,000 rpm for 10–15 min in the PR-2 International centrifuge, and then centrifuged at 111,000 × *g* for 60 or 90 min in the SW-39 rotor of a Spinco model L ultracentrifuge. Pellets were resuspended in 0.1–0.2 ml of 1% ammonium acetate, negatively stained with 2% phosphotungstic acid (PTA) at *p*H 5.0 or 7.0, and spread on formvar-coated copper grids [7]. All electron micrographs were taken with a Siemens Elmiskop 1A at magnifications of × 40,000–80,000.

Viruses and sera. Dr. Dorothy Hamre kindly supplied 229E virus and guinea pig antiserum. The virus had been purified by the terminal dilution technique in WI38 cultures.

Chloroform sensitivity, 5-iodo-2-deoxyuridine

[2] A. Z. Kapikian, H. D. James, Jr., S. J. Kelly, K. McIntosh, and R. M. Chanock, "Etiology of upper respiratory tract illnesses among civilian adults" (in preparation).

sensitivity, and acid lability tests. These tests were performed as previously described [16, 19–22]. The 229E-related viruses isolated in HEI cells were adapted to HDCS WI38, and tests were performed in tube cultures of HDCS WI38.

Infectivity titrations. HEI or WI38 cultures were used in infectivity titrations. Tenfold dilutions of virus were made in Hanks' BSS containing 0.5% gelatin, 100 units of penicillin per milliliter, and 100 μg of streptomycin per milliliter. Two-tenths milliliter of the appropriate virus dilution was inoculated into each of 2–4 tube cultures; the cultures were examined for the appearance of CPE 3 times weekly for approximately 2 weeks. Infectivity titers were based on CPE and calculated by the method of Reed and Muench [23].

Inoculation of suckling mice. Swiss mice of the CD-1 strain were obtained from Charles River Mouse Farms, Incorporated, Wilmington, Massachusetts. Retired breeders from this mouse colony were tested and found to be free of CF antibody to MHV, strain A59 [24]. One litter each of suckling mice, 0–3 days old, was inoculated intracerebrally with 0.01 ml of tissue culture harvests of viruses 489, 511, 515, and 844 and observed for 21 days. If a mouse developed illness, it was sacrificed and brain suspension was passaged to additional suckling mice intracerebrally and intraperitoneally; in addition, such suspensions were inoculated into HDCS WI38 cultures.

Complement fixation (CF) tests. 229E virus was inoculated into 32-oz bottles of WI38 cultures and allowed to adsorb for 1 hr. Fluids harvested after 2 freeze-thaw cycles, about 48 or 72 hr after inoculation, were employed as CF antigen. CF tests with appropriate controls were performed as previously described [7]. CF tests involving large numbers of paired sera could not for practical reasons be completed in a single day of testing. Therefore, such CF results reported in this study were from tests performed several days in succession.

Neutralization tests. Equal volumes of virus and fourfold dilutions of inactivated serum (56 C for 30 min) were incubated at room temperature for 2 hr. Two-tenths milliliter of the mixture was inoculated into each of 2 WI38 cultures. These were examined for CPE at a time when a simultaneous titration indicated that approximately 32–320 TCD_{50} of virus were present. The cultures were examined at 2–3-day intervals for approximately 2 weeks, since CPE with the 229E-related viruses did not appear before the fifth or sixth day. Serum neutralizing end points were calculated according to the method of Reed and Muench and were expressed as initial serum dilutions [23].

Results

Isolation and growth in tissue culture. The 3 nasal wash specimens which yielded 229E-related viruses from the 1962–1964 study were obtained within a 7-week period, March 2 to April 13, 1964, while the 6 virus-positive nasal wash specimens from the 1965–1967 study were obtained during the first 3 months of 1967 (table 1). The specimens were inoculated into roller tube cultures as stated in the Methods section. None of the inoculations into rhesus MK, Hep-2, HDCS WI26 or WI38, AT-39, or HEK cultures gave CPE. However, all 9 specimens produced CPE on initial passage in HEI tissue culture. The CPE was characterized by a gradual elongation of the cells throughout the monolayer beginning on the fifth or sixth day after inoculation; specific foci were not evident. Gradually, small granular round cells appeared throughout the monolayer. The cell sheet was rarely destroyed completely. At times uninoculated HEI tissue cultures appeared somewhat "stringy," and for this reason recognition of virus-specific CPE was occasionally difficult, especially at the beginning of the study.

Table 1. Source of "IBV-like" viruses recovered in human embryonic intestine cultures from nasal washings taken from patients with upper respiratory illnesses

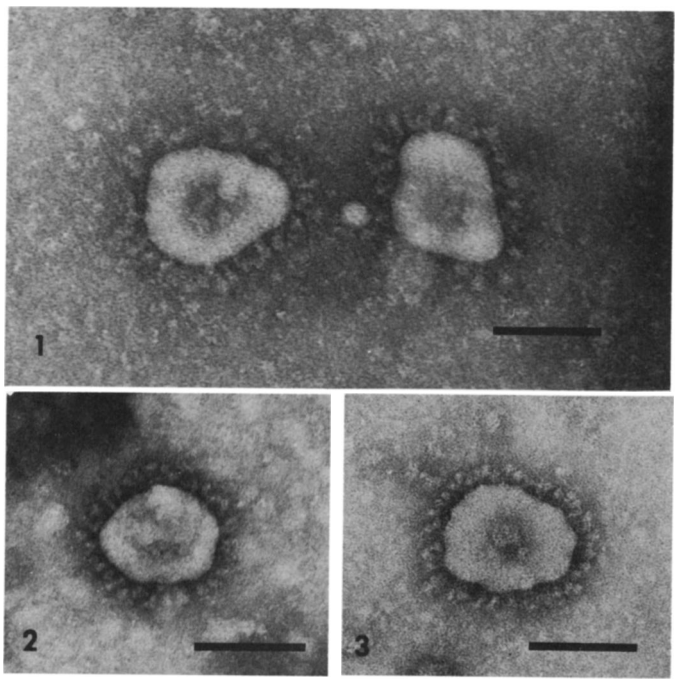
Patient number	Age	Sex	Date of specimen
489	21	female	3/2/64
511	33	female	4/9/64
515	24	female	4/13/64
840	39	male	1/23/67*
844	41	male	1/30/67
862	31	male	3/3/67
865	54	female	3/7/67
868	36	female	3/9/67
879	21	female	3/16/67

*CPE was visualized in HEI_1, but specimen was not available for further testing. Therefore WI_1, which did not show CPE, was passaged to HEI, which demonstrated typical CPE.

Electron microscopic and filtration studies. HEI culture harvests were clarified and concentrated by ultracentrifugation and examined by electron microscopy. Figure 1 shows representative particles seen in the harvests of isolate 489. Morphologically similar particles were seen in harvests of each of the other 8 isolates. A comparison of the isolates with strain 229E (figure 2), and avian IBV (figure 3), revealed their remarkable similarity. The particles usually appeared round or elliptical, but many were somewhat pleomorphic. A characteristic feature of the particles was the widely spaced club- or pear-shaped surface projections, which were narrow at the base, about 10 mμ wide at the outer edge, and approximately 20 mμ in length. The mean of the largest diameter (including projections) of 67 samples of the 9 isolates was 152 mμ ± 6 mμ (i.e., ±2 × standard error [SE] of mean) with a range of 104–250 mμ; the mean of the shortest diameter of these 67 samples was 107 mμ ± 4 mμ (i.e., ±2 × SE of mean) with a range of 76–160 mμ. Details of the morphology of this group of viruses have been described elsewhere [7–10, 25]. In filtration studies, in which Swinnex-25 filter units attached to 20-ml vacuum tubes were employed, virus strain 844 passed through a 450-mμ, 220-mμ, and 100-mμ but not a 50-mμ or 10-mμ Millipore filter [26, 27].

Attempts to grow agents in organ culture (O.C.). Attempts were made to cultivate these agents in human embryonic tracheal O.C. and to detect them by electron microscopy using methods described previously [7]. Three passages of the original specimens obtained from 5 of the patients with serologic evidence of 229E virus infection (in the 1962–1964 study) were made in human embryonic tracheal O.C. Virus particles could not be visualized in the third passage O.C. harvests by electron microscopy. HEI-positive harvests of viruses 489, 511, 515, and 844 were also passaged 3 times in O.C., and again virus particles could not be visualized by electron microscopy. Ciliary action of the organ cultures was unaffected by these 4 isolates during the 3 passages. Titrations in HEI or WI38 tissue cultures of harvests of first, second, and third O.C. passages revealed that growth in O.C. of low-titered inocula of strains 489, 511, and 515 could not be detected, while a higher-titered inoculum of strain 844 appeared to initiate replication at a low level (table 2).

Determination of nucleic acid type. Viruses 489, 511, 515, and 844 were tested for nucleic



Figures 1–3. Figure 1: two particles found in tissue culture harvests of isolate 489, negatively stained with PTA. The bar in all 3 figures represents 100 mμ. ×144,000. Figure 2: 229E virus, negatively stained with PTA. ×144,000. Figure 3: infectious bronchitis virus, Beaudette 42 strain, negatively staind with PTA. ×144,000.

Table 2. Attempts to grow 229E-related viruses in organ culture

Strain	Passage history of material inoculated into O.C.	Titer* in TCD_{50}/0.2 ml (log_{10})							Visualization of particles by electron microscopy of	
		Inoculum	Pooled harvests 1st passage in O.C.†	Pooled harvests 2d passage in O.C.†	Harvests from indicated day of 3d passage in O.C. (days)				Pooled 3d passage O.C. harvests	Tissue culture harvests (passage level) (titer* in TCD_{50}/0.2 ml [log_{10}])
					4	7	12	2–12		
489	HEI_3	2.5	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	No	Yes (HEI_2) (3.5)
511	HEI_3	3.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	No	Yes (HEI_2) (4.0)
515	HEI_2	2.5	1.5	<1.0	<1.0‡	<1.0	<1.0	<1.0	No	Yes (HEI_1) (2.0)
844	HEI_3WI38_1	5.0	2.0	3.0	N.T.	N.T.	N.T.	0.5	No	Yes (HEI_3WI38_1§) (5.0)

* Titrations of viruses 489, 511, and 515 were performed in HEI tube cultures, whereas titrations of virus 844 were performed in HDCS, WI38 tube cultures.

† Pools of fluids harvested at 2–3 day intervals for 12 or 13 days after inoculation.

‡ Day 5.

§ The same passage as that inoculated into O.C.

Table 3. Effect of 5-iodo-2-deoxyuridine (5-IUDR) and 5-IUDR plus thymidine on virus multiplication

Virus	Infectivity titer in indicated medium*		
	Maintenance medium (MM)†	MM + $10^{-4.3}$ M 5-IUDR	MM + $10^{-4.3}$ M 5-IUDR & $10^{-4.0}$ thymidine
489	3.5	3.5	4.0
511	4.0	4.0	4.0
515	4.0	3.5	3.5
844	3.5	4.0	4.0
Polio virus type 1 (LSC-1)	≥6.5	≥6.5	≥6.5
Vaccinia	4.5	<0.8	4.5

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

† MM as described in Materials and Methods.

Table 4. Stability of 229E-related viruses

Virus	Chloroform sensitivity test Infectivity titer (TCD_{50}/0.2 ml [log_{10}])		Acid lability test Infectivity titer (TCD_{50}/0.2 ml [log_{10}])	
	Treated with chloroform	Untreated	*p*H 2.7*	*p*H 7.0*
489	<0.8	2.3	<0.8	3.0
511	<0.8	4.8	<0.8	4.5
515	<1.0	5.0	<0.8	4.5
840	<1.0	≥3.5	<1.0	3.0
844	<0.8	2.3	<0.8	4.5
862	<1.0	≥3.5	<1.0	2.5
865	<1.0	≥3.5	<1.0	2.5
868	<1.0	2.5	<1.0	2.5
879	<0.8	3.0	<1.0	3.0
Rhinovirus 1A	≥3.5	≥3.5	≥1.5	≥3.5
Herpes simplex	<1.0	3.5	N.T.	N.T.
Polio virus type 1 (LSC-1)	N.T.	N.T.	≥5.5	≥6.0

* 3 hr at 25 C.

acid type. Multiplication of these 4 viruses was not inhibited by $10^{-4.3}$ M 5-iodo-2-deoxyuridine (5-IUDR), a concentration inhibitory for DNA viruses. As seen in table 3, 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 10^{-4} M thymidine was added to the maintenance medium containing 5-IUDR. These findings suggest that the nucleic acid core of these 4 viruses was RNA.

Chloroform sensitivity. Table 4 shows that all 9 virus strains were inactivated by chloroform. The table also shows that a control virus, rhinovirus 1A (a known chloroform-resistant virus) was resistant to inactivation by chloroform, while another control virus, herpes simplex (a known chloroform-sensitive virus) was inactivated by chloroform.

Acid lability. Each of the 9 virus isolates exhibited at least a 100-fold reduction in infectivity titer after exposure to *p*H 2.7 for 3 hr at 25 C (table 4). The table also shows that rhinovirus 1A (a known acid-labile virus) demonstrated at least a 100-fold reduction in infec-

tivity titer, while the infectivity of poliovirus type 1 (a known acid-resistant virus) was unaffected after exposure to *p*H 2.7 for 3 hr.

Pathogenicity in mice. Virus strains 489, 511, 515, and 844 were inoculated into suckling mice as described in Materials and Methods. No illness was observed in mice inoculated with strains 489, 515, and 844; 4 of 8 mice inoculated with virus 511 died on the fourth day after inoculation. However, brain suspensions passaged intracerebrally and intraperitoneally to additional suckling mice failed to produce illness; in addition, such suspensions inoculated into WI38 cultures failed to produce CPE.

Serologic studies. 10–32 TCD_{50} of each of the 9 isolates were neutralized by a 1:160–1:1280 dilution of 229E guinea pig antiserum which had a homologous titer of 1:320–1:1280 against 32–100 TCD_{50}. This would indicate that each of the 9 isolates was similar, if not identical, to 229E virus.

Table 5 shows that 8 of the 9 patients from whom the 229E-related viruses were isolated had a significant CF antibody response to 229E virus. In neutralization tests, 3 of the 9 virus-positive patients exhibited significant increases in antibody to both strain 229E and their own isolate; in addition, 2 patients had such rises in antibody to strain 229E alone, while 2 others had such rises only to the strain which they shed.

Efficiency of techniques for detection of virus infection. The CF test was a more sensitive index of 229E infection than virus isolation. Table 6 shows that, during the period of 229E prevalence in 1966–1967, 22 of 89 individuals exhibited evidence of infection; 21 of the 22 developed a fourfold or greater CF antibody rise, whereas only 9 of the 22 shed virus detectable in HEI cultures.

Clinical findings. Each of the 9 virus-positive patients was cultured either on the day of onset (1 of 9) or the day after onset of symptoms. Their average age was 33 years, with a range of 21–54 years. The most common symptoms recorded were coryza (in all 9), nasal congestion (in 8), sneezing (in 7), and sore throat (in 5). Less common were headache (in 4), cough (in 3), muscle or general aches (in 3), and chills and feverishness (in 2). The chief complaint in 8 of the 9 patients was coryza or nasal congestion; 1 patient's chief complaint was sneezing. None of the patients had an abnormal temperature elevation on the day of examination.

Epidemiologic observations. Sera obtained from pediatric patients with acute respiratory illnesses from several populations were tested by the CF technique for evidence of 229E infection. Only 1 of 892 paired sera obtained from October, 1962, through August, 1965, from infants and children admitted to Children's Hospital, Washington, D.C., for predominantly lower respiratory tract illness, exhibited serologic evidence of 229E infection. In a similar study of 222 infants and young children admitted to Children's Hospital from December, 1966, through April, 1967, none of these patients developed an antibody rise for 229E virus. In addi-

Table 5. Serologic response of individuals from whom 229E-related viruses were recovered

Patient number	Serum tested	Reciprocal of neutralizing antibody titer against 32 TCD_{50} of 229E virus	Reciprocal of neutralizing antibody titer against indicated TCD_{50} of patient's isolate	Reciprocal of CF antibody titer to 229E virus
489	Acute	<4	<4 (32)	<8
	Conv.	16	6 (32)	16
511	Acute	24	<4 (1,000)	2
	Conv.	64	12 (1,000)	16
515	Acute	8	6 (32)	<2
	Conv.	64	24 (32)	8
840	Acute	4	4 (32)	4
	Conv.	32	24 (32)	32
844	Acute	<4	<4 (32)	8
	Conv.	16	24 (32)	≥32
862	Acute	<4	24 (32)	<4
	Conv.	6	48 (32)	16
865	Acute	<4	6 (10)	<4
	Conv.	12	16 (10)	16
868	Acute	24	8 (32)	8
	Conv.	64	24 (32)	16
879	Acute	24	<4 (10)	<4
	Conv.	24	8 (10)	8

Table 6. Evidence of infection with 229E-related viruses during period of prevalence (December, 1966 through April, 1967)

	Number of individuals		Total
	Virus isolated	Virus not isolated	
CF antibody rise	8	13	21
No CF antibody rise	1	67	68
Total	9	80	89

Table 7. Serologic (CF) evidence of 229E virus infection by month and year in civilian adults with upper respiratory illnesses

Months	1962		1963		1964		1965		1966		1967	
	No. tested	No. with ≥4-fold rise	No. tested	No. with ≥4-fold rise	No. tested	No. with ≥4-fold rise	No. tested	No. with ≥4-fold rise	No. tested	No. with ≥4-fold rise	No. tested	No. with ≥4-fold rise
Jan.–Feb.	...*	...	24	0	28	2 (7%)	...	...	38	1†	26	5 (9%)
March–April	...	...	54	0	22‡	3 (14%)	...	...	30	0	46	13 (9%)
May–June	...	...	27	0	1	0	...	...	18	0	8	0
July–Aug.	...	...	24	0	...	...	...	...	19	0	9	0
Sept.–Oct.	4	0	25	0	...	...	40	1	35	0	...	...
Nov.–Dec.	28	0	19	0	...	...	37	0†	21	3 (14%)	...	...

NOTE. For 1962–1964, 5 (2%) of 256 had rises; for 1965–1967, 23 (7%) of 317 had rises.

* Not studied.

† From December, 1965, through February, 1966, 18 (31%) of 59 patients developed ≥4-fold rises to NIH O.C. virus strains OC38 (664) and OC43 (690); 5 NIH O.C. viruses (663, OC38, OC43, 691, 703) were recovered in O.C. from 5 of these 18 patients [7].

‡ NIH O.C. virus 501 recovered in O.C from 1 patient who did not develop 229E CF antibody rise [7].

tion, none of 261 paired sera obtained from June, 1964, through June, 1965, from infants and young children with predominantly lower respiratory tract illnesses studied in Jamaica, Trinidad, Hong Kong, Cairo, Singapore, or New Delhi as part of a World Health Organization collaborative program, demonstrated evidence of 229E infection [28].

In the first period of the cross-sectional study of acute respiratory illness among NIH employees which extended from October, 1962, to May, 1964, infection with strain 229E was not detected for the first 15 months of the investigation (table 7). During the next 4 months, 5 of 50 patients studied developed a CF antibody rise to 229E virus. Over-all, in the first period, 5 (2%) of 256 patients exhibited serologic evidence of 229E virus infection. During the second period, which extended from September, 1965, through August, 1967, 229E virus infection was detected during 2 separate 4- and 5-month intervals. Infection was infrequent during the first interval, from October, 1965, through January, 1966; but during the second interval, from December, 1966 through April, 1967, 21 (24%) of 89 patients developed a rise in CF antibody to strain 229E. Over-all, during the 1965–1967 period, 23 (7%) of 317 patients exhibited serologic evidence of infection. During the interval in which 229E was prevalent, infections with rhinovirus and other cytopathic or hemadsorbing viruses were uncommon. Detailed data will be published in the future. As indicated previously, serologic evidence of infection with 229E virus was not detected among pediatric patients hospitalized with predominantly lower respiratory tract illness in the Washington, D.C., area during the interval when 229E virus was prevalent among adults with upper respiratory tract illness.

During 1965–1967, 152 (48%) of 317 adults studied had detectable CF antibody (1:4 or greater) for 229E virus, while 48 (15%) had serum antibody levels of 1:8 or greater. In contrast, only 4 (2%) of 222 infants and young children studied during the period December, 1966, through April, 1967, possessed serum CF antibody at a titer of 1:4 or greater.

Discussion

Viruses resembling avian IBV and MHV in morphology have recently emerged as possible important etiologic agents of acute upper respiratory illnesses in adults. Only 12 isolations from natural infections have been reported: B814, the first "IBV-like" virus isolated from man could be cultivated only in human embryonic tracheal O.C. [5]; 229E and 4 serologically identical strains were recovered in HEK cultures [6]; and 6 NIH O.C. strains (501, 663, OC38 [664], OC43 [690], 691, and 703) were isolated in human embryonic tracheal O.C. [7]. Two of the 6 NIH O.C. strains (OC38 and OC43) were subsequently adapted to suckling mouse brain, but none of the 6 strains grew in monolayer tissue culture [29]. The 9 strains recovered in this study

were found to be similar, if not identical, to strain 229E by one-way neutralization tests and, in addition, to possess similar morphologic and biophysical properties.

The 9 229E-related strains were recovered on initial passage in semi-continuous HEI tissue cultures; they could not be isolated on initial passage in conventional tissue cultures. In addition, since the technique of O.C. passage followed by electron microscopic examination of pooled, concentrated O.C. harvests had proved to be a sensitive system for the recovery of NIH O.C. viruses, this technique was applied both to several clinical specimens yielding agents resembling strain 229E and to several tissue-culture-adapted strains as well. In all cases, attempts to detect virus by electron microscopy in O.C. harvests failed, although titration of such harvests showed in 1 case that low-grade replication of virus probably occurred. It appears, therefore, that HEI tissue culture is preferable to O.C. for isolation of certain strains of "IBV-like" viruses such as these 229E-related agents. It should be noted, however, that the 6 "IBV-like" strains recovered in O.C. in this laboratory could not be adapted to grow in HEI tissue culture [K. McIntosh, unpublished studies]. Therefore, for isolation of "IBV-like" viruses, it would appear to be necessary to employ both HEI tissue culture and O.C. However, additional ways to facilitate the growth of "IBV-like" viruses such as 229E and others must be found since HEI cultures, although more sensitive than any conventional tissue culture or O.C. system, were much less sensitive than the measurement of CF antibody rises for detection of 229E infection.

The serologic survey of 229E virus infection in various population groups revealed that infection with this agent was rare in infants and children with lower respiratory tract illnesses. In the 1962–1964 study of upper respiratory tract illness among adults, 5 (2%) of 256 individuals had serologic evidence of 229E infection. It was noteworthy that these 5 infections occurred from January through April, 1964, when rhinovirus and other known respiratory virus infections were uncommon but upper respiratory tract illnesses were prevalent; during this period 229E virus infection was associated with 5 (10%) of the 50 illnesses sampled. In the 1965–1967 study of upper respiratory tract illness in adults, 23 (7%) of 317 patients developed serologic evidence of 229E infection. It was striking that 21 of the 23 infections occurred during a 5-month period (December, 1966–April, 1967) when infections with rhinoviruses and other cytopathic or hemadsorbing viruses were uncommon while upper respiratory tract illness remained prevalent. During this 5-month period, 21 (24%) of 89 patients studied developed serologic (CF) evidence of 229E infection. It was previously reported that 5 NIH O.C. viruses were recovered in O.C. from specimens obtained from 9 patients during a 3-month period of the previous year (December, 1965–February, 1966) [7]. In addition, in a serologic survey using as antigen 2 serologically identical NIH O.C. viruses (strains OC38 and OC43) originally recovered in O.C., and subsequently adapted to grow in the brain of suckling mice, 18 (31%) of 59 patients (including the 5 patients above who yielded NIH O.C. viruses) developed serologic evidence of infection with these viruses during this 3-month period (December, 1965–February, 1966) [29]; only 1 of the 59 patients developed a CF antibody rise to 229E virus during this period. It was of interest that, in these CF antibody surveys, patients with serologic evidence of 229E infection rarely developed concurrent CF antibody rises to the OC38 and OC43 mouse brain antigens [30].

These observations suggest that "IBV-like" viruses may be etiologic agents of a portion of respiratory illnesses which occur during the winter season, when the prevalence of rhinovirus and other known respiratory virus infections is often low, but that of upper respiratory tract illnesses quite high [2–4].

The occurrence of a typical common-cold-like illness in the 9 patients from whom 229E-like viruses were isolated is consistent with the clinical findings in volunteer studies with B814 and 229E viruses [5, 11].

Summary

Nine virus strains resembling "IBV-like" virus 229E of Hamre were recovered in human fetal intestine fibroblast cultures from nasopharyngeal washings of adults with acute upper respiratory tract disease. Eight of the 9 virus-positive individuals developed serologic (CF) evidence of 229E virus infection. The virus isolates exhibited properties typical of the "IBV-like" virus group: distinctive morphology, resistance to 5-IUDR, and chloroform sensitivity. Measurement of CF

antibody response was found to be approximately twice as sensitive as virus recovery for detection of infection. Standard monolayer tissue cultures as well as human fetal tracheal O.C. were ineffective for recovery and recognition of the 229E related isolates. A seroepidemiologic survey indicated that 229E virus infection was rare in children with lower respiratory tract disease. However, such infection occurred in 10%–24% of adults with upper respiratory tract illnesses during 2 of 4 winters—a season when rhinovirus infection was uncommon but respiratory disease morbidity was high.

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