Recovery of New Viruses (Coryzavirus) from Cases of Common Cold in Human Adults. (26962)

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Although the common cold continues to be one of the most prevalent of all the illnesses of man, it remains one of the least understood in terms of its etiology. A number of families of viral agents have been shown to be important in causing acute respiratory illnesses of man, especially among children. None of these viruses, however, account for the great mass of acute infections of the upper respiratory tract, especially in adults, which are characterized by a prominent coryza accompanied with mild constitutional symptoms and little or no fever and which are generally classed as the common cold.

What might constitute a breakthrough in the field of the common cold has been reported by Tyrrell *et al.*(1-6). These workers have recorded recovery in primary cell cultures of human fetal kidney and in monkey kidney of a spectrum of viruses which exhibit heterogeneity with respect to cultivability, cytopathogenicity and antigenic composition. In their virus cultivation procedure, Tyrrell *et al.*(1-6) have emphasized the need for maintaining a low pH (0.03% sodium bicarbonate) and low temperature. These agents, collectively called "Salisbury viruses" have been shown to induce cold-like illnesses in human volunteers. Additional viruses were isolated by Hobson *et al.*(7) using similar techniques.

During the past 3 years, our laboratory has been engaged intensively in studies of respiratory diseases of man with special reference to acute respiratory illnesses in children and the common cold in adults. For virus recovery attempts, we chose to employ strains of serially passaged human fibroblastic cells of fetal origin and with diploid karyotype which were recently developed and described by Hayflick *et al.*(8,9) of the Wistar Inst. of Anatomy and Biology.

Through use of such cultures, we have recovered from cases of common cold a group of viruses of small size which are strongly cytopathic for the diploid human fetal lung and kidney cell cultures. These agents are immunologically distinct from the prototype F.E.B. and H.G.P. strains of "Salisbury" viruses and, for purpose of designation, are called coryzavirus because of their association with coryzal disease in man.

The present preliminary report describes the findings to date for 17 coryzaviruses recovered from cases of common cold, and describes the utility of diploid human fetal lung and kidney cells for propagating a variety of viruses. Finally, data are presented which show the need for standard conditions in measuring virus particle size through use of Elford's collodion membranes.

Materials and methods. The patients were employees of Merck & Co. and all but one (M.R.H.) were located in the plant at Rahway, N.J. All were seen by physicians at the plant dispensary. The patients were selected on the basis of having an acute respiratory illness of 4 days or less duration judged on clinical grounds to be of probable viral etiology. Included for control purpose were "normal" individuals who visited the dispensary for some other cause, such as injury. Each patient was examined clinically, was queried concerning recent vaccinations, and the findings were recorded. Two dry cotton swab specimens were collected from the throat. These were extracted immediately into 5 ml of Difco veal infusion broth in a screw cap tube, frozen in dry ice and transmitted to the laboratory where they were thawed, distributed, and stored frozen in dry ice in flame-sealed glass containers until tested up to 1½ years later. A 20 ml sample of blood was taken at the time of the first visit and again 3 to 4 weeks later. The sera were stored frozen at −20°C until tested.

Tissue cultures. Diploid human fetal fibroblast cultures. Strain WI-10 of human fetal

kidney and strain WI-26 of human fetal lung were obtained from Dr. L. Hayflick of the Wistar Inst. of Anatomy and Biology(8,9). The cells were propagated in Eagle's medium in Earle's balanced salt solution(10) containing 10% of unheated calf serum. Conventional trypsinization techniques were employed for subculture. During viral propagation, the cultures were maintained in Eagle's medium with 2% of inactivated horse serum or, where noted, in a serum-free medium. The maintenance medium contained 0.006M tris (hydroxymethyl aminomethane) buffer plus 0.09% added sodium bicarbonate. The initial pH was usually around 7.2 to 7.4. *HeLa cell cultures* were grown in Eagle's medium with 10% of inactivated calf serum. During viral growth, the cells were maintained in Eagle's medium with 2% of inactivated horse serum. *Grivet kidney cell cultures.* Trypsinized suspensions of grivet renal cells (*Cercopithecus aethiops*) were planted in Melnick's(11) lactalbumin-yeast extract medium containing 2% of inactivated calf serum. For maintenance purpose, Eagle's medium containing 2% of inactivated chicken serum was used. Penicillin and streptomycin at concentrations of 100 units and 100 μg per ml, respectively, were included in all culture media.

Virus studies. Virus isolation. The throat specimens were diluted 1:2 in Eagle's medium containing 100 units or 100 μg each of penicillin, streptomycin and mycostatin and were spun for 30 minutes at 2000 rpm in the horizontal centrifuge. Supernate was inoculated in 0.25 ml amount, in triplicate, into diploid fetal lung or kidney, HeLa and grivet renal cell cultures. The diploid fetal cultures were incubated at 33°C on the roller drum and the other cell cultures were incubated on the drum at 36°C. All cultures were observed daily for cytopathic effect (CPE) for 10 to 14 days. A single blind passage was made of all cultures which failed to show CPE. Tubes which exhibited CPE were harvested and the contents were stored frozen at −20°C. *Infectivity titrations of Coryzaviruses.* Serial 10-fold dilutions of coryzaviruses recovered in diploid lung or kidney cell cultures were titrated in 0.2 ml amounts in diploid fetal lung cultures containing 2.0 ml of maintenance medium and were incubated on roller drums at 33°C. The titer was the highest initial dilution of virus which caused a CPE by the eighth day post-inoculation. *Serum neutralization test.* Seed stocks of coryzaviruses were prepared in cultures of diploid fetal lung cells. The infected cultures were harvested when CPE was complete (days 3 to 5), were frozen and thawed once, and were clarified by low speed centrifugation. The titers were usually $10^{3.0}$ to $10^{5.0}$ TCD_{50} per 1.0 ml. For neutralization titrations, serial 2-fold dilutions of inactivated serum (56°C, 30 minutes) were incubated for one hour at room temperature with an equal volume of virus diluted to contain 100 TCD_{50} of virus per 0.2 ml. Diploid fetal lung cultures were inoculated in duplicate with 0.2 ml of the serum-virus mixtures and incubated at 33°C on the roller drum. The tests were read on day 3 or 4 of incubation when control cultures showed degeneration of 25 to 50% of the cell sheet. The titer was the highest initial dilution of serum which showed complete suppression of viral CPE.

Biophysical and biochemical procedures. Gradocol membrane filtration. Coryzavirus was propagated in diploid fetal lung cell cultures which were maintained without added serum. The harvested fluid was clarified by low speed centrifugation, 10% of sterile veal infusion broth was added to the supernate, and the mixture was homogenized with 10% of fluorocarbon 113 for 2 minutes at top speed in an Omnimix homogenizer in ice. Final clarification was carried out at 2000 rpm for 10 minutes in the horizontal centrifuge. Aliquots of the material were filtered through Elford's gradocol membranes of various pore size as stated in the text and the filtrates were titrated in quadruplicate in tubes of diploid fetal lung cell cultures. *Sedimentation studies.* Coryzavirus, poliovirus III (Saukett) and adenovirus 2 were spun simultaneously for various time periods at 20°C in the SW-39 head of the model E Spinco ultracentrifuge at an average centrifugal force of 123,600 $\times$ g. Following centrifugation, the top 1.0 ml of supernate was carefully sampled and titrated, at 6 tubes per dilution, in the appropriate cell cultures.

The viruses employed in the experiments were propagated in grivet or in diploid fetal lung cell cultures maintained in medium 199 or in Eagle's medium without added serum. Hence, the density and viscosity of the virus-containing fluids were roughly the same. *Electron micrography.* Diploid fetal lung cells infected with coryzavirus were processed according to Palade(12). The cells were embedded in 80% butyl, 20% methyl methacrylate polymerized at 55°C overnight. Sections were cut with a Porter-Blum ultramicrotome equipped with a diamond knife and were examined with a Philips EM-100 electron microscope. *Nucleic acid determination.* The coryzavirus nucleic acid type was determined according to Salzman(13). By this method, tests are made for inhibition of viral synthesis by FUDR (5-fluorodeoxyuridine). For this purpose, diploid fetal lung cell cultures were prepared with Eagle's medium (without serum) containing, in final concentration, 10^{-6}M FUDR, 10^{-5}M FUDR, or 10^{-6}M FUDR plus 10^{-5}M thymidine. Coryzavirus (unknown), vaccinia (known DNA-containing virus) and encephalomyocarditis virus (known RNA-containing virus) were titrated, in quadruplicate, in cultures maintained with each of the above media or with Eagle's medium alone. Determination of nucleic acid type was based on significant suppression of multiplication of DNA virus by FUDR and reversal of this effect by thymidine.

Results. Recovery of coryzaviruses. Throat specimens from 110 patients with mild acute upper respiratory illness and from 45 control cases were specially selected for virus isolation attempts in diploid fetal kidney or lung, in HeLa and in grivet renal cell cultures. The cases were selected on the basis of negative serodiagnostic complement-fixation or hemagglutination-inhibition test findings for influenza A_1, A_2, B, C, parainfluenza 1, 2, 3, adenovirus (group), reovirus (group) and respiratory syncytial virus.

Seventeen throat specimens (15%) from respiratory disease cases and one specimen from a control case yielded cytopathic agents in diploid fetal human cell cultures. These cytopathic effects were first noted between the third and seventh days of cultivation. Subculture did not increase the number of isolates. HeLa cell cultures and grivet renal cell cultures inoculated at the same time with the same throat specimens failed to reveal any cytopathic change indicative of the presence of a virus. Uninoculated or broth inoculated control cell cultures of all types revealed no cytopathic effect. Viruses recovered in the diploid cell and which could not be propagated in HeLa or grivet renal cell cultures were tentatively designated coryzaviruses.

Clinical findings in patients from whom coryzaviruses were recovered. The patients, at time of examination, revealed a clinical picture typical of the common cold. The clinical findings were coryza with nasal edema and discharge. Pharyngitis, which sometimes was painful, and edema of the pharynx were commonly present. About ⅓ of the patients complained of cough or hoarseness. Cervical nodes were present in a small portion of the cases. None of the patients had clinically significant fever and there were no outstanding changes in the total or differential white blood count.

Serologic findings in the patients. The findings in serum neutralization tests with paired sera from 10 cases from whom coryzaviruses were isolated are presented in Table I. The serologic response was generally poor as measured by the neutralization test procedure employed. Only 4 of the 10 patients showed an antibody response against their homologous virus and, in these instances, the convalescent serum titers were only 1:10 or 1:20. No pre-existing homologous antibody was demonstrated in any of the acute phase sera tested. Additionally, as stated above, none of these patients' sera showed diagnostic findings for influenza, parainfluenza, adenovirus, reovirus or respiratory syncytial virus.

Attempts to demonstrate complement-fixing antibody in patients' sera tested with homologous tissue culture coryzavirus fluids gave uniformly negative results.

Biological properties of the coryzaviruses. Cytopathology. All coryzaviruses recovered to date have shown a similar cytopathic effect and this is indistinguishable in diploid fetal kidney and lung cultures. Typical changes

TABLE I. Serum Neutralization Test Findings in Ten Patients from Whom Coryzaviruses Were Recovered.

Case no.	Day of disease	Titer against homologous virus
68	3	<5
	24	20
MRH	2	<5
	27	20
54	1	<2.5
	22	10
211	1	<5
	21	10
1	1	<2.5
	28	<2.5
6	2	<2.5
	25	<2.5
7	2	<2.5
	22	<2.5
53	2	<2.5
	22	<2.5
181	2	<2.5
	25	<2.5
204	1	<2.5
	21	<2.5

in wet and Giemsa stained preparations of diploid lung cell cultures infected with coryzavirus strain 68 are shown in Fig. 1. Cytopathic effect usually began within 2 days post-inoculation and was most often complete within 5 days. Uninoculated diploid fetal cells were typically elongate. Coryzavirus infection caused the cells to separate. Early in the process, a few of the cells assumed a rounded, oval or angular form, became refractile, and some exhibited swelling. This change ultimately occurred throughout the cell sheet. The cells finally were lysed leaving a finally granular debris, naked nuclei, and filamentous structures which might represent disrupted cell membranes. In Giemsa stained preparations, the nuclei showed distortion and wrinkling and stained either more intensely or less intensely than normal cell nuclei. Margination of chromatin and other disruption of nuclei was apparent but no clearly defined inclusion bodies were seen. Cell lysis was clearly shown.

Host range. None of the 10 coryzavirus isolates shown in Table I caused significant cytopathic effect on 2 serial passages in HeLa and grivet renal cell cultures incubated on roller drums at 33°C or at 36°C. No virus could be recovered on subculture into diploid fetal lung cultures of the second passage HeLa and grivet culture material. Groups of one day-old suckling white Swiss mice inoculated intraperitoneally with the 10 coryzavirus strains which titered $10^{3.0}$ to $10^{4.5}$ per ml failed to develop overt signs of illness within 30 days following inoculation. Additionally, the 68 and M.R.H. strains of coryzavirus failed to induce illness within 30 days in 10-12 g mice inoculated intraperitoneally and intracerebrally or in young adult guinea pigs inoculated by the intraperitoneal route. Mice weighing 10-12 g failed to develop illness or grossly detectable lung lesions following intranasal inoculation with these 2 strains. Neither strain 68 nor M.R.H. coryzavirus caused visible pathology on primary passage or on subculture in 7 day-old embryonated chicken eggs inoculated via the yolk sac nor could the virus be recovered in tissue culture from the egg passage material. Embryonated chicken eggs inoculated via the allantoic and amniotic routes and incubated 3 days failed to show pathology or to develop agglutinins for chicken or guinea pig erythrocytes, on primary passage or on subculture, and the virus could not be recovered in tissue culture. None of the strains of coryzavirus showed growth on ordinary bacterial culture media and pleuropneumonia-like organisms could not be cultured from aliquots of strain 68.*

Optimal propagation temperature. No attempt was made to recover coryzaviruses from throat specimens in cell cultures at 36°C rather than at 33°C. It was found, however, that after an initially low titer on first passage at 36°C, strains 68 and M.R.H. of coryzavirus rapidly increased in titer on serial passage giving fluids with infectivity titers as high as were obtained on incubation at 33°C.

Hemagglutination and hemadsorption. None of the 10 agents listed in Table I caused hemagglutination of guinea pig, rooster or type "O" human red blood cells when incubated at room temperature or at 4°C. Additionally, none of the infected dip-

* These tests were performed by Dr. L. Hayflick.

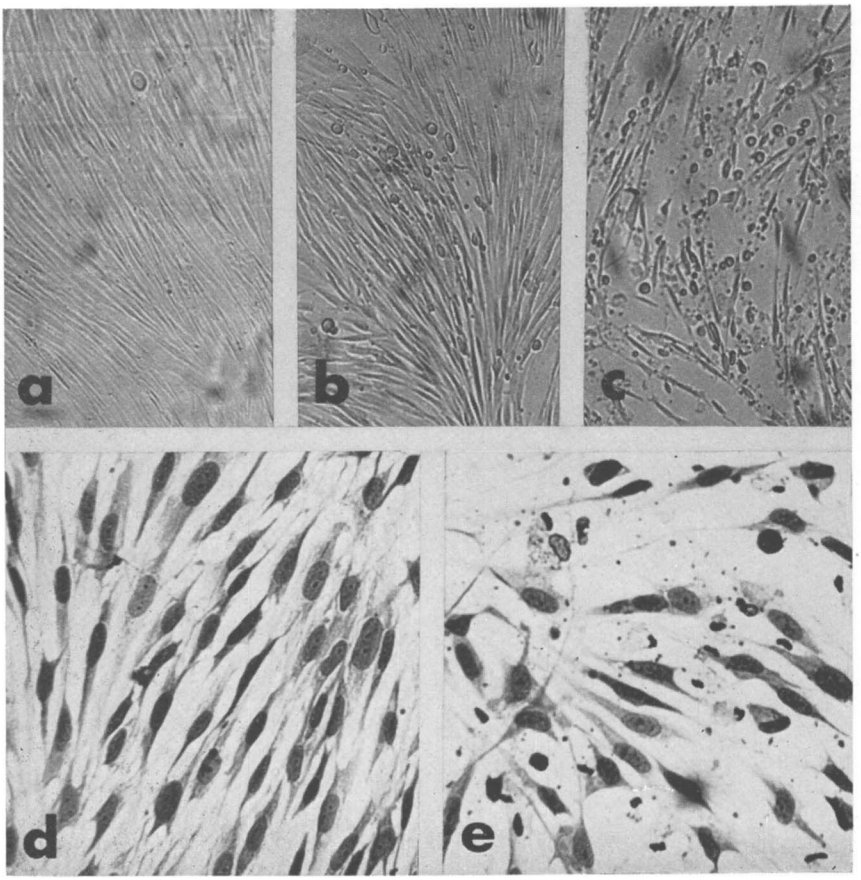

FIG. 1. Cytopathology of coryzavirus group 1 strain 68 in cell cultures of diploid human fetal lung. Wet preparations:
a. Control b. Early CPE c. Advanced CPE × 48 Geimsa stained preparations:
d. Control e. Post infection—24 hours × 170

loid fetal lung cultures showed hemadsorption for guinea pig or rooster erythrocytes at 4°C or at room temperature when tested at a time after cytopathic change had occurred in the cultures.

Antigenic heterogeneity among coryzavi-

TABLE II. Evidence for Serologic Heterogeneity Among Coryzaviruses.

Patient's serum	Serum sample	Coryzavirus isolate									
		Group 1					Group 2	Group 3	Group 4		
		68	54	7	6	53	MRH	211	204	1	181
Group 1											
68	Acute	<5	<5	<5	<5	<5	<5	<5	<5	<2.5	5
	Conv.	*20*	20	10	10	20	<10	<10	<10	<2.5	10
54	Acute	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	2.5	<2.5	<2.5	<2.5
	Conv.	5	*10*	5	10	5	<5	5	<5	<5	<2.5
Group 2											
MRH	Acute	<5	<5	<5	<5	<5	<5	5	<5	<5	<5
	Conv.	<10	<10	<10	<10	<10	*20*	10	<10	<10	<10
Group 3											
211	Acute	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<5	<2.5	<2.5	<2.5
	Conv.	<5	<5	<5	<5	<5	<2.5	*10*	<5	<5	<5

ruses. Paired sera from cases 68, 54, M.R.H. and 211, which showed a neutralizing antibody increase, were titrated with the 10 isolates presented in Table I. The findings are summarized in Table II. The viruses were separated into 4 distinct groups designated 1, 2, 3 and 4. Group 1 strains 68, 54, 7, 6 and 53 appeared to be alike antigenically. Group 2 and 3 strains M.R.H. and 211 appeared to be represented by only a single example. No titer increases were shown against group 4 strains so that degree of homogeneity could not be measured.

Lack of antigenic relationship of coryzaviruses to known viruses. Antisera† against the prototype F.E.B. and H.G.P. strains of "Salisbury" viruses failed to neutralize any of the 10 isolates shown in Tables I and II when used at serum dilutions which completely neutralized the homologous agents. Additionally, the F.E.B. and H.G.P.† viruses were not neutralized by groups 1, 2 or 3 sera from patients 68, 54, M.R.H. and 211. None of the 10 coryzavirus isolates were neutralized by hyperimmune animal antisera of known potency against ECHO virus types 11, 20, 22 or 28 or by Coe (Coxsackie A 21) (14) or Pett(15) strains of virus. Finally, neither coryzavirus strain 68 nor M.R.H. was neutralized by potent antisera against the remainder of the presently recognized ECHO virus types.

Biophysical and biochemical properties of coryzaviruses. Gradocol membrane filtration. Strain 68 of coryzavirus prepared in diploid fetal lung was used. This material was largely freed of extraneous proteinaceous material by centrifugation and by fluorocarbon treatment. Initial experiments, in which 10 ml volumes of virus were filtered, showed passage of the virus through 760 mμ but not 320 or 220 mμ average pore diameter (APD) membranes. Early centrifugation data suggested a smaller particle size than indicated by filtration and it appeared likely, therefore, that the virus was being adsorbed to the filters. To overcome this, larger quantities of virus-containing fluid were passed. The importance of volume of virus filtered is clearly shown in Table III. In

† These viruses and antisera were kindly furnished by Dr. D. A. J. Tyrrell.

TABLE III. Gradocol Membrane Filtration of Strain 68 Coryzavirus.

Avg. pore dia. of membrane, mμ	Sample tested		Result CPE	Result TCD_{50}/ml
Exp. 1		ml		
320	1st	10	neg.	
220	1st	10	"	
100	1st	10	"	
	2nd	10	pos.	
85	1st	10	neg.	
	2nd	10	pos.	
61	1st	10	neg.	
	2nd	10	pos.*	
	3rd	10	"	
40	1st	10	neg.	
	2nd	10	pos.*	
	3rd	10	"	
33	1st	10	neg.	
	2nd	10	"	
	3rd	10	"	
	4th	10	pos.*	
Exp. 2				
61	1st	30		$10^{4.0}$
	2nd	20		$10^{5.2}$
40	1st	30		$10^{2.2}$
	2nd	20		$10^{3.2}$
33	1st	30		$10^{1.2}$
	2nd	20		$10^{3.2}$
27	1st	30		neg.
	2nd	20		Trace (3/6 tubes pos.)

* Appearance of CPE delayed 3 days.

Exp. 1, based on a 10 ml filtration sample, the virus failed to pass even a 320 mμ APD membrane. Membranes of 100, 85, 61 and 40 mμ APD required the filtration of 20 ml of fluid before virus could be detected in the filtrate, and the 33 mμ membrane required 30 ml of fluid. These relationships are exemplified further in Exp. 2 in which infectivity titrations were carried out. It is seen that a small amount of virus was detected after filtration through the 27 mμ APD membrane. Considering that only a trace of virus was detected in the 27 mμ filtrate and applying Black's factor of 0.64(16) to convert pore diameter to virus particle size, the virus diameter is estimated to be about 17 mμ.

Centrifugation.‡ A second measure of

‡ The authors are indebted to Dr. A. A. Tytell and to Mr. D. S. Spicer for assistance in performing the centrifugation runs.

TABLE IV. Comparison of Sedimentation of Coryzavirus 68, Poliovirus III and Adenovirus 2 at 123,600 × g, According to Time.

Virus*	Log_{10} per ml reduction in TCD_{50} when centrifuged for time periods				
	0	15 min.	30 min.	45 min.	60 min.
Coryzavirus—68	0	$10^{0.3}$	$10^{0.2}$	$10^{0.4}$	$10^{1.4}$
Poliovirus III (30 mμ dia.)	0	$10^{0.4}$	0	$10^{1.5}$	$10^{2.2}$
Adenovirus 2 (70 mμ dia.)	0	$10^{1.8}$	$10^{1.4}$	$10^{2.6}$	$10^{2.6}$

* Initial virus titers, Coryzavirus 68, $10^{4.5}$ TCD_{50}/ml; poliovirus III, $10^{6.9}$; adenovirus 2, $10^{7.1}$.

particle size of strain 68 coryzavirus was achieved by comparing the sedimentation rate of this virus with that of poliovirus III, with known particle size of about 30 mμ, and that of type 2 adenovirus, with known particle size of about 70 mμ. The experiments were conducted as described in the materials and methods and the findings (Table IV) are expressed in terms of reduction in infectivity in the top 1 ml of supernate, according to length of centrifugation period. It is seen that more than 90% of the adenovirus was removed from the upper 1 ml of supernate within 15 minutes. Forty-five minutes were required to accomplish this for poliovirus and 60 minutes for coryzavirus. Assuming similar density, this places the size of coryzavirus at something less than poliovirus.

Electron micrography§. Thin sections were prepared of diploid fetal lung cultures infected with coryzavirus 68 and of uninfected cultures. An electron micrograph of an infected cell is shown in Fig. 2. Spherical structures about 18 mμ in diameter were present in a crystalline array. No such structures were seen in uninoculated cells. These particles are tentatively ascribed to coryzavirus.

Nucleic acid typing. The findings of the experiments conducted according to Salzman (13) are shown in Table V. Coryzavirus 68 was not suppressed by FUDR and hence is classed as an RNA virus. Vaccinia virus, known to be of DNA type, was inhibited by FUDR but this was reversed by added thymidine. Encephalomyocarditis virus, known

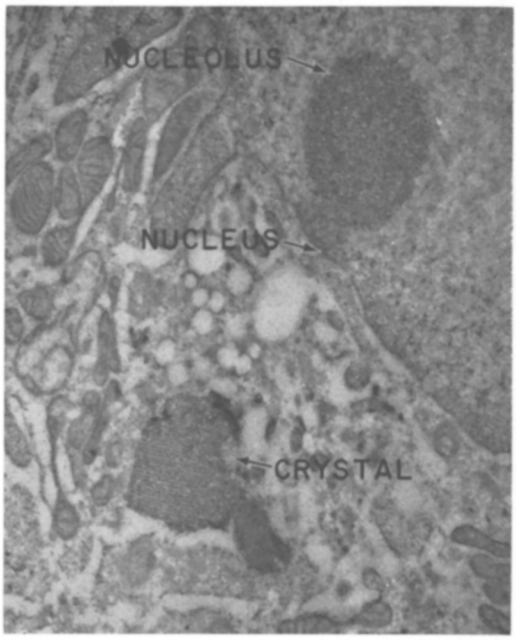

FIG. 2. Electron micrograph of a thin section of a diploid cell infected with coryzavirus group 1 strain 68. × 17,400.

TABLE V. Determination of Nucleic Acid Type of Coryzavirus 68 Through Use of FUDR.**

Virus	Additive to culture medium				Virus nucleic acid type
	None	FUDR 10^{-6}M	FUDR 10^{-5}M	FUDR 10^{-6}M plus thymidine 10^{-5}M	
Coryzavirus 68	$10^{3.4}$*	$10^{3.7}$	$10^{3.4}$	$10^{3.4}$	RNA
Encephalomyocarditis	$10^{7.4}$	$10^{7.4}$	$10^{7.7}$	—	RNA
Vaccinia	$10^{6.7}$	$10^{3.2}$	—	$10^{6.7}$	DNA

* Infectivity titers expressed as TCD_{50} per ml.
** 5-fluorodeoxyuridine.

§ The authors are indebted to Dr. A. I. Schepartz and to Mr. R. W. Ziegler for preparing the sections and for the electron micrography.

TABLE VI. Summary of Presently Known Biological and Physical Properties of Coryzavirus.

A. Clinical
   1. Recovered from patients with common cold.
   2. Patients develop antibody in response to infection.
B. Biological properties
   1. Grow and produce CPE in diploid human fetal kidney and lung cell strains.
   2. Neither propagate nor produce significant CPE in HeLa or in grivet renal cell cultures.
   3. Readily recovered in diploid human fetal cell cultures incubated at 33 ° C; also propagate at 36 ° C.
   4. Cytopathic effect is primarily cell rounding, swelling, lysis. No inclusion bodies seen.
   5. Fails to grow or cause detectable damage in suckling or adult mice, guinea pigs, or embryonated chicken eggs by usual routes of inoculation.
   6. Fails to produce hemagglutinins and fails to cause hemadsorption.
   7. Antigenically heterogeneous; at least 4 groups recognized (groups 1, 2, 3 and 4).
   8. Serologically unrelated to "Salisbury" prototypes FEB and HGP; unrelated to presently recognized ECHO, Coe, Pett and Reoviruses.
   9. Fails to propagate on ordinary culture media, including media for pleuropneumonia.
C. Biophysical and biochemical properties
   1. About 17 to 18 mμ as estimated from gradocol membrane filtration, centrifugation and electron micrography data.
   2. Resistant to ether and to fluorocarbon 113.
   3. RNA virus as determined by lack of FUDR inhibition.

to be of RNA type, was not affected by FUDR.

Ether resistance. Coryzavirus 68, prepared with serum-free medium and titering $10^{4.2}$ TCD_{50} per ml, was treated with 20% diethyl ether. There was no loss of infectivity titer on storage overnight at 4°C.

Summary of known properties of coryzavirus. Table VI summarizes the known biological, biophysical and biochemical properties of coryzavirus. Most of the data were collected using strains 68 and M.R.H. of coryzavirus.

Viral susceptibility spectrum of diploid fetal cell cultures. Diploid fetal lung and kidney cell cultures appeared equally sensitive to coryzaviruses and equally suitable for work with these agents. In studies conducted in collaboration with Dr. L. Hayflick, the susceptibility of diploid fetal kidney strain WI-10 to a variety of viral agents was measured. The findings are presented in Table VII.

In virus recovery attempts from human respiratory specimens of known content, the fetal kidney failed to detect parainfluenza 2, respiratory syncytial or adenovirus 5 viruses. By contrast, these were detected in cultures of HeLa or grivet renal kidney or both. This indicates a lesser sensitivity for detection even though these same viruses with the exception of parainfluenza 2, appear to propa-

TABLE VII. Susceptibility of Diploid Human Fetal Kidney Cell Cultures (Strain WI-10) to a Variety of Viral Agents as Measured by Development of Cytopathology or of Hemadsorption Effect.

Virus	Effect	Virus	Effect
Enterovirus		Coryzavirus	yes
Poliovirus I (Mahoney)*	yes	Reovirus 1	"
I (Chat)*	"	Myxovirus	
II (TN)*	"	parainfluenza 1	no
III (Fox)*	"	2	"
Coxsackie A_1*	no	3	"
A_9	yes	Adenovirus 2*	"
A_{13}*	"	12*	"
A_{21} (Coe)	"	Respiratory syncytial	"
B_1*	no	Measles*	"
ECHO 9*	yes	Varicella*	"
11	"	Vaccinia*	"
20	"	Arborvirus	
21*	"	WEE*	"
22	"	Yellow fever (17D)*	yes
28	"	Encephalomyocarditis	
Pett	"	EMC*	yes
Salisbury viruses		Mengo*	"
FEB	yes	Rabies*	yes (Partial)
HGP	"	Poiyoma	no

* Results obtained by Dr. Hayflick. Presented by permission.

gate in the fetal kidney cells. It would appear, therefore, that the diploid fetal cells should not be used alone for diagnostic test purpose.

Discussion. The findings presented reveal that viruses have been recovered from cases of common cold in the human adult. This illness has been outstanding in the past for its failure to yield to studies of its etiology. Recent studies, not reported herein, have shown recovery also of similar viruses from children with respiratory illness. Recovery of the new viruses was made possible by the use of newly developed cell cultures derived from human fetal kidney and lung and maintained in serial passage in a diploid state(8,9). Because of their association with illness of predominantly coryzal nature, these agents are called coryzaviruses.

The extent to which coryzaviruses are responsible for or are involved in cases of common cold cannot be stated from the present data. The recovery of coryzavirus from 15% of 110 cases of common cold in contrast to only one of 45 (2%) "normal" persons, together with the demonstration of development of antibody in a portion of cases during convalescence, provides evidence for the etiologic role of these agents in the common cold. Failure to recover virus or to demonstrate antibody increase against other known respiratory viruses lends additional support for the primary role of coryzavirus in the etiology of cases studied.

The weak antibody response or the failure to respond serologically to homologous coryzavirus, among the cases studied, might be due to a relative insensitivity of the neutralization technique employed to detect antibody. Alternatively, it might reflect a poor antibody response to a possibly superficial infection with coryzavirus and may, in turn, be responsible in part at least for the apparent transient immunity in certain common colds.

The coryzaviruses appear to be unique in their limited host range, causing CPE in diploid fetal cells but failing to propagate or show demonstrable effect in HeLa and grivet kidney cells as well as in a variety of common laboratory hosts. The size of the virus appears to be very small. Coryzavirus shares with many other agents, *e.g.*, enteroviruses, the property of ether resistance and RNA. The agent has failed, to date, to show hemagglutination or hemadsorption.

The possible relationship of the coryzaviruses to certain of the "spectrum" of "Salisbury" viruses cannot be ascertained with certainty at the present time. The coryzaviruses differ immunologically from the prototype F.E.B. and H.G.P. Salisbury agents and are readily distinguished from H.G.P. prototype by failure to grow and produce CPE in grivet kidney cell cultures. Tyrrell *et al.* (1-6) emphasize the importance of reduced bicarbonate and lowered temperature for growth of the Salisbury strains. Ordinary bicarbonate concentrations, used in conjunction with tris buffer, were employed for isolation and propagation of the coryzaviruses. Although propagated routinely at 33°C, coryzavirus was readily adapted to multiplication at 36°C. Coryzavirus, like the Salisbury viruses, was ether and fluorocarbon resistant. The H.G.P. prototype of Salisbury virus was held back by a 69 mμ APD collodion filter(3).

In the present studies, a minimal detectable amount of coryzavirus strain 68 passed through a 27 mμ APD Elford collodion filter, and this became apparent only after 30 ml had already been filtered through the membrane. Determination of particle size by collodion membrane filtration is clearly influenced by several vagaries, most important of which are the relative purity of the material being filtered, concentration of virus in the fluid, and volume of fluid passed. Applying Black's factor of 0.64(16), the size of coryzavirus 68 is estimated to be about 17 mμ diameter. This is in good agreement with the electron microscope and centrifugation data.

The propagation in human diploid fetal cells of coryzaviruses raises the question of vaccines. Utilizing frozen cell banks, the diploid cell provides a potential for almost unlimited quantities of tissue culture material. The retention by such cells of a diploid karyotype and of the other properties considered characteristic of "normal" cells (9), answers most of the objections raised in the past to employment of serially passaged cell cultures for human vaccine purpose. The degree of diversity of antigenic types

in the coryzavirus group and the extent to which these agents contribute to the common cold must be the subject of further research. The poor antibody response to the natural infection with coryzavirus should encourage the use of a killed vaccine in which an adequate immunizing dose of antigen can be applied.

Summary. Viruses have been recovered in diploid cell cultures of human fetal lung or kidney from cases of common cold in human adults and children. These agents, which comprise at least 4 serologic groups, appear to be related etiologically to the common cold in man. Because of their association with coryzal disease and for purpose of designation, the agents are called coryzavirus. Coryzavirus has failed to propagate in HeLa or grivet renal cell cultures, did not produce pathology in common laboratory hosts, and appears to be unrelated to the known respiratory viruses of man. Similarities to and differences from the "spectrum" of "Salisbury" agents are discussed. The agent appears to be of very small particle size and is estimated to be about 17 to 18 mμ in diameter. Other biological, biophysical and biochemical properties of coryzavirus are presented.

ADDENDUM

A number of strains of coryzavirus were recovered from cases of colds both in adults and in children. Cross-neutralization tests with animal antisera have revealed at least 6 distinct serotypes of coryzavirus. Only one-third of strains recovered to date fall into these 6 types. The low incubation temperature (33°C) initially used to recover virus in the diploid cells was found unnecessary for at least 3 of the serotypes and routine 36°C temperature of incubation was often employed in the later work.

The final taxonomic position of the coryzaviruses must await further study. The agents herein designated coryzavirus are similar to the enteroviruses with respect to size, ether resistance, and RNA content and may fall within the general enterovirus group as presently defined. The possible relationship of the coryzaviruses to certain of the Salisbury agents cannot be decided until further cross-serologic tests are made and until precise and comparable biophysical test results are available. The degree of homogeneity among both groups of agents has not been defined.

Andrewes *et al.* (*Virology,* 1961, v15, 52) in a recent publication have proposed to call the Salisbury cold viruses rhinoviruses and to place them in a suggested family of "Naniviruses" which would include the present enteroviruses, foot and mouth disease virus, Teschen disease, encephalomyocarditis and a number of other viruses. Johnson and associates (unpublished) have recently recovered agents from cases of common cold in primary human cell cultures and in the diploid human fetal cell cultures employed in the present investigations. These workers have recognized at least 6 distinct serotypes and have proposed that their viruses be included as types of enterovirus.

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