of radioactivity in the skin§. The wide variations observed in the choice of various forms of cobalt for preferential localization among the body tissues, the different times at which such localizations occur, and also the vast differences in rate and preferred mode of excretion followed by these chelates, all suggest that the cobalt travels from one tissue to another in the form in which it is administered, perhaps in combination with some body constituents (for example, blood proteins), but never splitting up into the ionic form. This possibility is being investigated by studying the state in which the cobalt-60 exists in the various tissues and in excretion. Investigations on the uptake of cobalt-60 by liver slices *in vitro* indicate that cobalt is taken up by a passive process, which is enhanced by SDDC, but inhibited by most of the other chelating agents, EDTA being the most potent inhibitor.§

Summary. The path of cobalt-60, administered alone as cobaltous chloride or with any one of the chelating agents 1-nitroso 2-naphthol, SDDC, rubeanic acid, BAL, EDTA or cysteine, is traced by studying the tissue distribution at various times after intravenous administration to albino rats. With ionic cobalt and with a number of chelates, an initial accumulation of radioactivity in the liver is noted, even though subsequent distribution varies widely. The 1-nitroso 2-naphthol chelate follows the route Blood → Liver → Skin, Bones and Muscles → Excretion. EDTA- and cysteine-chelates exhibit a marked apathy for the liver, but preferentially concentrate in the skin and bones. Quantitatively, the liver, skin, bones and muscles are the chief tissues of physiological importance in cobalt metabolism.

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Characterization and Classification of ECHO 28-Rhinovirus-Coryzavirus Agents. (27662)

A. KETLER, V. V. HAMPARIAN AND M. R. HILLEMAN

Division of Virus and Tissue Culture Research, Merck Institute for Therapeutic Research, West Point, Pa.

Several viruses or groups of viruses have been recovered in tissue culture from cases of common cold. The first of these were the 2060 and JH viruses discovered independently by Pelon *et al.*(1) and by Price(2) and now designated ECHO virus type 28(3). Additionally, there are the rhinoviruses described by Tyrrell, Taylor-Robinson and coworkers(4) and shown to consist of more than 6 immunologically different strains, certain of which grew only in human cell cultures (H strains) while others were propagated in monkey renal (M strains) as well as human cells; the coryzaviruses described by Hamparian *et al.*(5) and shown to comprise at least 4 distinct groups; viruses recovered by Johnson *et al.* (unpublished) and stated to consist of 6 distinct serotypes of the general enterovirus family; and viruses recovered by Hamre *et al.* (6) and by Hobson *et al.*(7).

The present report describes the isolation and immunologic differentiation of 20 distinct

serotypes of coryzavirus. The results of extensive investigations of the immunological and the biological-biophysical properties of the ECHO 28, rhinovirus and coryzavirus agents show differences from other known viruses and provide a basis for including them in a single group.

Materials and methods. Clinical specimens. Details concerning the child and adult populations from which the viruses were recovered have been described(5,8,9). Methods for handling of specimens were the same as described before.

Tissue cultures. Cultures of the *WI-26* strain of diploid human fibroblasts(10) and of *grivet renal cells* were prepared and handled as described earlier(5). The maintenance medium for the WI-26 cultures was modified to contain a greater amount of sodium bicarbonate (0.14%) and was adjusted to pH 7.4 with tris (hydroxymethyl aminomethane) buffer. *HeLa* cell cultures were grown in Eagle's medium with 5% calf serum and were maintained with the same medium. The *KB* cell cultures were grown and maintained in Melnick's lactalbumin-yeast extract medium(11) containing 5% calf serum. Penicillin and streptomycin at 100 units or 100 μg per ml were present in all media and, except for WI-26, all culture media contained 0.24% sodium bicarbonate without added tris buffer. The WI-26 cultures were held at 33°C or at 36°C. The cultures maintained better at the lower temperature.

Viruses. Except for the change in culture media referred to earlier, the *coryzaviruses* were recovered and propagated as previously described(5). The *rhinoviruses* were obtained from Dr. D. Taylor-Robinson either as human embryo kidney or as monkey kidney tissue culture passage material and were propagated either in WI-26 or in HeLa cell cultures in this laboratory. All *other viruses* were from the files of this laboratory and were identified in tests with reference antisera from reliable sources.*

Antisera. Infected *coryzavirus* and *rhinovirus* preparations containing 10^3 to $10^{5.5}$ TCD_{50} per 0.2 ml were treated once with fluorocarbon 113 and were emulsified with an equal volume of Arlacel A (10%) - Drakeol (90%) adjuvant. Groups of 8 guinea pigs were given two 1 ml injections of the antigen 6 weeks apart and the animals were exsanguinated 2 weeks after the second injection. The homologous neutralizing antibody titers ranged from 1:20 to 1:640. *Enterovirus* antisera were prepared in this laboratory from documented strains or were kindly furnished by reliable sources.* *Neutralization tests* were performed by usual procedures using 200 to 2000 TCD_{50} of virus and incubation of the virus-serum mixtures at room temperature for 60 minutes prior to inoculation. The titer was the highest initial dilution of serum which caused 75% or greater suppression of cytopathology at a time when the controls showed 75-100% involvement of the cell sheet.

Biophysical and biochemical procedures. pH stability. The ECHO 28-rhinovirus-coryzavirus agents to be tested were diluted 1:10 in Eagle's medium adjusted to pH 2.8 or to pH 7.2 with tris buffer. The remaining viruses were diluted to contain about 10^3 to 10^4 $TCID_{50}$/0.2 ml. After 3 to 4 hours standing at room temperature, the residual virus content of each was determined by infectivity titration in appropriate tissue culture systems. pH measurements of the samples at the beginning and at the finish of the tests were pH 2.9 $\pm$ 0.1 and pH 7.2 $\pm$ 0.2. *Heat stability.* Duplicate aliquots of virus were incubated at room temperature or submerged in a water bath at 50°C in stoppered Wassermann tubes using due care to avoid viral contamination of the upper portions of the tubes. After incubation, the virus infectivity titers were determined in appropriate cell culture systems. At least one coryzavirus and one enterovirus were included in each test to control the adequacy of the heating procedure. *Nucleic acid typing.* The FUDR (5-fluorodeoxyuridine) virus inhibition method of Salzman(12) was employed as described previously(5). Additionally, infectious nucleic acid was extracted by the cold phenol method of Gierer and Schramm(13) as modified by Alexander *et al.*(14) and was identified in tests using RNA'se and DNA'se. *Sedi-*

* Drs. H. A. Wenner, F. Lehman-Grube, A. B. Sabin, William McD. Hammon, Microbiological Associates.

TABLE I. Origin, 37 Coryzavirus Strains Comprising 20 Serotypes.

Coryzavirus		Patient		Diagnostic neutralization tests	
Type	Strain	Age, yr	Diagnosis	Acute	Convalescent
Adults					
11	6	29	URI	<2.5	<2.5
"	7	47	"	<2.5	<2.5
"	23	61	"	<2.5	<2.5
"	27	28	"	<2.5	10
"	53	21	"	<2.5	<2.5
"	54	46	"	<2.5	10
"	68	40	"	<5	20
"	89	40	"	<2.5	20
"	116	37	Not ill	—	—
12	MRH	41	URI	<5	20
13	211	49	"	<5	10
14	43	42	"	<2.5	40
"	204	44	"	<2.5	10
15	1	52	"	<2.5	<2.5
16	181	31	"	<2.5	<2.5
18	17	20	"	<2.5	10
"	93	45	"	<2.5	<2.5
19	15	31	"	<2.5	640
20	21	23	"	<2.5	40
21	47	41	"	<2.5	80
22	127	58	"	<2.5	160
Children		Age			
14	5812	4 yr	Croup	—	40
"	5961	4 mo	URI	<2.5	10
16	5126	2 yr	"	<2.5	20
"	6079	2 "	"	<2.5	20
17	5647	5 mo	"	<2.5	80
"	5882	2 "	Bronchopneu.	<2.5	40
"	5986	7 yr	Bronchitis	<2.5	40
18	6072	5 "	"	<2.5	40
23	5007	7 "	"	<2.5	320
24	5124	6 "	URI	—	—
25	5146	7 "	"	<2.5	<2.5
26	5426	3 "	"	<2.5	160
27	5660	6 "	"	5	160
28	5870	6 mo	"	<2.5	20
29	6101	10 "	"	<2.5	10
30*	5582	2 yr	Not ill	<2.5	10

* M strain. All others were H strains.

mentation. For each determination, aliquots of 2 coryzavirus strains were centrifuged simultaneously with an aliquot of adenovirus type 5 at 123,600 × *g* for 15 minutes in the SW39 head of the model E Spinco ultracentrifuge. All viruses tested were in media of identical composition. The upper 1 ml of supernate was sampled for simultaneous titration with control samples which were not centrifuged.

Results. Recovery of coryzavirus strains. As seen in Table I, 20 coryzavirus strains including 11 coryzavirus types were recovered in isolation attempts made with throat secretions from 140 cases in adults with common cold, which were designated clinically as acute upper respiratory illness (URI). A single type 11 coryzavirus strain was recovered from one of the 48 persons who served as normal controls. Type 11 virus was most commonly found. Fifteen coryzavirus strains representing 11 coryzavirus types were isolated from secretions of 263 cases of respiratory illnesses among children aged 4 months to 7 years. A single strain (5582) of type 30 virus was recovered from 81 control cases tested. Most of the children from whom coryzaviruses were isolated showed mild upper respiratory illness typical of "colds". Five of these, however, showed lower respiratory tract involvement, 1 with croup, 3 with bronchitis and 1 with bronchopneumonia. Twenty-five of 33 respiratory

disease cases (76%) showed an increase in neutralizing antibody during convalescence while 8 of 33 (24%) failed to do so. The child control case also showed a titer increase. Serologic responses appeared to be more consistent in children than in adults. Half of the adults who failed to respond were type 11 cases. All of the cases were negative, serodiagnostically, for influenza, parainfluenza, adenovirus, respiratory syncytial and reovirus infection(9).

In the isolation attempts, additionally, two type 1, seven type 2, three type 3 and four type 5 adenoviruses and five herpes simplex viruses were recovered from the respiratory cases. Two type 2 and one type 5 adenovirus plus two herpes simplex strains were isolated from controls.

Tissue culture. All the coryzavirus strains propagated well and caused marked cytopathology in the WI-26 strain of diploid human fibroblast. All the types were tested and, in contrast to earlier findings(5), were found to propagate readily and to cause cytopathic changes in KB and in HeLa cell cultures. Certain coryzavirus strains were also recovered on serial passage of throat washings in KB cell cultures. Type 30 strain 5582 recovered from a control case was shown to cause weak cytopathology in grivet renal cell cultures and, hence, was classed as a monkey (M) strain. None of the remaining types caused cytopathic change or propagated in cell cultures of grivet kidney under the conditions employed nor could they be adapted to growth in these cells. These were regarded as human (H) strains.

Serologic investigations. Table II presents the findings in neutralization tests using the 20 coryzavirus types, the 6 rhinoviruses, and all of the ECHO, Coxsackie and reovirus types. All the sera used were prepared in animals. There were no cross-reactions between any of the coryzavirus types and none of the coryzavirus antisera reacted with any of the 6 rhinovirus strains. The 2 rhinovirus antisera reacted only with the homologous agents and did not cross-react with any of the coryzaviruses. None of the prototype ECHO, Coxsackie or reovirus antisera reacted with any of the coryzaviruses or rhinoviruses when used at a dilution containing a minimum of 20 neutralizing antibody units.

Cross-neutralization tests were also carried out using the paired sera and virus strains from patients representing 10 different coryzavirus types (Table III). There was a striking degree of type specificity and lack of cross-reaction. In only 1 instance, as seen in the table, *i.e.*, type 23 paired sera *vs* type 18 virus, was there any detectable cross-reaction.

Biochemical investigations. Nucleic acid. Tests of FUDR inhibition (Table IV) established that all 20 coryzavirus types, all 6 rhinoviruses and ECHO virus 28 were of RNA type. Table V shows that the infectious nucleic acid extracted from strain 68 of type 11 coryzavirus was destroyed by RNA'se but not by DNA'se. This supported the nucleic acid determination with FUDR.

TABLE II. Summary Results, Neutralization Tests, Using Coryzavirus, Rhinovirus, ECHO, and Coxsackie Types.

	Neutralization test results with sera *vs*							
		Rhinovirus				Coxsackie		
Viruses	Coryzavirus 11-30	Sal./1/58H	Sal./1/57M	ECHO 28	ECHO 1-8, 11-27	A1-20b, 21-24	B1-6	Reovirus 1-3
Coryzavirus 11-30 (individually)	Homol. 1:5 or > Heterol. 0*	0	0	0	0	0	0	0
Rhinovirus								
Sal./1/58H (FEB)	0	1:5 or >	0	0	0	0	0	0
Shef./1/60H	0	0	0	0	0	0	0	0
Sal./1/59H	0	0	0	0	0	0	0	0
Sal./1/51H	0	0	0	0	0	0	0	0
Sal./1/57M (HGP)	0	0	1:5 or >	0	0	0	0	0
Sal./1/60M	0	0	0	0	0	0	0	0

* 0 = <1:5.

TABLE III. Cross-Neutralization Tests with Paired Sera and Viruses from Cases Representing 10 Coryzavirus Types.

Virus type	Patient Case No.	Age (yr)	Serum sample	Neut. titers obtained in tests with virus type and strain: 11 (89)	14 (43)	17 (5582)	18 (17)	19 (15)	20 (21)	22 (127)	23 (5007)	26 (5426)	27 (5660)
11	89	40	Ac.	< 5	< 5	5	5	< 5	< 5	< 5	< 5	< 5	< 5
			Conv.	*20*	< 5	< 10	5	< 5	< 5	< 5	< 5	< 5	< 5
14	43	42	Ac.	< 5	< 5	5	20	10	5	< 5	< 5	< 5	< 5
			Conv.	< 10	*20 or >*	< 10	40	10	< 10	< 10	< 10	< 10	< 10
17	5882	2 mo	Ac.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
			Conv.	< 10	< 10	*20 or >*	< 10	< 10	< 10	< 10	< 10	< 10	< 5
18	17	20	Ac.	< 5	< 5	< 5	< 5	< 5	< 5	10	< 5	< 5	< 5
			Conv.	< 5	< 5	< 10	*10*	< 5	< 5	10	< 5	< 5	< 10
19	15	31	Ac.	< 5	< 5	< 5	< 5	< 5	< 5	10	< 5	< 5	< 5
			Conv.	< 10	< 10	< 10	< 10	*20 or >*	< 10	20	< 10	< 10	< 10
20	21	23	Ac.	< 5	< 5	5	< 5	< 5	< 5	< 5	5	< 5	< 5
			Conv.	< 10	< 10	10	< 10	< 10	*20*	< 10	< 10	< 10	< 10
22	127	58	Ac.	5	5	5	< 5	< 5	< 5	< 5	< 5	5	< 5
			Conv.	< 10	< 10	10	< 10	< 10	< 10	*20 or >*	< 10	10	< 10
23	5007	7	Ac.	< 5	< 5	< 5	< 5	< 5	5	< 5	< 5	< 5	< 5
			Conv.	< 10	< 10	< 10	10	< 10	10	< 10	*20 or >*	< 10	< 10
26	5426	3	Ac.	< 5	< 5	< 5	< 5	5	< 5	< 5	< 5	< 5	< 5
			Conv.	< 10	< 10	< 10	< 10	10	< 10	< 10	< 10	*20 or >*	< 10
27	5660	6	Ac.	< 5	< 5	10	40	5	10	10	< 5	< 5	< 5
			Conv.	< 10	< 10	20	40	10	20	20	< 10	< 10	*40 or >*

pH stability. In the course of investigations to concentrate coryzavirus by precipitation with nucleic acid at low pH, it was found that coryzaviruses, unlike poliovirus, were highly acid labile. For this reason, coryzaviruses, rhinoviruses, ECHO viruses and representative Coxsackie and reovirus strains were tested for loss of infectivity titer after incubation at pH 3.0. The data in Table VI show that all the coryzaviruses and rhinoviruses were extremely acid labile in contrast to the ECHO, Coxsackie and reovirus agents. ECHO 28 was a notable exception to the other ECHO agents in that it, too, exhibited

TABLE IV. Establishment of RNA Type of Coryzaviruses and Rhinoviruses through Use of FUDR.*

Virus	Neg. $\log_{10}$ virus titer: FUDR alone	FUDR + thymidine	Virus nucleic acid type
Coryzavirus			
Type 11	4.0	4.2	RNA
12	5.0	5.5	"
13	4.0	4.2	"
14	4.7	5.2	"
15	4.2	5.0	"
16	5.0	5.5	"
Types 17-30	Results similar to above		"
Rhinovirus			
Sal./1/58H(FEB)	4.7	5.2	"
Shef./1/60H	2.5	2.5	"
Sal./1/59H	2.7	3.0	"
Sal./1/51H	3.0	3.2	"
Sal./1/57M(HGP)	5.7	5.7	"
Sal./1/60M	4.0	4.0	"
ECHO 28	4.5	4.7	"
Controls†			
Vaccinia	1.7	5.2	DNA

* 5-fluorodeoxyuridine.

† Type 11 strain 68 of coryzavirus was used as the known RNA control in all tests (see Table V).

acid lability. Additional tests were performed in which representative ECHO, coryzavirus and rhinovirus types propagated in WI-26 cell cultures were tested simultaneously for pH lability. The findings were in agreement with those shown in the table and indicated that the tissue culture source of the virus did not affect the results.

Ether stability. All the coryzaviruses were stable on treatment with ether.

Thermal stability. The coryzaviruses, rhinoviruses and representative ECHO, Coxsackie and reovirus types were tested for stability to heat when exposed to 50°C temperature for 30 minutes. All coryzavirus and rhinovirus H viruses were very stable on heating and showed a maximum loss of log 1.8 of virus infectivity titer (Table VII). By contrast, the ECHO (exclusive of ECHO 28) and Coxsackie viruses were very heat labile and showed a minimum loss in infectivity titer of log 3.5 on heating. The coryzavirus M, rhinovirus M, and ECHO 28 M strains were intermediate and showed extreme variability in results in repeat tests with the same strain which ranged from almost total heat stability to near complete heat lability. Such variable results were not referrable to the kind of cell culture used since comparative tests of representative strains, all of which were propagated in WI-26 cell cultures, exhibited similar inconsistency of result in repeat tests.

TABLE V. Recovery of Infectious RNA from Coryzavirus Type 11 (Strain 68).

Preparation	Infectivity test
Original virus	Titer $10^{-3.5}$/0.2 ml
Phenol extract	
Untreated	Positive*
DNA'se treated	"
RNA'se "	Negative

* Positives showed typical degeneration in 75-100% of cells by day 5. DNA'se treated sample was subcultured and identified serologically as coryzavirus 11.

Sedimentation. Previous studies(5) with strain 68 of coryzavirus 11 revealed a small size based on filtration, sedimentation and electron micrography.

Coryzavirus types 16, 18, 22, 23 and 26 were also examined for sedimentation property. Table VIII shows that these coryzavirus types, like type 11, sedimented very slowly. Assuming a density equal to that of type 11, it may be concluded that the remaining coryzavirus strains were also of small particle size.

Discussion. These findings revealed that coryzaviruses are of importance in respiratory disease in children as well as in adults. The extent to which coryzaviruses are responsible for or are involved in cases of respiratory illnesses of children and adults cannot now be assessed with certainty since the sensitivity of the coryzavirus isolation methodology as a diagnostic tool is not known. However, such virus was recovered from 6% of 263 cases of respiratory illness among children and from 14% of 140 cases of respiratory illness in adults. The fact that coryzavirus was recovered only once from 81 normal children and once from 48 normal adults provides substantial evidence for the role of these agents in causing illnesses in man.

All of the isolations from adults were from cases of common cold. It is significant that 5 of the 15 children from whom coryzaviruses were recovered exhibited lower respiratory tract involvement with croup, bronchitis or

TABLE VI. Differentiation of Coryzaviruses, Rhinoviruses and ECHO Virus 28 from ECHO and Coxsackie Viruses Based on Stability at pH 3.0.

Virus		Virus propagated in:	Infectivity titer*		Virus		Virus propagated in:	Infectivity titer	
Group	Type		pH 3.0	pH 7.2	Group	Type		pH 3.0	pH 7.2
Coryzavirus	11 (H)	WI-26	<1.0	4.0	ECHO	6	GMK	7.5	7.5
"	12 "	"	"	6.0	"	7	"	6.5	6.0
"	13 "	"	"	4.0	"	8	"	7.5	7.0
"	14 "	"	"	4.0	"	11	HeLa	5.5	5.0
"	15 "	"	"	4.0	"	12	GMK	7.5	8.0
"	16 "	"	"	5.0	"	13	"	6.5	7.0
"	17 "	"	"	3.0	"	14	"	5.0	5.0
"	18 "	"	"	4.0	"	15	"	6.0	6.0
"	19 "	"	"	5.5	"	16	WI-26	5.5	5.5
"	20 "	"	"	3.0	"	17	GMK	5.5	4.5
"	21 "	"	"	3.0	"	18	"	4.5	4.5
"	22 "	"	"	3.0	"	19	"	6.5	6.0
"	23 "	"	"	4.0	"	20	HeLa	5.5	5.5
"	24 "	"	"	4.0	"	21	GMK	6.0	5.5
"	25 "	"	"	3.0	"	22	"	4.5	4.5
"	26 "	"	"	3.0	"	23	"	5.5	4.5
"	27 "	"	"	3.5	"	24	"	5.5	6.0
"	28 "	"	"	3.0	"	25	"	5.5	5.5
"	29 "	"	"	3.0	"	26	"	5.5	5.5
"	30 (M)	"	"	4.0	"	27	"	4.5	5.0
Rhinovirus†	Sal./1/58H (FEB)		"	5.0	Coxsack.	A- 7	HeLa	7.0	7.5
"	Shef./1/60H		"	3.0	"	A- 9	"	7.5	8.0
"	Sal./1/59H		"	3.0	"	A-10	GMK	6.0	6.5
"	Sal./1/51H		"	3.5	"	A-13	HeLa	6.0	6.5
"	Sal./1/57M (HGP)		"	6.5	"	A-14	GMK	4.5	5.0
"	Sal./1/60M		"	4.5	"	A-21	HeLa	6.5	7.0
ECHO	28 (M)	HeLa	"	5.5	"	A-23 (ECHO 9)	GMK	5.0	5.5
"	1	GMK‡	7.0	7.0	"	Pett	HeLa	6.0	6.5
"	2	"	6.5	7.0	"	B- 1	"	7.5	8.0
"	3	"	6.0	6.0	"	B- 3	"	7.5	8.0
"	4	"	5.0	5.0	"	B- 6	GMK	6.5	6.5
"	5	"	7.0	7.0	Reovirus (Control)	1	"	6.5	6.5

* Titer = neg. $\log_{10}$/0.2 ml and reflect the extrapolation of the diluted test samples to original titer levels.
† Sal./1/57M was propagated in HeLa cell cultures, all other strains in WI-26.
‡ GMK = grivet monkey kidney.

TABLE VII. Tests for Thermal Stability (50°C for 30 Min.) of Coryzavirus, Rhinovirus, ECHO, Coxsackie and Reovirus Strains.

Virus Strain	T. culture source	Neg. $\log_{10}$ virus titer: Not heated	Heated	Titer reduct.	Relative thermostability
Coryzavirus type 11 (H)	WI-26	4.7	3.5	1.2	Stable
" 12 "	"	3.7	2.7	1.0	"
" 13 "	"	3.7	2.5	1.2	"
" 14 "	"	4.2	4.2	0	"
" 15 "	"	3.5	3.2	.3	"
" 16 "	"	4.5	3.7	.8	"
" 17 "	"	1.5	.7	.8	"
" 18 "	"	4.0	3.0	1.0	"
" " "	HeLa	3.5	1.7	1.8	"
" types 19-29 "	WI-26		As above		"
" 30 (M)*	"	3.0	1.7	1.3	Variable
" "	"	4.2	<1	>4.2	"
Rhinovirus					
" Sal./1/58H (FEB)	WI-26	4.7	4.7	0	Stable
" Shef./1/60H	"	2.5	2.2	.3	"
" Sal./1/59H	"	3.0	3.0	0	"
" Sal./1/51H	"	3.5	3.0	.5	"
" Sal./1/60M	"	4.2	3.5	.7	Variable
" Sal./1/57M (HGP)	"	3.7	<1.0	>3.7	"
" " "	HeLa	5.7	5.0	.7	"
ECHO type 28 (M)	"	5.5	3.5	2.0	"
" 2	GMK	7.0	<2.0	>5.0	Labile
" 11	HeLa	5.0	<2.0	>3.0	"
" 16	WI-26	4.7	<1.0	>3.7	"
" 20	HeLa	6.0	2.5	3.5	"
" 26	GMK	6.7	3.2	3.5	"
" 27	"	5.7	<3.0	>2.7	"
Coxsackie A- 7	HeLa	8.5	<4.0	>4.5	"
" A- 9	"	8.2	<4.0	>4.2	"
" A-13	"	6.2	<3.0	>3.2	"
" A-21	"	7.2	<1.0	>6.2	"
" Pett	"	4.5	<0	>4.5	"
" B- 1	"	8.2	<4.0	>4.2	"
Reovirus 1 (Control)	"	6.2	6.2	0	Stable

* This was the only strain of coryzavirus which propagated in grivet renal kidney. M strains grew in monkey kidney as well as in human cell cultures while H strains propagated only in human cells. Findings with M strains were highly variable from test to test, hence, exemplary test results are shown for these viruses.

bronchopneumonia. Detailed clinical findings are to be recorded elsewhere(15). The finding of lower respiratory tract involvement in children is consistent with the pattern for other viruses(16) in which the clinical consequence of first infection is more severe than that seen following repeated infections with the virus. All of the children who were studied were outpatients. Studies in hospitalized cases of respiratory illness might well have been overshadowed by illnesses of other etiology and it is doubtful that the lower respiratory tract involvement in coryzavirus cases would have been found.

The great majority of persons responded serologically to coryzavirus infection with a neutralizing antibody response, usually of relatively low level but reaching titers as high as 1:640. Coryzavirus 11 appeared to be unusual in the failure in 4 of 8 cases to show a homologous antibody response. It was of interest, in studies not reported above, that cases 23 and 53 from which coryzavirus 11 was isolated, and which failed to show a homologous response, did show a heterotypic serologic response to coryzavirus types 17 and 21. The significance of these findings is not clear.

Serologic investigations have established clearly the lack of demonstrable immunologic

TABLE VIII. Establishment of Slow Sedimentation Rate for Several Representative Coryzavirus Strains.

Virus Type and strain	Infectivity titer Neg. $\log_{10}$/0.2 ml Original	Supernate	Reduct. in titer, log
11 – 68	3.8	3.5	.3
16 – 181	4.0	4.5	+ .5
Adenovirus 5 (control)	5.5	2.2	3.3
18 – 93	5.2	5.0	.2
22 – 127	3.5	3.5	0
Adenovirus 5 (control)	5.5	2.5	3.0
23 – 5007	3.5	4.2	+ .7
26 – 5426	4.0	3.7	.3
Adenovirus 5 (control)	5.5	2.5	3.0

relationship between any of the coryzavirus types or between the coryzaviruses and the rhinoviruses and the lack of demonstrable immunologic relationship of the coryzaviruses and rhinoviruses to the ECHO, Coxsackie and reovirus agents was revealed. The high degree of specificity of the coryzavirus types shown in tests with guinea pig antisera was confirmed in tests with paired sera from human cases. The multiplicity of antigenic types and the striking lack of immunologic crossing between types goes far toward explaining the recurrence of the common cold and points out the difficulties likely to be encountered in preparing polyvalent vaccines of adequate protective value.

All of the 20 coryzavirus types and the 6 rhinoviruses proved to be ether stable and of RNA type. The RNA type was confirmed in the case of type 11 by extraction of infectious ribonucleic acid. These properties are shared by the ECHO and Coxsackie viruses.

Table IX summarizes certain of the known properties of coryzaviruses, rhinoviruses, ECHO viruses and Coxsackie viruses. All these agents are of RNA type, are ether stable and are of small size. The coryzaviruses, rhinoviruses, and ECHO 28 stand out, however, contrast sharply to the remaining ECHO and Coxsackie agents in their lability to acid. The coryzavirus and rhinovirus H strains were remarkably stable to heating at 50°C for 30 minutes in contrast to the relative lability of the ECHO 1-27 and Coxsackie agents. No

TABLE IX. Summary, Comparison of Properties of Coryzaviruses, Rhinoviruses, ECHO and Coxsackie Viruses.

Property	Virus group: Coryzavirus 11-29 (H)	Coryzavirus 30 (M)	Rhinovirus H	Rhinovirus M	ECHO 28	ECHO 1-27	Coxsackie A	Coxsackie B
Propagability–Human cells	Yes	Yes	Yes*	Yes†	Yes	Yes(18)	Usually yes (19)	Yes(18)
Monkey kidney	No	"	No	"	Yes	Yes	Usually no (20)	Yes(18)
Stability–pH (3.0)	No	No	No	No	No	Yes	Yes	Yes
Heat (50°C, 30 min.)	Yes	Variable	Yes	Variable	Yes	No	No	No
Size	Type 11, 17-18 mμ(5)	—	<64 mμ(22)	<64 mμ(22)	16 mμ(3)‡	25-30 mμ(21)	25-30 mμ(20)	25-30 mμ(20)
" 18, 26-28 mμ(24)								
Nucleic acid type	RNA	RNA	RNA	RNA	RNA	RNA	RNA	RNA
Ether stability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Clinical illness	Adult—"colds" (URI)	—	"Colds"	"Colds"	"Colds"			
Child— "								
" Bronchitis								
" Bronchopneu.								

* Sal./1/58H (FEB) tested only. † Sal./1/57M (HGP) tested only. ‡ Numbers in parentheses are reference numbers.

explanation was found for the extreme variability in heat sensitivity of coryzavirus and rhinovirus M strains. One possibility may have been concerned with variability in content of various components in the virus-containing tissue culture fluids as, *e.g.*, amount of cystine(22). The coryzaviruses, rhinoviruses and ECHO 28 cause infections of the respiratory tract with apparent infrequent infection of the gastrointestinal tract. In the present 15 coryzavirus cases in children, *e.g.*, no such virus was recovered from the stools.

Based on pH stability findings and, less definitively, on heat stability and clinical results, there would appear to be a valid basis for including ECHO 28, the rhinoviruses and the coryzaviruses in a single group with the 2060 strain of ECHO 28 designated as the first agent of the group to be definitively isolated and characterized. Present data suggest also that the strains of virus recovered by Hamre *et al.*(6), by Hobson *et al.*(7) and the strains recovered by Johnson *et al.* and designated as enteroviruses (unpublished) should be included in the same group. The group has been divided(4) into 2 subgroups based on propagability or lack of propagability in monkey renal cell cultures. Of the 20 coryzavirus types reported herein, type 30 alone was propagable in grivet monkey renal cell cultures.

The exact position of the ECHO 28-rhinovirus-coryzavirus group to the family of enteroviruses(23) cannot be definitively stated. The former group shares with the enteroviruses the properties of small size, RNA type, and ether resistance. The property of stabilization by molar Mg^{++} to 50°C for 60 minutes(24) was tested with a few strains. The coryzavirus-rhinovirus agents proved to be of variable stability on heating alone under these conditions. However, strains of coryzavirus and rhinovirus which proved sufficiently heat sensitive to permit testing were stabilized by Mg^{++}. The acid lability, relative heat stability and clinical properties of the ECHO 28-rhinovirus-coryzavirus group differentiate them clearly from the ECHO 1-27 and Coxsackie viruses. The precise size and structural properties of the ECHO 28-rhinovirus-coryzavirus group agents are not definitively established. Preliminary data for one coryzavirus, *i.e.*, strain 68 of type 11, indicated(5) a smaller size (17-18 mμ) than generally ascribed to enteroviruses (25-30 mμ). The filtration and centrifugation methods employed were not sufficiently precise to provide a definite answer. The small size particles seen in crystals inside infected cells may have been subject to shrinkage and compression distortion during fixation. Howatson and Almeida(25) demonstrated particles in HeLa cells infected with strain 93 of type 18 coryzavirus stained with phosphotungstic acid. These particles measured 26-28 mμ in diameter and appeared to have a definite substructure. The coryzavirus particles appeared to be similar to poliovirus particles observed for control purpose. More revealing structural studies with capsomere counts should facilitate in clarifying the relationships of these agents. In the present study, the coryzavirus types were designated 11-30 to permit strains of earlier recovery by other workers to be designated 1-10.

Like many newly discovered agents, these viruses suffer from excessive nomenclature. It seems desirable, as suggested by Tyrrell (17), that another single name should be sought which is based perhaps on other than clinical properties. Such a name might well await the precise definition of size, shape and structural characteristics of the agents. Meantime, we have found it convenient to refer to the ECHO 28-rhinovirus-coryzavirus agents as ERC viruses until a final name is selected.

Summary. Thirty-five coryzavirus strains were recovered from cases of common cold in adults and from "colds" (upper respiratory illness), croup, bronchitis and bronchopneumonia in children. Two additional viruses were recovered from clinically well persons. These viruses were divided into 20 distinct serotypes based on tests with specific guinea pig antisera. Nineteen of the 20 types grew only in human cell cultures (H) while a single type (30) propagated also in grivet monkey kidney cells (M). None of the coryzavirus strains was related immunologically to any of the rhinoviruses, or to the ECHO, Coxsackie A or B, or reoviruses. The coryzaviruses shared properties with the ECHO 28 and

rhinoviruses which provided a basis for the designation of an ECHO 28-rhinovirus-coryzavirus (ERC) group and for differentiating them from the remaining ECHO and Coxsackie A and B viruses. The ERC agents share with ECHO-Coxsackie A and B agents the properties of small size, RNA type, and ether stability, but differ from them in acid lability and in infection of the respiratory tract of man in contrast to the usual habitat in the gut of the ECHO and Coxsackie agents. The H strains of coryzavirus and rhinovirus also differed from the ECHO 1-27 and Coxsackie viruses in their relative stability to heat. The M strains of rhinovirus and coryzavirus and ECHO 28 were intermediate and showed highly variable heat stability properties. The exact position and nomenclature of the ERC viruses remain to be determined.

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