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Contributions to Characterization and Classification of Animal Viruses. (28247)

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The virus "explosion" of the past two decades has emphasized the need for systematic organization of the known viruses. Such information is of importance not only from the standpoint of taxonomy and identification, but to provide inferential knowledge of properties of viruses based on known qualities of related agents.

From the purely taxonomic standpoint, viruses, like other microbes, might be classified best according to their chemical and physical properties such as size, shape, chemical composition, and the like. Certain of these qualities, however, may not be easy to measure and may be of little use for identification in the routine laboratory. Classification systems, therefore, which embrace easily measured basic properties provide a bonus in being useful in the practical sense.

The ECHO 28 - rhinovirus - coryzavirus (ERC) group(1-3) of agents associated etiologically with the common cold(4) is included presently in the Picornavirus family and shares with the enterovirus subgroup (poliovirus, Coxsackie A and B, ECHO) the properties of small size, RNA type, and ether stability. During studies with the ERC agents in our laboratory(1,2), it was found that measurement of lability at pH 3.0 provided a simple and reliable means for separating these agents from the enteroviruses. Further studies have shown that the quality of acid lability or stability is an apparently stable property of viruses which is measured easily and is of very great value as a criterion for secondary separation of virus groups. Tests for nucleic acid type, for stability or instability to lipid solvents (ether or chloroform) and for acid stability or lability were found to provide a simple and easy means for primary subgrouping of viruses. Further subgrouping into families generally required an

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additional measurement such as for size, shape or for heat stability or lability.

This report summarizes the results of tests of a number of viruses for acid lability, for nucleic acid type, and for ether or chloroform sensitivity. These data, together with other known properties of viruses, have been assembled to extend classification schemes or to give a new slant to recent efforts of others(5,6) in the area of viral nomenclature. The study and review make no presumption for completeness. Instead, they are exemplary and place special emphasis on agents likely to be encountered in a virus diagnostic laboratory where human materials are under investigation.

Materials and methods. Cell cultures. Cultures of the WI-26 strain of diploid human fibroblasts(7) and of grivet renal cells were prepared and handled as described earlier (1,7). HeLa cell and human heart cultures and the WISH continuous line of human amnion(8) were propagated in Eagle's medium containing 5% of inactivated calf serum. Primary cell cultures of rabbit kidney and hamster kidney were prepared from trypsinized cell suspensions and were grown in Eagle's medium containing 10% of inactivated calf serum. Cultures of mouse embryo cells were prepared as previously described(9) except that 10% of inactivated horse serum was substituted for the human serum. Chick embryo cell cultures were prepared according to Katz *et al.*(10) except that medium 199 with 2% of inactivated calf serum was substituted for the bovine amniotic fluid medium. During virus propagation, the monkey and rabbit kidney cell cultures were maintained with Eagle's medium containing 2% of inactivated chicken serum. The remaining kinds of cell cultures were maintained with the same medium used for growth, but with reduced amount of serum in most instances. Penicillin and streptomycin at 100 units and 100 μg per ml, respectively, were included in all media.

Viruses. Most of the viruses employed were from the virus seed stock collection regularly maintained in this laboratory. The chick adapted strain of Flury rabies virus was obtained from Dr. T. J. Wiktor of Wistar Institute of Anatomy and Biology. The AD 169 strain of cytomegalovirus was obtained from Dr. W. Rowe of National Institutes of Health and the hamster kidney adapted OCT 541 strain of Japanese B encephalitis was sent by Dr. W. McD. Hammon of the University of Pittsburgh. The Moses strain of rabbit myxoma was furnished by Dr. R. E. Shope of Rockefeller Institute and the strain of Rous sarcoma virus was obtained from Dr. W. Ray Bryan of the National Cancer Institute. All the monkey kidney-adapted influenza viruses were obtained from Dr. W. J. Mogabgab of Tulane University and the CL strain of vaccinia was purchased from the Viral and Ricksettsial Registry. The MI strain of vaccinia virus was derived from commercial calf lymph vaccine and was passed 11 times in embryonated chicken eggs, 15 times in KB cell cultures, and 1 to 3 times in HeLa cell cultures prior to use in the study.

Test for lability at pH 3.0. The method for measuring lability at pH 3.0 has been described(1,2). In certain instances, *i.e.*, rabies and LCM, viral infectivity titrations were carried out in mice. *Nucleic acid type.* Inhibition of DNA virus synthesis was measured according to Salzman(11). By the direct method(1,2), viral suppression was measured in comparative titrations for infectivity in cell cultures in presence of FUDR (5-fluoro-2′-deoxyuridine) and in presence of FUDR plus thymidine. By the indirect method, groups of cell cultures were treated with FUDR or with FUDR plus thymidine and inoculated with a constant amount of virus. After allowing time for virus adsorption, the cell sheet was washed to remove residual virus and refed with fresh medium containing drug or drug plus thymidine. Following a suitable incubation period the cell cultures were harvested and titrated for virus content. In the tests, BUDR (5-bromo-2′-deoxyuridine) sometimes was substituted for FUDR. *Lipid solvents.* Tests for ether sensitivity were according to Andrewes and Horstmann (12) and employed absolute diethyl ether. Chloroform sensitivity was measured by the method of Feldman and Wang(13).

TABLE I. Determination of Acid Sensitivity of Several Animal Viruses.

Family or group	Type and strain	Cell culture system: Virus prep'n	Cell culture system: Virus tit'n	Infectivity titer, neg $\log_{10}$: pH 3.0	Infectivity titer, neg $\log_{10}$: pH 7.2	Conclusion
Adenovirus	3	HeLa	HeLa	2.5	2.5	Stable
	7	"	"	3.0	3.5	"
	5	"	"	5.5	5.5	"
	12	"	"	3.0	2.7	"
Papovavirus						
SV_{40}		Grivet kidney	Grivet kidney	5.0	5.5	"
Polyoma	SE	Mouse embryo	Mouse embryo	4.7	4.7	"
Herpesvirus-like						
Herpes simplex	McIntyre	Rabbit kidney	HeLa	<2.0	4.5	Labile
Varicella	Shaffer	WI-26	WI-26	6/10 pos	10/10 pos	" (tentative)
Cytomegalovirus	AD-169	WI-26	WI-26	<0.0	1.5	"
Poxvirus						
Vaccinia	CL	HeLa	WI-26	<3.0	5.5	"
Myxoma	Moses	Rabbit kidney	Rabbit kidney	1.5	4.5	"
Reovirus	1	Grivet kidney	Grivet kidney	6.5	6.5	Stable
Picornavirus						
Enterovirus						
ECHO	1	" "	" "	7.0	7.0	"
"	16	WI-26	WI-26	5.5	5.5	"
Coxsackie	A- 7	HeLa	HeLa	7.0	7.5	"
	A-21	"	"	6.5	7.0	"
	B- 1	"	"	7.5	8.0	"
ERC (ECHO-rhinovirus-coryzavirus)	ECHO 28	"	"	<1.0	5.5	Labile
	H-11	WI-26	WI-26	<1.0	4.0	"
	M-30	"	"	<1.0	4.0	"
Arbovirus						
Group A — EEE	823A	Chick embryo T.C.	Chick embryo T.C.	3.5	7.7	Labile
" B — Jap. B	OCT 541	Hamster kidney	Hamster kidney	<2.0	5.5	"
Myxovirus						
Influenza	A1	Grivet kidney	Grivet kidney	<2.0	4.5	"
	A2	" "	" "	<2.0	3.0	"
	B1	" "	" "	<2.0	5.0	"
	B2	" "	" "	<2.0	4.2	"
Parainfluenza	1	" "	" "	<1.0	5.0	"
	2	" "	" "	<1.0	6.0	"
	3	" "	" "	<1.0	5.5	"

In all titrations, 4 to 8 tubes were used per dilution.

Results. Tests for pH 3.0 lability. The results of tests to determine the sensitivity of a number of viruses to a low pH are summarized in Table I. A reduction in titer of 1 $\log_{10}$ or more of virus on treatment at pH 3.0 for 30 minutes was interpreted as acid lability while retention of full titer or loss of less than 1 $\log_{10}$ of activity was considered indicative of acid stability. Adenoviruses, papovaviruses, reovirus, and enteroviruses were stable while the herpesvirus-like agents (herpes simplex, varicella, cytomegalovirus), poxviruses (vaccinia, myxoma), ERC viruses (ECHO 28, rhinovirus, coryzavirus), arbovirus A and B, and Myxoviruses (influenza, parainfluenza) or Myxovirus-like agents (measles, respiratory syncytial, rabies, Rous sarcoma), encephalomyocarditis and lymphocytic choriomeningitis were labile. The varicella virus used was, by necessity, of low titer before treatment and could not be tested in the absence of cells or cell particulates. Hence, the conclusion must be regarded as tentative. The LCM virus, unlike all the other viruses which were grown in cell cultures, was propagated in animal tissues and the agent might have been "aggregated" by low pH to give lower apparent than real titers. For this virus also, the conclusion must be regarded as tentative.

Tests for nucleic acid type. Table II shows the results of tests to determine the nucleic acid type for 9 different viruses with findings as anticipated. The indirect test was generally as reliable as the direct assay. It was worthy of note that the FUDR test was not applicable to the monkey kidney cell system, possibly because the compound is broken down in certain primary cell cultures(14). BUDR gave reliable results in monkey renal cell cultures† due to the fact that this compound does not suffer the same fate at FUDR.

Tests for destruction by organic solvents. Diethyl ether long has been employed for purpose of ascertaining whether a particular virus contains an essential lipid(12). Feldman and Wang(13) have suggested substitu-

† Tytell, A. A., unpublished.

TABLE I (continued).

Family or group	Type and strain	Cell culture system: Virus prep'n	Cell culture system: Virus tit'n	Infectivity titer, neg $\log_{10}$: pH 3.0	Infectivity titer, neg $\log_{10}$: pH 7.2	Conclusion
Measles	Edmonston	Human amnion (WISH)	Human amnion (WISH)	<1.0	3.5	"
Respiratory syncytial	Long	HeLa	HeLa	<1.0	3.7	"
Rabies	Flury	Chick embryo T.C.	Newborn mouse	<1.6	4.5	"
Rous	Bryan	Wing web tumor	Chick embryo T.C.	<3.0 ffu	$1.1 \times 10^{7.0}$ ffu	"
Miscellaneous						
Encephalomyocarditis	Strain B	Human heart T.C.	WI-26	3.5	7.0	"
Lymphocytic choriomeningitis	EW	Adult mouse brain	Adult mouse	3.2	4.8	" (tentative)

TABLE II. Determination of Nucleic Acid Type of Several Animal Viruses.

Virus	Cell culture test system	Kind of test	Infectivity titer, neg $\log_{10}$ FUDR	FUDR + thymidine	Nucleic acid type
Adenovirus 5	WI-26	Indirect	3.0	6.5 or >	DNA
Vaccinia	"	Direct	1.7	5.2	"
Herpes simplex	"	"	3.5	5.7	"
	"	Indirect	2.7	4.5	"
	Grivet kidney	Direct	5.2	4.7	See text
	" "	Indirect	6.2	6.5	" "
Cytomegalovirus	WI-26	Direct	<1.0*	1.5	DNA
ECHO 28	"	"	4.5	4.7	RNA
Measles	"	Indirect	2.7	3.0	"
Respiratory syncytial	"	"	1.7	1.5	"
Rabies	Chick embryo	"	3.1*	2.7	"
Encephalomyocarditis	WI-26	Direct	7.0	6.7	"

* 5-bromo-deoxyuridine was substituted for FUDR.

tion of chloroform for this purpose because of its greater efficiency as a lipid solvent. A special utility of chloroform for detecting essential lipid in poxgroup viruses was found in the present study and is illustrated in Table III for vaccinia. It is seen that significant loss of titer, *i.e.*, 1 $\log_{10}$ or greater, was more consistent and of greater mean magnitude when chloroform was employed than when ether was used.

Discussion. Bases for classification. pH 3.0 lability or stability appears to be a stable basic property of animal viruses which, in most instances, can be measured with simplicity and certainty. Hence, it has importance as a basis for virus classification and utility for identification of agents in the routine laboratory. The method is most applicable to viruses which can be grown in cell culture systems and can be tested relatively free of cellular constituents.

Within the limits of present experience, neither media composition nor viral passage history has shown any apparent effect on the property of acid lability. Such possibility may exist, however, and it may be necessary eventually to settle on a standard diluent for conduct of tests and to evaluate findings in relation to history of the virus in the laboratory.

In situations in which suspensions of infected tissue are employed for test or in which the virus grown in culture is strongly cell-associated, as for example varicella, the method is of less value. Among the viruses considered in the present study, and except for

TABLE III. Comparison of Sensitivity of Vaccinia Virus to Diethyl Ether and to Chloroform.

Vaccinia virus strain	Virus* titration system	Infectivity titer, neg $\log_{10}$ Untreated	Treated Ether Titer	Ether Loss	Chloroform Titer	Chloroform Loss
MI	HeLa	4.8	4.7	.1	<2.0	>2.8
"	WI-26	5.2	4.2	1.0	2.7	2.5
"	"	5.0	3.0	2.0	3.9	1.1
"	"	5.2	4.0	1.2	—	—
"	"	4.2	<2.0	>2.2	—	—
"	HeLa	5.3	3.6	1.7	—	—
"	WI-26	5.6	—	—	<2.0	>3.6
CL	HeLa	5.3	5.5	0	3.8	1.5
"	"	5.3	4.7	.7	—	—
"	WI-26	5.3	4.0	1.3	—	—

* All test viruses propagated in HeLa cell cultures. For infectivity titrations, serial 10-fold dilutions were used and 8 cultures were employed per dilution.

the ERC agents, there was close correlation between the quality of ether stability and of acid stability.

The determination of *nucleic acid type* by FUDR or by BUDR inhibition, whether by the direct or indirect method, appears to be a simple and reliable criterion which can be obtained rapidly. The workability of the test in the particular cell systems employed always was controlled by including known RNA and DNA viruses. The hazard of lack of controls was exemplified clearly in the present studies in which tests with FUDR in the grivet system indicated that herpes simplex virus was of RNA type. This clearly was not the case and the findings readily were explainable on the basis of the ability of monkey renal cells to break down FUDR(14) rendering it non-inhibitory for this DNA virus.

Measurement of *essential lipid* by tests for sensitivity to organic solvents must employ solvents which are sufficiently efficient to provide definitive and reproducible results. For most viruses, tests with ether are adequate. As shown in the present study, with vaccinia virus, chloroform gives far more consistent and reliable results than does ether.

Measurement of *heat stability or lability* may prove valuable as a criterion for separating certain subgroups such as for differentiating reovirus from enterovirus, but may give variable results as with the H strains of ERC group viruses(1,2) and may be influenced by the quantity of certain media constituents such as L-cysteine(15) which is difficult to control. It was found in the present study (not presented above) that vaccinia, SV_{40} and reovirus 1 were relatively stable on heating at 50°C for ½ hour while herpes simplex, adenovirus type 5, measles and parainfluenza 1, 2 and 3 viruses were not. For certain viruses, tests for heat stabilization by divalent cation may be of value. Melnick and coworkers(16,17) have shown that the enteroviruses and reoviruses were so stabilized while adenoviruses, papovaviruses, herpesviruses, Myxoviruses, arbovirus and poxvirus were not.

Tests for inhibition of virus by 2-(α hydroxybenzyl)-benzimidazole (HBB) and by guanidine HCl have been used by Tamm and Eggers(18) in the monkey kidney cell culture system for viral differentiation within the Picornavirus group. Thus: (a) poliovirus, Coxsackie B and the majority of ECHO viruses were inhibited both by HBB and by guanidine HCl; (b) Coxsackie A and HGP (M strain Sal/1/57M of rhinovirus) viruses are inhibited by guanidine HCl but not by HBB; (c) ECHO 28 and B 632 (M strain Sal/1/60M of rhinovirus) and ECHO 22 and 23 viruses are not inhibited by either drug.

Tests for HBB sensitivity of the ERC viruses in monkey kidney cultures have limited utility for diagnostic virologic purpose since the majority of the known types are of H (human) variety and grow in human but not in monkey kidney cells. In studies not recorded in the text and in which WI-26 cell cultures were used, the results were variable. It was found that all H and M strains of ERC virus tested [ECHO 28 (M), rhinovirus HGP (M) and FEB (Sal/1/58H), and coryzavirus types 11 and 18 (H strains)] were inhibited in one experiment or another by HBB but there was no dependable differential quality since ECHO 16 and Coxsackie A7 and 13 were also inhibited. In the HeLa cell system, ECHO 28, rhinovirus HGP (M) and FEB (H), coryzavirus 11, and Coxsackie A21 were not inhibited by HBB but Coxsackie B1 was. These studies indicate that HBB presently is of little use for simple differentiation of ERC viruses from enteroviruses in the routine laboratory.

System for classification. Cooper(5) and Andrewes *et al.*(6) have emphasized recently the importance of stable physical and chemical properties such as nucleic acid type, size, structure, and ether sensitivity, as prime characters to be used for virus differentiation. Cooper used nucleic acid type as the first criterion, ether sensitivity as the second criterion, with size and immunologic characters as tertiary characters. Fig. 1 presents a classification scheme for certain viruses based on Cooper's system(5) but using acid lability as the third criterion after nucleic acid type and ether stability. Findings in the present stud-

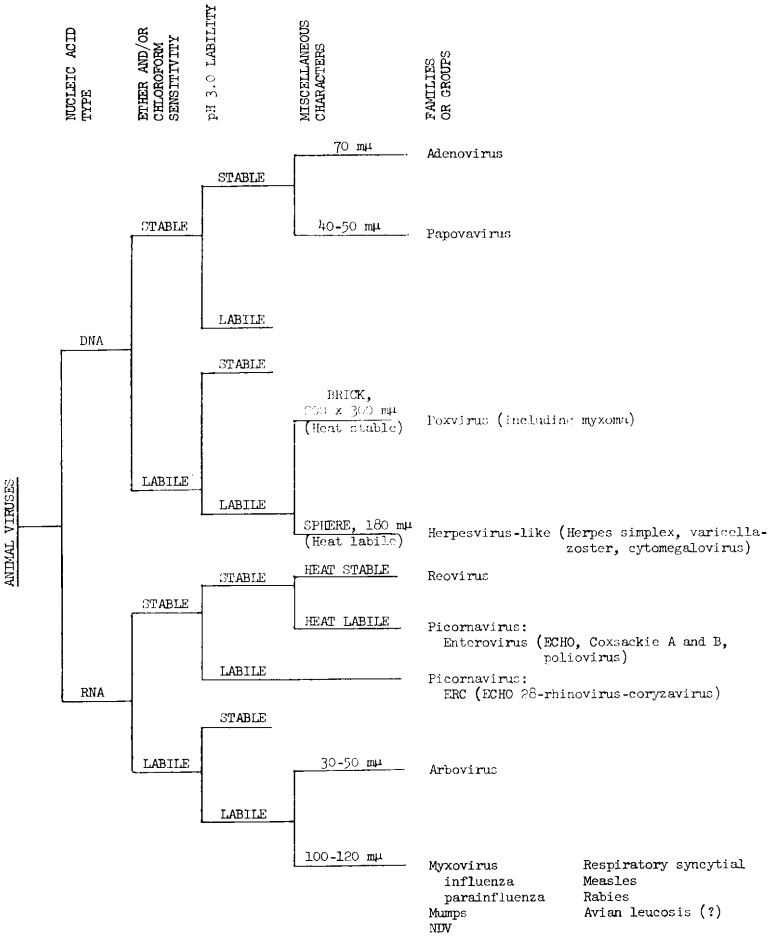

FIG. 1. Schematic position of several animal virus families or groups.

ies were included in preparing the Figure.

Certain of the natural relationships between viruses merit discussion in the light of the presentation in Fig. 1. In this, no indication of completeness is intended and general or review references have been used wherever possible. Additionally, properties of single or several viruses of a particular group have been regarded as characteristic of the entire group even though such properties of all the individual viruses presently may not be known.

Adenoviruses. The adenoviruses are a clearly defined group of viruses(19). These agents are of DNA type, are ether resistant, and all presently accepted members share a common group CF antigen. The agents of the group are about 70 mμ in diameter and show an icosahedral form(20,21) with 252 capsomeres(21) in the capsid. Multiplication is nuclear and there is no membrane outside the capsid. Latent and chronic infections are common. These viruses are not stabilized by divalent cation(17) on heating at 50°C.

Papovaviruses. Melnick(22) recently has pointed out the similarities of rabbit and human papilloma, mouse polyoma and vacuolating agent (SV_{40}) viruses which seem to form a natural group of tumor viruses. These viruses are of double-stranded DNA type, are ether resistant, and are stable on treatment at pH 3.0. The multiplication site is in the nucleus. There is disagreement as to whether these agents have 42(22) or 92(23) capsomeres. The viruses are stable on heating at

50°C in water but are labile in the presence of divalent cation.

Differentiation between adenoviruses and papovaviruses can be accomplished readily in tests for adenovirus group CF antigen. In the present study, adenovirus type 5 was found to be labile on heating at 50°C for ½ hour, while SV_{40} virus was not. If the other agents in these groups react similarly, then heat sensitivity tests may provide an additional technique for differentiating the two groups. Size differentiation is more difficult to achieve. Cytopathology is of considerable help in that SV_{40} virus causes vacuolization, polyoma viruses cause cell necrosis and pyknosis and human wart virus is reported to cause ballooning and vacuolization(24). By contrast, adenoviruses typically cause cell rounding followed usually by formation of clusters of affected cells.

Poxviruses. The poxviruses(25-30) are large (200 × 300 mμ) DNA viruses which share a common nucleoprotein antigen. Multiplication is in the cytoplasm. Presently included in the group are the viruses of smallpox, vaccinia, cowpox, rabbitpox, ectromelia, rabbit myxoma, rabbit fibroma, the avian poxviruses, monkeypox and bovine papular stomatitis. Also included may be the viruses of molluscum contagiosum, milker's nodules (? cowpox), ovine pustular dermatitis, and certain other poxviruses of domestic and wild animals not as yet well enough studied to ascertain whether they belong to the group. Present evidence suggests that reliable demonstration of essential lipid for certain members of the group depends on use of chloroform.

Herpes-like viruses. Herpes simplex, varicella-zoster, and cytomegalic inclusion disease viruses share many properties which presently suggest, but do not establish, that they may comprise a single family of viral agents. All are of DNA type, are ether and acid labile, and form eosinophilic type A intranuclear inclusions surrounded by marginated chromatin (31-38, present data). Herpes simplex virus consists of a core or nucleoid (78 mμ) surrounded by a capsid (105 mμ) of icosahedral structure and enclosed in an envelope (180 mμ). The capsid consists of 162 capsomeres which show 5:3:2 axial symmetry in their arrangement. The capsomeres appear to be hollow structures. The virus may appear in cut sections in a variety of forms. Essentially, however, the nucleoid and capsid appear to be formed in the nucleus and the envelope, consisting of one or more membranes, is acquired prior to or during passage into the cell cytoplasm. Virus B of monkeys is related closely to herpes simplex. Varicella virus shows essentially identical morphology to herpes simplex virus(34). Varicella and herpes zoster viruses are generally regarded to be but a single agent recovered from 2 different clinical manifestations of viral infection(35). Less is known concerning cytomegalovirus. The gross structure of the virus, however, is like that of herpes simplex (36) and the virus appears to be formed in the cell in a manner similar to that of herpes simplex(36-38). Pereira(39) has pointed out that in addition to the recognized members of the herpesvirus group (herpes simplex, pseudo-rabies, virus B and virus III) and those mentioned above, there are sufficient resemblances shown by other agents to suggest a possible relationship to the group. These include infectious bovine rhinotracheitis, feline rhinotracheitis, infectious laryngotracheitis, equine abortion viruses, and two groups of agents recovered from lumpy skin disease of cattle.

Perhaps the simplest means for differentiating between the herpes-like and the poxviruses is the demonstration of eosinophilic type A nuclear inclusions in the former and eosinophilic masses (Guarnieri bodies) in the latter. Vaccinia virus was found in the present study to be relatively heat stable while the herpes-like viruses were not. If heat stability is a general quality of poxviruses, then tests for heat sensitivity may provide an additional means for differentiating the two groups. Cytopathology caused by herpes simplex virus consists predominantly of focal necrosis, amitotic cell division, and formation of giant cells. For certain strains, large syncytia may be produced. For poxviruses, diffuse cell degeneration with rounding, swell-

ing, clumping, cytoplasmic granulation and frequent formation of multinucleated giant cells is characteristic(39).

Picornavirus. The picornavirus group presently includes those viruses designated as enteroviruses [ECHO (exclusive of ECHO 28), Coxsackie A and B, and poliovirus](40) plus the ERC (ECHO 28, rhinovirus, coryzavirus) agents(1,2,41,42) which appear to be the primary cause for most "common colds" in human adults(3,43,44) and which cause "colds" as well as lower respiratory tract illnesses in children(3). The first ERC viruses, *i.e.*, 2060 (45) and JH(46) to be recovered were placed in the enterovirus group and were designated ECHO 28(47). There is now a valid basis for placing ECHO 28 with the ERC agents (1).

The picornaviruses are small RNA viruses and contain no essential lipid. The enterovirus subgroup can be distinguished readily from the ERC viruses by the stability of the former and instability of the latter to treatment with acid at pH 3.0 for 30 minutes. The enteroviruses measure 25-30 mμ in diameter. The enteroviruses are relatively labile to heating at 50°C for ½ hour. The H types of the ERC group, *i.e.*, strains which grow in human cells but not in monkey renal cells, are relatively stable to such treatment. The M types of ERC agents, by contrast, are variable in their sensitivity to heat, even in repeat tests with the same strain of virus. The particle size for the ERC viruses has not been established fully to date(1,2,44). Type 11 was found to measure 17-18 mμ in crystals and type 18 was shown to be 26-28 mμ in diameter when measured as free particles.

Andrewes *et al.*(6) have included the viruses of foot-and-mouth disease, Teschen disease and probably encephalomyocarditis viruses in the picornavirus (formerly nanivirus) group. The acid lability of encephalomyocarditis virus would place it closer to the ERC agents than to enteroviruses.

Reovirus. The reoviruses(48,49), like picornaviruses, are of RNA type and are ether stable. The reoviruses differ from enteroviruses in that they are larger (70 mμ) and are stable on heating at 50°C(1). The reoviruses differ from the ERC viruses with respect to size. Perhaps the simplest means for distinguishing the reoviruses from ERC agents is in the acid stability of the former. Additionally, hemagglutination of type O human erythrocytes, propagation in newborn mice, and distinctive cytoplasmic inclusions are properties that the reoviruses possess but the ERC agents do not. Further, it may be noted that all reovirus types grow and produce cytopathology in monkey renal cell cultures while the H types of ERC virus do not. The reovirus types share a group-specific complement-fixing antigen. The reoviruses are presumed to be of icosahedral shape with 5:3:2 symmetry(49).

Arbovirus. These viruses(50-52) are of RNA type, are small (30-50 mμ)(5), and are ether labile(12) and acid labile. There are presently at least 125 viruses in the family in 5 or more subgroups. The most outstanding distinctive quality for these viruses is their multiplication in an arthropod vector. Cytopathology is principally necrotic(53) in contrast to the prominent syncytium formation noted for the Myxovirus. Arboviruses usually cause encephalitis in suckling mice and this property is useful for differentiation from most myxoviruses.

Myxoviruses. The Myxovirus group(6,54) generally is held to be a family of viruses which are of medium size, of RNA type, are sensitive to destruction by ethyl ether, and consist of a central core which is surrounded by a membrane. To this can be added the property of acid lability. The Myxoviruses appear to mature at the cell surface and not to exist in complete form inside the cell. The Myxoviruses exhibit properties of hemagglutination, of hemadsorption and of virus elution. Cytopathology, when present, consists mainly of cell lysis with formation of giant cells or syncytia.

Influenza, parainfluenza, Newcastle Disease virus, and simian virus 5 (SV_5) clearly belong in the Myxovirus group. Certain influenza viruses of animals including swine influenza, fowl plague, and equine influenza belong to influenza type A based on their common complement-fixing antigen(55).

Respiratory syncytial virus(56-59), like the Myxoviruses, is of medium size, is of RNA type (present report), is destroyed by ethyl ether, and is acid labile. It propagates in cell cultures ordinarily used to grow Myxoviruses and causes the characteristic syncytial changes. Unlike most Myxoviruses, the respiratory syncytial agent fails to propagate in embryonated hen's eggs, fails to cause overt disease in common laboratory animals, and fails to cause hemagglutination or hemadsorption.

Measles virus shares the general properties of Myxoviruses including medium size(60, 61), RNA type (present report), destruction by diethyl ether(60,61), and acid lability. It causes the characteristic syncytial changes (61), propagates in embryonated eggs, causes hemagglutination and hemadsorption(62), and shows a structure not unlike that of certain Myxoviruses(60). Further, it causes hemolysis(61) as is shown for mumps, NDV, and certain parainfluenza viruses. Distemper and rinderpest viruses are very similar to measles and show antigenic crossing(61,63).

Rabies virus is also of medium size(64), is of RNA type (present study), is destroyed by diethyl ether(65) and is acid labile (present study). Almeida and coworkers(64) have shown recently a structure typical of that of the Myxoviruses, particularly the mumps, parainfluenza, and NDV agents. The agent fails to cause cytopathology when grown in cultures of hamster kidney(66).

Andrewes *et al.*(6) have proposed that the ether sensitive, RNA type avian leucosis viruses probably are related closely to the Myxoviruses. Rous sarcoma virus, a member of the leucosis group, was shown in the present study to share the property of acid lability.

Lymphocytic choriomeningitis. Relatively little is known about the biological position of this agent. It is in the small size range (33-60 mμ), is ether labile(12) and is relatively heat sensitive(67). It appears to be acid labile.

Summary. Systematic organization of the known viruses is important from the taxonomic viewpoint and in the practical sense in providing inferential knowledge based on known qualities of related agents. Determination of nucleic acid type, sensitivity to organic solvents, pH 3.0 lability, size, and structure provide excellent basic criteria for grouping viruses. Further subgrouping can be based on factors such as heat stability, sensitivity to drugs, cytopathology, and type of inclusions. Acid lability tests and nucleic acid typing were carried out for a number of viruses and methods for determining essential lipid, drug sensitivity and heat lability were explored. A simplified working scheme for classification and identification of viruses is presented.

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