VIRUSES *of* VERTEBRATES

Sir CHRISTOPHER ANDREWES

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Sir CHRISTOPHER ANDREWES
M.D., F.R.C.P., F.R.S.
Late Wellcome Trust Fellow, Late Deputy-Director
National Institute for Medical Research
Mill Hill, London

and

H. G. PEREIRA
M.D.
National Institute for Medical Research
Mill Hill, London

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Viruses of Vertebrates

Viruses of Vertebrates has become the standard work of reference on the properties and characteristics of viruses affecting man, domestic animals, birds and other vertebrates. In this new edition systematic arrangement of the contents reflects developments in classification, and unclassified viruses are now relatively few. Some rearrangement has also been possible in the enlargement of generic descriptions of morphology and chemical and physical properties so that facts are not repeated in the descriptions of individual viruses.

Painstaking study and condensation of essential information have been necessary to keep the extent of the new edition within reasonable bounds, and the authors have achieved a clear and readable prose style coupled with a very considerable factual content. The continuing increase in the use of laboratory animals and tissue cultures, the development of electron microscopy, and recognition of the importance of the zoonoses, all increase the likelihood of workers being faced with a new or unfamiliar virus. The virologist, the student and researcher in allied fields of microbiology will find this edition of 'Andrewes and Pereira' an invaluable addition to their library shelves.

Preface to the Third Edition

Since 1967, when the Second Edition of this book was published, it has become steadily clearer that most viruses can be logically placed into genera. These genera have morphological, chemical and physical characters in common, so that it is justifiable to deal with these in a generic description and to avoid repeating the facts under accounts of individual viruses. The reader will therefore have to seek more information under generic descriptions at the beginning of each chapter. The International Committee for the Nomenclature of Viruses has erected a number of new genera, so that there are eleven more here than in the Second Edition of this book. Further a move has been made towards creating higher taxa: two families, Picornaviridae and Papovaviridae, with three and two genera respectively have been created. A third family, Togaviridae, proposed by an Arbovirus Study Group, although not officially approved by the ICNV, will be adopted to cover the genera Alphavirus and Flavovirus.

The wholly unclassified viruses have become steadily fewer; they are now dealt with in one chapter of 15 pages, compared with six chapters of 72 pages in the First Edition and five chapters of 46 pages in the Second Edition.

To avoid overloading the book it has been necessary to be very selective in the matter of references. We have therefore included those likely to be most helpful, either because they afford the fullest account of the facts, or because they describe recent work not referred to in other books or reviews. We have accordingly frequently omitted reference to the first reported description of a new finding, though a few classical papers are mentioned.

C. H. ANDREWES
H. G. PEREIRA

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Preface to the First Edition

There are books on the principles of virology and books on virus diseases, especially those of man. This volume deals rather with the viruses themselves, their properties and their relations to one another. Their pathogenic effects are, in principle, considered only so far as is necessary to identify a virus and to indicate its importance. In practice an account of the disease-producing powers of viruses will be found to occupy considerable space, for it is largely by their deeds that we know them.

Viruses attacking only insects are not considered nor are plant viruses. The book does, however, deal not only with viruses of man but also with those affecting other vertebrates. The wider view is necessary if one is to consider viruses from the point of view of taxonomy. Moreover, with the widespread use of various laboratory animals and of tissue cultures derived from various species, workers are bound to encounter viruses of unknown origin. The importance of the zoonoses—diseases of other animals transmissible to man—also is increasingly recognized.

The book will, it is hoped, serve three purposes. By the orderly arrangement of the known facts about viruses it should help the enquirer to discover rapidly whether this or that is known about a particular virus, and guide him to fuller sources of information on the matter. Secondly it is intended to help him, as Bergey's manual helps the bacteriologist, to identify an unknown virus which he may encounter. Thirdly, there is, I hope, a long-term scientific justification for the book. We do not know enough to classify all viruses in an orderly manner. A partial attempt to do so is made in Parts I and II by arranging into groups such viruses as seem ripe for such an attempt. I have tried to avoid what I feel is a pit-fall, the proposal of a classification in advance of adequate knowledge: so Part III contains the viruses which we cannot yet classify rationally, arranged according to the species they attack. It is hoped that as knowledge grows, one may be able, in possible

future editions, to promote more and more viruses from Part III to a more satisfactory status in Parts I and II.

Finally I apologize, particularly to many veterinary friends, for the presumptuous attempt of an individual virologist to deal with all viruses of vertebrates.

January 1964 C. H. ANDREWES

Abbreviations

The following conventional abbreviations have been used:

BUDR = 5-bromo-2′-deoxyuridine
CAM = chorio-allantoic membrane
CE = chick embryo
CF(T) = complement fixation (test)
CNS = central nervous system
CPR = cytopathic effect
CSF = cerebrospinal fluid
DNA = deoxyribonucleic acid
EM = electron microscope (or microscopic)
EMC = encephalomyocarditis
FUDR = 5-fluoro-2′-deoxyuridine

$$\text{GC content} = \text{moles} \frac{\text{guanine} + \text{cytosine}}{\text{total nucleotides}} \times 100$$

HA = hæmagglutination
HAI = hæmagglutination-inhibition
IB = inclusion body
IC = intracerebral
IM = intramuscular
IN = intranasal
IP = intraperitoneal
IUDR = 5-iodo-2′-deoxyuridine
IV = intravenous
MK = monkey kidney
MW = molecular weight
NDV = Newcastle disease virus
RBC = red blood cell
RDE = receptor destroying enzyme
RNA = ribonucleic acid
SV = simian virus
TC = tissue culture
VN(T) = virus neutralization (test)
UV = ultraviolet

TABLE 1

PROVISIONAL VIRUS CLASSIFICATION

Nucleic acid	RNA				DNA		
Symmetry	Cubical		Helical	Uncertain	Cubical		Uncertain
Presence of outer membrane	0 (ether-stable)	+ (ether-labile)	+ (ether-labile)	+ (ether-labile)	0 (ether-stable or -labile)	+ (ether-labile)	+ (ether-stability varies within the group)
	Picornaviridae	Togaviridae	Orthomyxovirus Paramyxovirus Rhabdovirus	Leukovirus Arenavirus Coronavirus	Parvovirus Papovaviridae Adenovirus Iridovirus	Herpesvirus	Poxvirus
	Reovirus						

Contents

PART I

RNA Viruses

1

Picornaviridae

Review: Plummer (1965).

This has been adopted as a family name to include the 3 genera enterovirus, rhinovirus and calicivirus. The family is characterized as follows: virions consist of a naked capsid with icosahedral symmetry, containing 20 to 30 per cent single-stranded RNA with a molecular weight of $2{\cdot}5 \times 10^6$ to $2{\cdot}8 \times 10^6$ daltons. Over-all diameter 30 to 40 nm. Ether-resistant. Viral replication not dependent on cellular DNA function. Viral synthesis and maturation in the cytoplasm.

ENTEROVIRUS

This genus includes viruses inhabiting preferentially the intestinal tract of vertebrate hosts, such as polioviruses, coxsackieviruses echoviruses, and a number of bovine, porcine, murine, avian and other viruses. Besides the habitat, the main character distinguishing these viruses from those of other genera of Picornaviridae is their stability at acid pH. Cationic stabilization to thermal inactivation (Wallis & Melnick, 1962) and inhibition of viral replication by 2-(α-hydroxybenzyl) benzimidazole (Eggers & Tamm, 1961) have also been suggested as characters peculiar to the genus, but exceptional behaviour is observed with some members (see Plummer, 1965).

Morphology. Electron microscopic examination of poliovirus (Dales et al., 1965; Mattern & Daniel, 1956; Horne & Nagington, 1959), coxsackieviruses A (Mattern & DuBuy, 1956) and B (Morgan et al., 1959) and echoviruses (Duffy et al., 1962; Rifkind et al., 1961; Jamison, 1969) reveals approximately spherical naked virions with uniform diameter variously estimated between 20 and 30 nm and a nucleoid 6 to 20 nm across. Variations in size have been reported by Jamison & Mayor (1966) who describe poliovirus 1 and echovirus 19 as being larger (mean diameter 23·5 nm) than other echoviruses and coxsackie B2 (mean diameter 21·0 nm). Finch & Klug (1959) suggested on the basis of X-ray diffraction studies that polioviruses have icosahedral symmetry with 60 protein sub-units 6 to 6·5 nm across forming

a shell around an RNA core. Icosahedral capsids made up of 32 (Mayor, 1964; Jamison, 1969) or 42 (Agrawal, 1966) capsomeres have been suggested on the basis of negative contrast pictures of poliovirus.

The morphogenesis of poliovirus (Mattern & Daniel, 1956; Dales et al., 1965), coxsackievirus (Stuart & Fogh, 1961) and echovirus (Rifkind et al., 1961; Duffy et al., 1962) takes place mainly in the cytoplasm although nuclear and nucleolar participation in viral synthesis has been suggested. Virions are often seen in association with small cytoplasmic vesicles or with fibrillar structures. Crystalline arrays of virions are often seen. The role of cytoplasmic membranes in poliovirus biosynthesis has been described by Caliguri & Tamm (1970).

Similar morphological features have been described for enteroviruses of murine (Leyon, 1951; Dales & Franklin, 1962), bovine (Polson & Kipps, 1965; Gralheer et al., 1965), avian (Richter et al., 1964) and porcine (Singh et al., 1961; Meyer et al., 1964) origins although it has been suggested that the last are slightly but significantly larger (diameter 32 to 36 nm) than human enteroviruses. McFerran et al. (1971) found no morphological differences between enteroviruses of porcine, bovine and ovine origins, all measuring 28 $\pm$ 3 nm in diameter.

Chemical composition. Infectious single-stranded RNA has been obtained from human (Colter et al., 1957a; Sprunt et al., 1959), porcine (Brown & Stewart, 1960), murine (Colter et al., 1957b; Ada & Anderson, 1959) and avian (Vindel, 1963) enteroviruses. An RNA content of 22 to 30 per cent has been estimated for polioviruses (Schwerdt & Schaffer, 1955) and 21 to 31·7 per cent for murine enteroviruses (Rueckert & Schäfer, 1965; Faulkner et al., 1961; Scraba et al., 1967; Burness, 1970). Recent MW estimates of the RNA of human (Tannock et al., 1970; Granboulan & Girard, 1969) and murine (Burness, 1970) enteroviruses give values of $2{\cdot}5 \times 10^6$ to $2{\cdot}7 \times 10^6$ which are higher than the previously accepted value of 2×10^6.

The base composition of the RNAs of representative enteroviruses is shown in Table 2. A study of polynucleotide sequences by annealing experiments revealed considerable areas of homology in RNAs of poliovirus types 1, 2 and 3 (Young et al., 1968).

Analysis of the protein composition of polioviruses (Maizel & Summers, 1968), coxsackieviruses (Kiehn & Holland, 1970), murine (Burness & Walter, 1967; O'Callahan et al., 1970) and bovine (Johnson & Martin, 1970) enteroviruses reveals 3 major structural polypeptides with molecular weights ranging from 24,000 to 35,000, one minor structural polypeptide in some strains and several nonstructural com-

ponents, the largest being a precursor cleaved into the structural polypeptides on assembly into virions (Jacobson & Baltimore, 1970).

Physico-chemical characters. Physical properties of representative enteroviruses are shown in Table 3. Enteroviruses are resistant to lipid solvents, survive well at −76°C in 50 per cent glycerol in the cold, but on the whole not very easily preserved by lyophilization. Most of the infectivity may be lost through this procedure, although the remaining fraction may survive well. Freeze-drying is withstood better in the

TABLE 2
BASE COMPOSITION OF ENTEROVIRUS RNAs

Virus	*Base composition (per cent)*				*Reference*
	G	*A*	*C*	*U*	
Poliovirus 1	24·7	28·5	21·7	25·0	Schaffer et al., 1960
Poliovirus 2	23·2	28·7	22·5	25·5	
Poliovirus 3	24·0	27·5	21·5	25·7	
Coxsackie A9	27·7	27·0	20·7	24·7	Mattern, 1962
Coxsackie A10	28·3	27·3	21·0	24·8	
Columbia SK (ME)	23·7	25·1	24·2	26·9	Rueckert & Schäfer, 1965
EMC	23·7	27·4	23·5	25·6	Faulkner et al., 1961
EMC	24·4	25·8	25·1	24·8	Burness, 1970
Bovine-VG-5-27	22·6	29·3	26·6	21·6	Martin et al., 1970
Bovine-VP-7-19	22·5	31·3	24·0	22·5	
Bovine-VC-65-182	23·1	31·0	25·0	20·5	

presence of 5 per cent glucose and 5 per cent dextran (Tyrrell & Ridgewell, 1965).

Other physico-chemical properties will be mentioned in the description of each member of the genus.

Antigenic structure. Over 60 antigenic types have been recognized among human enteroviruses. These are divided into 4 subgroups: (International Enterovirus Study Group, 1963): polioviruses (3 serotypes), coxsackieviruses A (24 serotypes), coxsackieviruses B (6 serotypes) and echoviruses (34 serotypes). It has been suggested that new human enteroviruses should be numbered sequentially from 68, irrespective of subgroups (Rosen et al., 1970).

Cultivation. Most, although not all, grow in tissue culture causing a type of cellular damage which is fairly characteristic. Cytological and

TABLE 3

BIOPHYSICAL PROPERTIES OF SOME ENTEROVIRUSES (INFECTIOUS VIRIONS)

Virus	*Particle weight (daltons)*	*Sedimentation coefficient* ($\times 10^{-13}$)	*Diffusion coefficient* (cm^2/sec)	*Frictional ratio*	*Water of hydration (g of water/ g dry virus)*	*Partial specific volume (ml/g)*	*Buoyant density (in CsCl or C_5CO_4) (g/ml)*	*Reference*
Poliovirus 1	$6{\cdot}8 \times 10^6$	160			0·3		1·34	Schwerdt & Schaffer, 1955
Poliovirus 2	$6{\cdot}8 \times 10^6$	158			0·28	0·64	1·34	Schaffer & Schwerdt, 1959
Poliovirus 3	$6{\cdot}4 \times 10^6$	157			0·37			Mattern, 1962
Echovirus 19		157					1·34	Fabiyi et al., 1964
EMC	$8{\cdot}5 \times 10^6$	162·3	$1{\cdot}44 \times 10^{-7}$	1·13	0·29	0·687	1·35	Burness & Clothier, 1970 Goodhart, 1965
Mengo	$8{\cdot}32 \times 10^6$	151	$1{\cdot}44 \times 10^{-7}$	1·1	0·23		1·32	Scraba et al., 1967
Teschen							1·34	Warrington, 1967
Bovine		156					1·34	Polson & Kipps, 1965

biosynthetic alterations associated with the growth of enteroviruses have been reviewed by Godman (1966). Virus is adsorbed to lipoprotein cell receptors present only in susceptible cells (Holland & McLaren, 1961). Cellular resistance to infection may be overcome by using infectious RNA rather than virus (Holland et al., 1959). Mechanisms of viral penetration and uncoating are unknown. Soon after infection the synthesis of cellular nucleic acids and proteins is inhibited. Virus replication is independent of cellular DNA function (Simon, 1961; Reich et al., 1961). Replication of poliovirus (Holland & Bassett, 1964; Crocker et al., 1964, coxsackievirus (Mattern & Chi, 1962), echovirus (Godman et al., 1964) and murine enteroviruses (Hausen, 1962; Franklin & Rosner, 1962) is entirely cytoplasmic. RNA replicative forms have been demonstrated in cells infected with poliovirus (Baltimore et al., 1964; Pons, 1964), EMC virus (Montagnier & Sanders, 1963) and murine encephalomyelitis virus (Hausen, 1965). Virus induced RNA-dependent RNA polymerases have been described during the replication of poliovirus (Baltimore & Franklin, 1963; Holland & Bassett, 1964), mengovirus (Baltimore & Franklin, 1963) and EMC (Horton et al., 1964). Synthesis of early and late proteins leads to the formation of 'procapsids' containing large polypeptides which are cleaved during the process of assembly with RNA (Jacobson & Baltimore, 1968). *In vitro* assembly of poliovirus has been described by Phillips (1969).

Habitat. As the name implies, enteroviruses are found primarily as inhabitants of the intestinal tract, particularly of young hosts. They commonly cause no illness, but may spread from the gut and cause destructive lesions in the central nervous system.

HUMAN ENTEROVIRUSES

Poliomyelitis

Synonyms: Acute anterior poliomyelitis. Infantile paralysis. Heine-Medin disease. *Poliovirus hominis.*

Reviews: Bodian & Horstmann (1965). Schaffer & Schwerdt (1959) (Physical properties).

Physico-chemical characters. Survives well at −20°C, and for 8 years at −70°C. As with many other picornaviruses, not readily preserved by freeze-drying, readily inactivated by heat—even 50°C for 30 minutes; but results vary according to strain and suspending medium; 30 minutes at 60°C seems always effective. The curve of inactivation by 0·1 per cent formaldehyde has been studied by several workers.