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PICORNAVIRUS INHIBITORS

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Abstract—Picornaviruses are among the best understood animal viruses in molecular terms. A number of important human and animal pathogens are members of the *Picornaviridae* family. The genome organization, the different steps of picornavirus growth and numerous compounds that have been reported as inhibitors of picornavirus functions are reviewed. The picornavirus particles and several agents that intereact with them have been solved at atomic resolution, leading to computer-assisted drug design. Picornavirus inhibitors are useful in aiding a better understanding of picornavirus biology. In addition, some of them are promising therapeutic agents. Clinical efficacy of agents that bind to picornavirus particles has already been demonstrated.

Keywords—Picornavirus, poliovirus, antiviral agents, drug design, virus particles, viral proteases.

CONTENTS

Abbreviations—2′-5′ A, ppp(A2′p5′A)$_n$A; BFA, brefeldin A; BFLA1, bafilomycin A1; dsRNA, double-stranded RNA; EMC, encephalomyocarditis; FMDV, foot-and-mouth disease virus; G413, 2-amino-5-(2-sulfamoylphenyl)-1,3,4-thiadiazole; HBB, 2-(α-hydroxybenzyl)-benzimidazole; HIV, human immunodeficiency virus; HPA-23, ammonium 5-tungsto-2-antimonate; ICAM-1, intercellular adhesion molecule-1; IP3, inositol triphosphate; M12325, 5-aminosulfonyl-2,4-dichorobenzoate; 3-MQ, 3-methyl quercetin; IRES, internal ribosome entry site; L protein, leader protein; RF, replicative form; RI, replicative intermediate; RLP, ribosome landing pad; SFV, Semliki forest virus; TOFA, 5-(tetradecyloxy)-2-furoic acid; VPg, viral protein bound to the genome; VSV, vesicular stomatitis virus.

1. INTRODUCTION

The *Picornaviridae* family has been the object of interest of many research groups for more than 30 years. As a result, members of this virus family are among the best understood animal viruses in molecular terms. A number of excellent reviews have been published covering different aspects of picornavirology (Putnak and Phillips, 1981; Koch and Koch, 1985; Carrasco and Castrillo, 1987; Krausslich and Wimmer, 1988; Rossmann and Johnson, 1989; Sonenberg, 1990; Palmenberg, 1990; Stanway, 1990; Racaniello, 1990; Lawson and Semler, 1990; Richards and Ehrenfeld, 1990; Minor, 1990; Sarnow *et al.*, 1990; Minor, 1992; Kirkegaard, 1992; Agol, 1993; Harber and Wimmer, 1993). Picornaviruses are structurally simple and appear as spherical particles of ~30 nm diameter that possess icosahedral symmetry. The genome is a molecule of single-stranded RNA of positive polarity. This family was first divided into four genera: *Enterovirus*, *Cardiovirus*, *Rhinovirus* and *Aphtovirus*. More recently, an additional genus, *Hepatovirus*, has been recognized on the basis of genome sequence and organization (Fig. 1). Some members of this group are of great importance in medicine and veterinary science, because they are pathogens of humans or of animals and, hence, of economic or ecological interest. Examples include poliovirus, the causative agent of poliomyelitis, coxsackie virus, a factor responsible for causing heart disease, and echo viruses that cause meningitis in humans, all three belonging to the *Enterovirus* genus. The main causative agents of common colds are rhinoviruses (genus *Rhinovirus*). Hepatitis A, a virus that causes hepatitis, was previously classified in the *Enterovirus* genus, but is now included in the *Hepatovirus* genus (Stanway, 1990; Harber and Wimmer, 1993). Finally, the infective agent for one of the most threatening afflictions of cattle, foot-and-mouth disease, is a member of the *Aphtovirus* genus. Although effective vaccines have been developed for some of these viruses, such as poliovirus or hepatitis A virus, the development of vaccines for other members of this group, such as rhinoviruses, has yet to be achieved (Minor *et al.*, 1990; Murdin *et al.*, 1990; Minor, 1990, 1992). Thus, it is important to develop antiviral agents to combat disease. Apart from the use of antiviral agents in this respect, these compounds play a pivotal role in molecular virology. Indeed, antiviral agents are very useful for elucidating numerous viral processes, if we can identify the exact target of the agent at the molecular level. In addition, antiviral agents provide clean and accurate tools that aid in the dissection of the complicated molecular steps of viral genome replication. Hence, antiviral agents are to molecular virology what other inhibitors are to molecular biology.

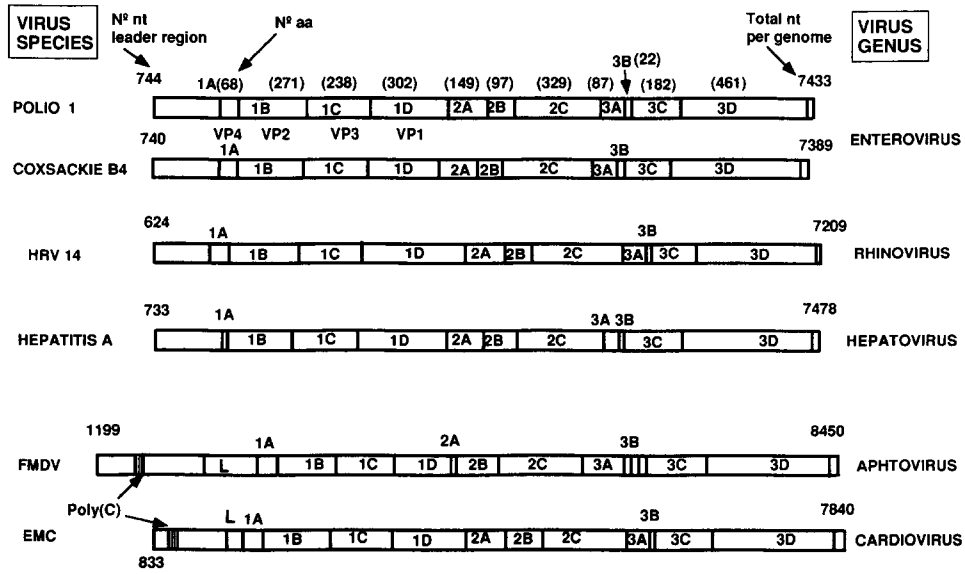


Fig. 1. Genome organization of different picornavirus species. Each protein is designated according to the actual nomenclature of picornavirus proteins. The number of nucleotides per genome and in the leader region, before the initiator AUG, are indicated. The numbers in parentheses represent the number of amino acids of each protein.

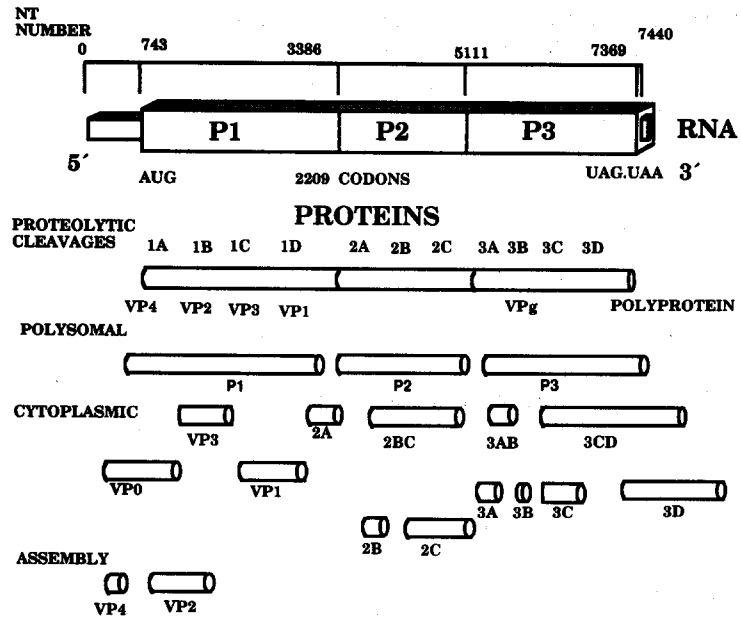


Fig. 2. Diagrammatic representation of the poliovirus genome and the proteolytic processing of poliovirus proteins. The three different types of proteolytic cleavages, polysomal, cytoplasmic and during assembly, are indicated.

Picornavirus inhibitors represent good examples of compounds that are not only useful in providing a better understanding of the biology of picornaviruses, but also in furnishing promising agents that are being assayed in clinical trials against the common cold, for instance. The large number of compounds now available for the selective blockade of different steps of the picornavirus replication cycle makes these agents invaluable tools for picornavirologists, but it is difficult to organize a comprehensive review covering all of them. Although I have tried to include as many examples as possible of the approaches used and the agents employed, inevitably, coverage will be incomplete. I apologise in advance to those whose work is not included, despite its merits.

1.1. Picornavirus Genome Organization

I shall not review here the structure of the picornavirus particle, or the different steps of its replication cycle; these topics will be covered at the beginning of each section dealing with specific picornavirus inhibitors. I shall briefly outline, instead, the structure of the genome by taking poliovirus as a representative member of this viral family.

Poliovirus was the first picornavirus and, in fact, the first animal virus with an RNA genome to be sequenced (Kitamura *et al.*, 1981; Racaniello and Baltimore, 1981a). Soon after this achievement, infectious cDNA was obtained, thus opening new possibilities for the genetic manipulation of this important human pathogen (Racaniello and Baltimore, 1981b; Omata *et al.*, 1984; Sarnow *et al.*, 1990; Kirkegaard, 1992). The RNA genome is a 7.544 stretch of nucleotides, of which almost 90% encodes for proteins (Sarnow *et al.*, 1990; Harber and Wimmer, 1993) (Fig. 2). The AUG that initiates protein synthesis is located at position 754 and is preceded by several AUGs (6–8, depending on the virus serotype), which are not recognized by the translation initiation complex (Meerovitch and Sonenberg, 1993). The initiator AUG is followed by 2209 codons that code for a polyprotein that is cleaved by virus-encoded proteases (Krausslich and Wimmer, 1988; Lawson and Semler, 1990; Palmenberg, 1990). Two termination codons are followed by 65 nucleotides, which precede a poly(A) stretch with an average length of 75 nucleotides (Yogo and Wimmer, 1972). This is genetically determined and encoded by the genome. After infection, the viral RNA contains longer poly(A) tails of 150–200 nucleotides that, presumably, are added after replication (Spector and Baltimore, 1975a, b; Spector *et al.*, 1975). The length of the genome and the disposition of the different viral proteins varies somewhat among the different picornavirus genera, as depicted in Fig. 1. Perhaps the most remarkable variations are the presence of an

additional L (leader) protein (Strebel and Beck, 1986) in foot-and-mouth disease virus (FMDV) and encephalomyocarditis (EMC) virus; the coding for three copies of the protein 3B (VPg), the virtual lack of protein 2A (16 amino acids) in FMDV (Stanway, 1990) and the presence of a poly(C) tract of variable length in the 5′ leader region in *Aphtovirus* and *Cardiovirus* (Clarke *et al.*, 1987; Stanway, 1990).

1.1.1. *The Non-Coding Regions*

The 754 nucleotides located at the 5′-terminus of the poliovirus genome have a number of functions, of which some have yet to be fully elucidated (Sarnow *et al.*, 1990; Kirkegaard, 1992). Among these, the following may be listed: (1) This region is necessary for replication of the RNA genome. (2) At least part of the 754 nucleotides is required to initiate the synthesis of the polyprotein. (3) This region is involved in genome packaging into virion particles. (4) Poliovirus neurovirulence mutants map in this region. In addition, the 5′ leader sequence may be involved in other processes required for virus replication. Thus, one of the viral proteases, protein 3C or its precursor 3CD, can interact with this region and, perhaps, regulate genome replication (Andino *et al.*, 1990a, b). Furthermore, a poliovirus–coxsackie hybrid has been generated that contains the 220–625 nucleotides from coxsackie virus, replacing the 220–627 nucleotides of poliovirus (Semler *et al.*, 1986). This hybrid is a ts mutant for genome replication!

One of the most salient features of the 5′-end of picornavirus mRNA is that it does not contain the cap structure typical of eukaryotic mRNA (Harber and Wimmer, 1993; Meerovitch and Sonenberg, 1993). Instead, the viral RNA terminates in pUpU. Within the virion particle, or during the replication of the viral genome, this end is covalently bound, in the case of poliovirus, to a virus-encoded peptide of 22 amino acids (Flanegan *et al.*, 1977; Lee *et al.*, 1977). This peptide is the viral protein 3B and is also known as VPg (viral protein bound to the genome) (Nomoto *et al.*, 1977a, b). Since picornavirus mRNA has no cap structure, the initiation of protein synthesis by these genomes is unusual (Meerovitch and Sonenberg, 1993). The initiation of translation in eukaryotic mRNAs commences by recognition of the cap structure by the initiator complex, a process involving cap-binding proteins (Kozak, 1989; Meerovitch and Sonenberg, 1993). This recognition is followed by the attachment and scanning of the 5′-end by the initiator complex, until an AUG is found in the correct context to start translation (Kozak, 1989; Cavener and Ray, 1991). In picornavirus mRNA translation, the initiator complex binds, instead, to an internal region located between nucleotides 140–630 (Pelletier and Sonenberg, 1988). In some picornaviruses, the initiator complex then scans the remaining 5′-end until the initiator AUG is found, or it might even jump or translocate directly to this AUG (Jackson *et al.*, 1990; Meerovitch and Sonenberg, 1993). There is a pyrimide stretch UUUCCUUUU in this region, which is conserved in picornaviruses (Beck *et al.*, 1983; Meerovitch and Sonenberg, 1993) from which nucleotides UUUCC (560–564) combined with the position of the seventh AUG located 150 nucleotides upstream of the initiator AUG are involved in the internal initiation of translation (Meerovitch *et al.*, 1991; Meerovitch and Sonenberg, 1993).

The 65 nucleotides located between the termination codons and the poly(A) tail play a role in the replication of viral RNA (Sarnow *et al.*, 1990). Indeed, a function of this region in the synthesis of negative-strand copies of RNA has been indicated (Sarnow *et al.*, 1986). The presence of a pseudoknot in this region (Iizuka *et al.*, 1987; Jacobson *et al.*, 1993) and the implication of these structures in the replication of viral RNAs has also been considered (Pleij, 1990). The poly(A) tail plays a function in stabilizing mRNAs, and the infectivity of poliovirus RNA without this tail is reduced (Sarnow, 1989). In addition, this poly(A) can participate in the replication of viral RNA (Richards and Ehrenfeld, 1990).

1.1.2. *The Coding Region*

Once the ribosome starts translation at the initiator AUG, it travels through the 2209 codons of the poliovirus genome and generates a polyprotein (Wimmer *et al.*, 1987; Harber and Wimmer, 1993). This polyprotein is not found as such in the infected cells, because it is cleaved while it is still on polysomes (Wimmer *et al.*, 1987; Lawson and Semler, 1990). The first cleavage is

accomplished by the viral protease 2A, releasing the precursor (P1) of the capsid proteins (see the diagram depicted in Fig. 2) (Toyoda *et al.*, 1986). Once the other viral protease (protein 3C) has been synthesized, it cleaves between P2 and P3 (Section 4.1) (Hanecak *et al.*, 1982). Therefore, the viral proteases are active as precursors and are able to cleave themselves in *cis*. Indeed, once the mature proteases have been generated during infection, they are also active in *trans* (Krausslich and Wimmer, 1988; Palmenberg, 1990; Lawson and Semler, 1990). For more details on the proteolytic events and the specificity of these proteases, the reader is referred to several excellent reviews (Palmenberg, 1987, 1990; Krausslich and Wimmer, 1988; Lawson and Semler, 1990; Hellen and Wimmer, 1992b). Apart from the cleavages accomplished by the viral proteases 2A and 3C, a final cleavage event occurs during the assembly process on protein VP0 to generate VP4 plus VP2 (Palmenberg, 1987, 1990). This cleavage is catalyzed by VP0 itself, in conjunction with the viral RNA (Arnold *et al.*, 1987; Palmenberg, 1987; Hellen and Wimmer, 1992b).

In the case of poliovirus, 11 mature proteins plus a different array of precursors are produced, thus making the analysis of protein synthesis in picornavirus-infected cells very complex (Jacobson and Baltimore, 1968; Summers and Maizel, 1968). Figure 3 shows the appearance of these polypeptides on two-dimensional polyacrylamide gels. The nomenclature of the picornavirus precursors and the mature proteins has been rationalized and is known as the L-4-3-4 nomenclature (Rueckert and Wimmer, 1984). As indicated in Section 1.1, some picornaviruses encode for a protein, preceding the capsid proteins, that is known as L protein. Synthesis of L protein is followed by that of four capsid proteins (P1 region) and seven polypeptides involved in vegetative functions (P2 and P3 regions). These three regions in the genome, P1, P2 and P3, are distinguished according to the two proteolytic cleavages that take place on the polyprotein at the polysomal level. The mature proteins encoded in each of these regions are numbered 1, 2 and 3, followed by a letter A, B, C or D. Thus, the eleven mature proteins are known as 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3A, 3B, 3C, 3D. The capsid proteins are also known by their original names: VP4 (or 1A), VP2 (or 1B), VP3 (or 1C) and VP1 (or 1D). Given the name of a viral protein, it is possible to identify its location on the genome. The precursor proteins are known by combinations of these names; for instance, precursor 3AB, 2BC or 2C3A. Although proteins 2C3A, or even 2B3A, are not found as such in infected cells, they can be generated by genetic engineering.

2. EARLY STEPS OF PICORNAVIRUS INFECTION

2.1. The Structure of Picornavirus Particles

Picornaviruses possess small virion particles whose structure at atomic resolution has been solved (Hogle *et al.*, 1985; Rossmann *et al.*, 1985; Luo *et al.*, 1987; Acharya *et al.*, 1989; Kim *et al.*, 1989; Rossmann and Johnson, 1989; Krishnaswamy and Rossmann, 1990; Yeates *et al.*, 1991; Grant *et al.*, 1992; Smyth *et al.*, 1993). The virions have a spherical shape and exhibit icosahedral symmetry (Rossmann and Johnson, 1989). They are composed of 60 copies of each structural protein VP1, VP2, VP3 and VP4, plus a copy of the genome RNA that contains one copy of the genome-bound viral protein (VPg) (Harber and Wimmer, 1993). This genomic protein is covalently attached to the 5′-end of the viral RNA through an ester bond between the tyrosine present in VPg and the 5′-OH of the last nucleotide in the viral RNA (Lee *et al.*, 1977). The virion is composed of 60 identical asymmetric units, each formed by a copy of each of the structural proteins, although only three (VP1, VP2 and VP3) are on the virion surface, with VP4 being located internally (Hogle *et al.*, 1985; Rossmann and Johnson, 1989).

Determination of the structures of picornavirus proteins revealed that the virion proteins VP1, VP2 and VP3 have different amino acid sequences, but similar tertiary structures (Hogle *et al.*, 1985; Rossmann *et al.*, 1985; Acharya *et al.*, 1989; Rossmann and Johnson, 1989; Grant *et al.*, 1992). This tertiary structure is also shared by the single protein of the capsid of many plant RNA viruses, suggesting that a kind of basic structure or "brick" can exist within different virion particles (Rossmann and Johnson, 1989). The protein fold that gives rise to this basic brick is depicted in Fig. 4. It is composed of two four-stranded β-sheets and is quite similar in different viruses (Hogle *et al.*, 1985; Rossmann *et al.*, 1985; Acharya *et al.*, 1989; Rossmann and Johnson, 1989). The minimum number of amino acids required to form a β-barrel is $\sim$150 (Rossmann and Johnson,

1989). There may be insertions of variable size between the strands at the cage of the traquetoid. VP1, VP2 and VP3 contain a common eight-stranded antiparallel β-barrel motif. Their amino termini intertwine to link them, thus forming a network in the interior of the capsid. Five VP3 amino termini form a five-stranded, helical β-cylinder in the virion interior (Hogle *et al.*, 1985;

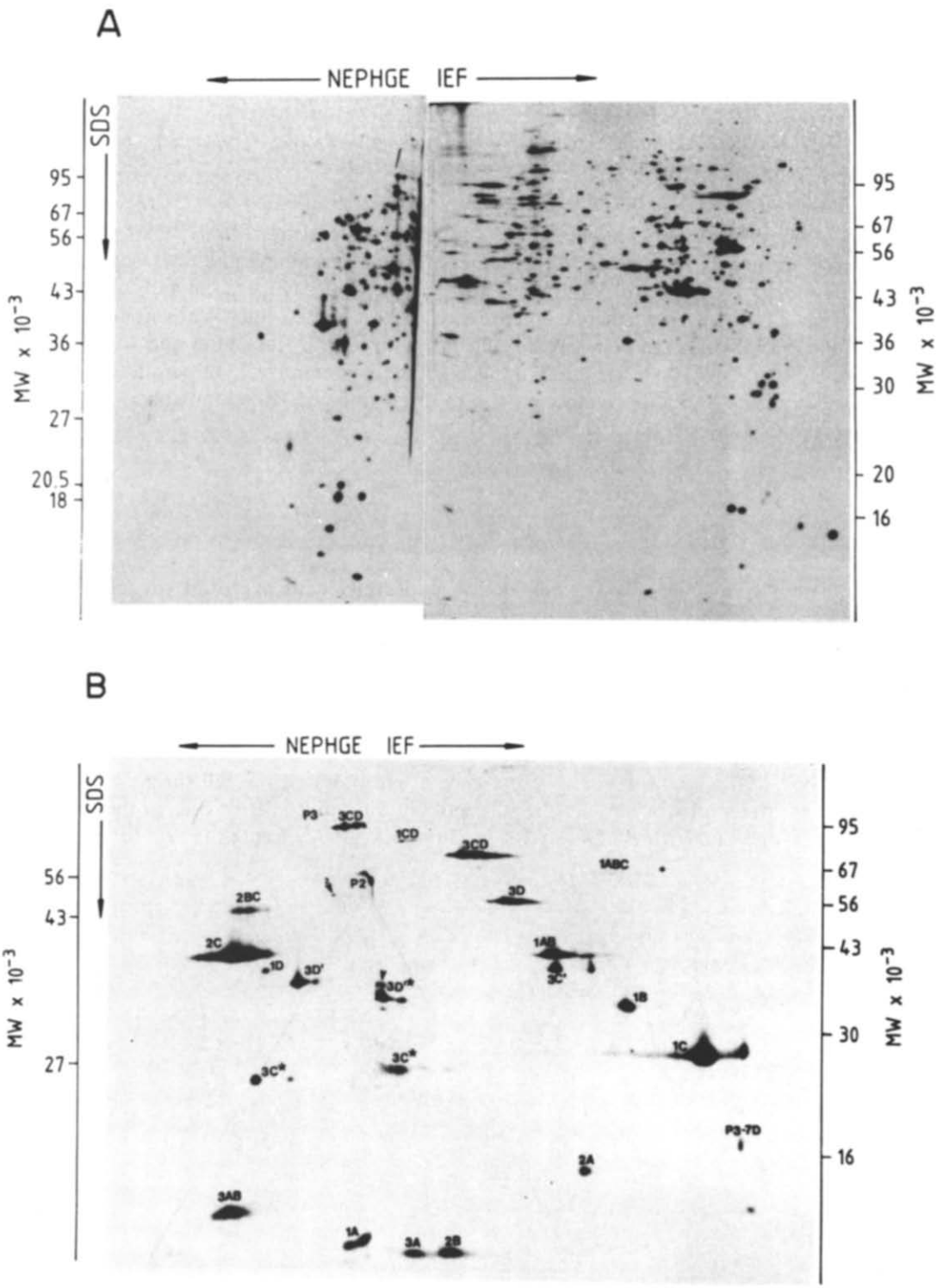


Fig. 3. Two-dimensional gel electrophoresis of [^{35}S]labelled proteins from uninfected (panel A) or poliovirus-infected (panel B) HeLa cells. The cells were labelled during one hour (4–5 hr p.i.) in methionine-free medium (Urzainqui and Carrasco, unpublished results).

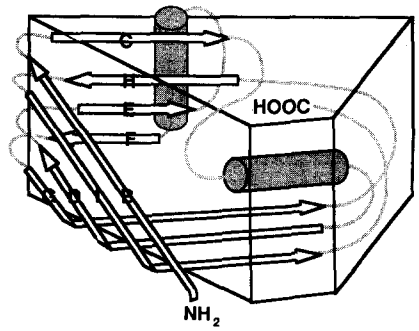


Fig. 4. Polypeptide fold of one protein subunit of poliovirus showing the secondary structural elements (modified from Hogle *et al.* (1985)).

Rossmann *et al.*, 1985; Acharya *et al.*, 1989; Rossmann and Johnson, 1989). VP4 is myristoylated at the amino terminus (Chow *et al.*, 1987; Paul *et al.*, 1987; Urzainqui and Carrasco, 1988). This myristoylate moiety lies inside the virion coat near the β-cylinder (Rossmann and Johnson, 1989). On the surface of each protomer there is a depression of 25 Å depth, which is absent in aphtovirions (Hogle *et al.*, 1985; Rossmann *et al.*, 1985; Acharya *et al.*, 1989; Rossmann and Johnson, 1989). This depression or "canyon" has been implicated in the interaction of virion particles with receptors; neutralizing antibodies cannot enter into this depression (Rossmann and Johnson, 1989; Rossmann, 1989b; Olson *et al.*, 1993a) (Fig. 5).

2.2. Picornavirus Receptors

Viral infection commences by the recognition and attachment of virion particles to specific molecules located on the surface of cells, the so-called receptors (Dales, 1973; Dimmock, 1982; Lentz, 1990). The chemical nature of the receptor molecules varies, depending on the species of virus that infects the cells, but many identified receptors are proteins and, more particularly, glycoproteins (Dales, 1973; Dimmock, 1982; Lentz, 1990). In principle, a single virus species can use different receptor molecules on a single cell type or on different kinds of cells. In addition, different virus species can use the same receptor to land on the cell that is to become infected (Dales, 1973; Dimmock, 1982; Lentz, 1990). The presence of receptors on the cell surface determines, in many instances, the viral tropism, i.e. the susceptibility of a given set of cells in the organism to infection by a particular virus (Dales, 1973; Dimmock, 1982; Lentz, 1990). In addition, the

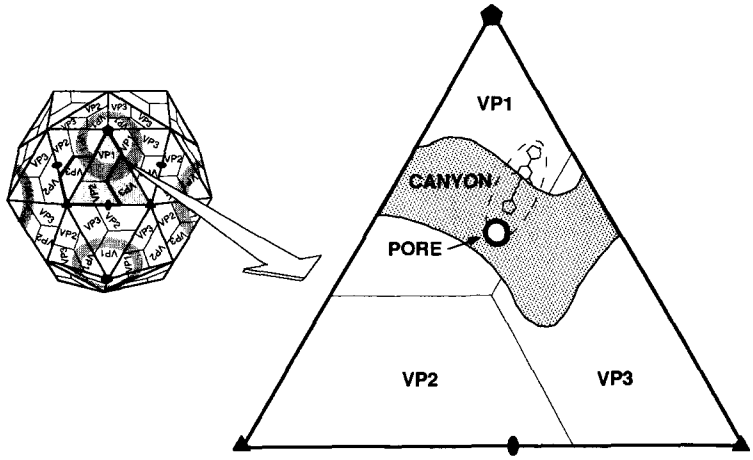


Fig. 5. Structure of a rhinovirus particle. The location of the viral structural proteins, VP1, VP2 and VP3, are indicated. The disposition of the canyon, the pore and a WIN compound is indicated (modified from Olson *et al.* (1993a)).

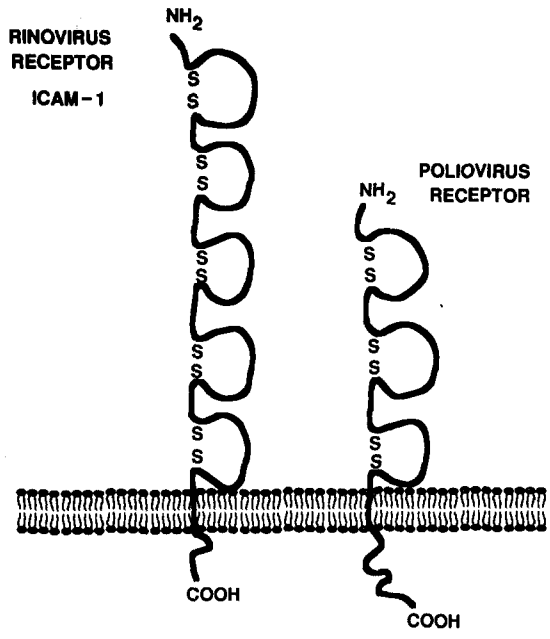


Fig. 6. Diagrammatic representation of the rhinovirus receptor (ICAM 1) and the poliovirus receptor molecules on the membrane.

interaction of viruses with cell surface receptors is one of the targets through which viral infection might be blocked (Lentz, 1990).

In the case of picornaviruses, the receptor molecules for poliovirus and rhinovirus have been identified, the genes encoding these glycoproteins cloned and their protein products expressed and characterized (Greve *et al.*, 1989; Mendelsohn *et al.*, 1989; Staunton *et al.*, 1989; Tomassini *et al.*, 1989). Both receptors belong to the superimmunoglobulin family of proteins (see reviews by Racaniello, 1990; Harber and Wimmer, 1993). The poliovirus receptor is composed of three immunoglobulin-like domains located on the surface of the cell, a hydrophobic transmembrane domain that anchors the receptor to the membrane, and a cytoplasmic C-terminal region (Mendelsohn *et al.*, 1989; Koike *et al.*, 1990) (Fig. 6).

The intercellular adhesion molecule-1 (ICAM-1) is the cellular receptor for rhinoviruses (Staunton *et al.*, 1989, 1990). This glycoprotein binds the lymphocyte function-associated antigen 1, an interaction involved in the immunological response (Staunton *et al.*, 1989, 1990). The receptor molecule ICAM-1 is composed of five immunoglobulin-like extracellular domains, a transmembrane domain of hydrophobic nature, and a short C-terminal cytoplasmic domain (Staunton *et al.*, 1989, 1990). Large amounts of truncated ICAM-1 lacking the hydrophobic and cytoplasmic domains have been obtained (Marlin *et al.*, 1990). These truncated forms are soluble and interact with rhinovirus particles (Marlin *et al.*, 1990; Hoover-Litty and Greve, 1993; Olson *et al.*, 1993a). The structure of the truncated ICAM-1 complexed with rhinovirus particles has been determined using cryoelectron microscopy, indicating that the receptor binds into the canyon (Olson *et al.*, 1993a, b).

The availability of cDNA clones coding for the poliovirus receptors made possible an analysis of the structure of the relevant gene and the transcripts derived from it in different cell lines (Koike *et al.*, 1990, 1991a; Morrison and Racaniello, 1992). The poliovirus receptor gene contains eight exons, and its transcription produces a number of splice variants of mRNAs (Koike *et al.*, 1990). A duplication of the poliovirus gene has been found in monkeys, the two genes being encoded at different loci of the genome (Koike *et al.*, 1992). Mouse L cells are infected by poliovirus when transfected with the gene encoding the poliovirus receptor (Mendelsohn *et al.*, 1989). Transgenic mice have been generated carrying the poliovirus receptor gene (Ren *et al.*, 1990; Koike *et al.*, 1991b; Ren and Racaniello, 1992). These mice are susceptible to poliovirus and could constitute a good animal model to test the efficacy of poliovirus vaccines and the antiviral activity of poliovirus inhibitors in animals.

The binding of virion particles to their receptors induces conformational changes in some virion proteins that could trigger the interaction of these proteins with the membrane (Fricks and Hogle, 1990; Hoover-Litty and Greve, 1993). Subsequently, the translocating activity of some of the virion proteins will be put in motion, thus inducing the passage of the virion through the lipid bilayer (Carrasco *et al.*, 1993; Pérez and Carrasco, 1993). Some virion proteins are, therefore, endowed with translocation activity similar to that displayed by some domains of polypeptide toxins (Carrasco *et al.*, 1993). However, this translocation capacity has not yet been assigned to any picornavirus protein.

In the model indicated above, receptor molecules are regarded as playing an active role during virus entry (Fricks and Hogle, 1990; Carrasco *et al.*, 1993; Hoover-Litty and Greve, 1993). The receptor binds the particle to the cell surface and this binding induces, in the virions, conformational changes that are required to uncoat the virion particle (Moore *et al.*, 1990; Hoover-Litty and Greve, 1993) (see Section 2.3). This process is required to allow penetration of the membrane and entry to the cytoplasm (Fricks and Hogle, 1990; Hoover-Litty and Greve, 1993). In agreement with this is the finding that binding of opsonized poliovirus to mouse L cells does not lead to infection (Mason *et al.*, 1993), indicating that the simple attachment of poliovirus to the cell surface does not suffice to initiate infection. Thus, the viral receptor in the cell plays a role beyond attachment (Mason *et al.*, 1993). Although lowering the pH in endosomes can contribute to the conformational changes that occur in virions, this process is not necessary in the case of poliovirus (Pérez and Carrasco, 1993) (see Section 2.3). Even in some enveloped viruses that have recently been investigated, binding of enveloped virions to receptors also induces conformational changes in the viral glycoproteins that are responsible for membrane fusion (Moore *et al.*, 1990; Meyer *et al.*, 1992). In principle, low pH would not be required to induce the conformational changes that lead to membrane fusion in the case of enveloped viruses, but would provide a proton-motive force to help the virion particle to trespass the membrane (Carrasco *et al.*, 1993).

2.3. Entry and Uncoating of Picornaviruses

The mechanism used by animal viruses to enter cells and to deliver their genomes in the cytoplasm is a subject of intensive research (Hoekstra and Kok, 1989; Marsh and Helenius, 1989). Animal viruses containing lipid membranes accomplish this task by fusion of the external envelope with cellular membranes, involving either the plasma membrane, as occurs with Sendai virus, herpesvirus or vaccinia virus, or the endosomal membrane, as is the case of semliki forest virus (SFV), vesicular stomatitis virus (VSV) or influenza virus (Hoekstra and Kok, 1989; Marsh and Helenius, 1989). In the latter case, fusion is promoted by conformational modifications of the viral glycoproteins after receptor binding and acidification of endosomes (Wiley and Skehel, 1987; White, 1990). Prevention of this acidification by lysosomotropic agents leads to inhibition of infection by SFV, VSV or influenza virus (Hoekstra and Kok, 1989). Models that explain the entry of animal virus particles devoid of a lipid envelope, such as picornaviruses, are more scarce (Hoekstra and Kok, 1989; Marsh and Helenius, 1989). In principle, these viruses could pass directly through the plasma membrane after receptor binding. Some evidence supports this mode of entry for certain viruses, such as adenoviruses (Brown and Burlingham, 1973), poliovirus (Dunnebacke *et al.*, 1969), rotaviruses (Kaljot *et al.*, 1988) and reovirus subviral particles (Borsa *et al.*, 1979). Alternatively, the viral particles can be internalized by receptor-mediated endocytosis and then cross the endosomal membrane (Everaert *et al.*, 1989; Marsh and Helenus, 1989; Kronenberger *et al.*, 1992). In both cases, the viral genome must pass across a lipid membrane, aided by the virion proteins. Most evidence indicates that the majority of naked animal virus particles follow the receptor-mediated endocytosis pathway to infect cells (Marsh and Helenius, 1989). Whether some of these viruses need a low pH step to trigger the crossing of the endosomal membrane, or enter cells by a pH-independent mechanism, remains open to debate (Brown and Burlingham, 1973; Madshus *et al.*, 1984c; Svensson, 1985; Sturzenbecker *et al.*, 1987; Gromeier and Wetz, 1990; Kronenberger *et al.*, 1991; Varga *et al.*, 1991). Two major questions, therefore, remain to be answered with respect to the mechanism of picornavirus entry into cells: Is a given picornavirus

species internalized through endosomes in a particular cell type?, or do endosomes need to be acidified for the virus to enter cells?

Attachment of poliovirus and its internalization in endosomes leads to conformational changes in the virion particle, particularly in VP1 (Fricks and Hogle, 1990), that allow interaction with the membrane and aid the insertion of the myristoylated protein VP4 into the membrane (Lonberg Holm *et al.*, 1975; Guttman and Baltimore, 1977; Fricks and Hogle, 1990). This insertion not only destabilizes the particle, but may also allow interaction with the membrane of some moieties of other viral proteins (Lonberg Holm *et al.*, 1975; Guttman and Baltimore, 1977; Fricks and Hogle, 1990) (Fig. 7). It can be speculated that the insertion of the virion proteins into the membrane would open a pore through which the genome is delivered. Alternatively, energy may be required in the form of ATP, or as the proton-motive (membrane potential and/or pH gradient) generated in endosomes by the activity of the proton-ATPase pump, that could be used to drive the nucleocapsid through the lipid barrier of the membrane (Carrasco *et al.*, 1993). A simple mechanistic model of viral particles opening pores in the membrane through which the genome passes is, however, inconsistent with the fact that the uncoating *per se* can occur *in vitro* in the presence of receptors (Hoover-Litty and Greve, 1993), whereas the entry of the genome into the cytoplasm requires a metabolically active cell (Hoekstra and Kok, 1989; Marsh and Helenius, 1989). The presence of ion channels in icosahedral virus particles (Kalko *et al.*, 1992) may indicate that insertion of these capsids into membranes opens the ion channels that are required to dissipate the ionic gradients between endosomes and the cytoplasm.

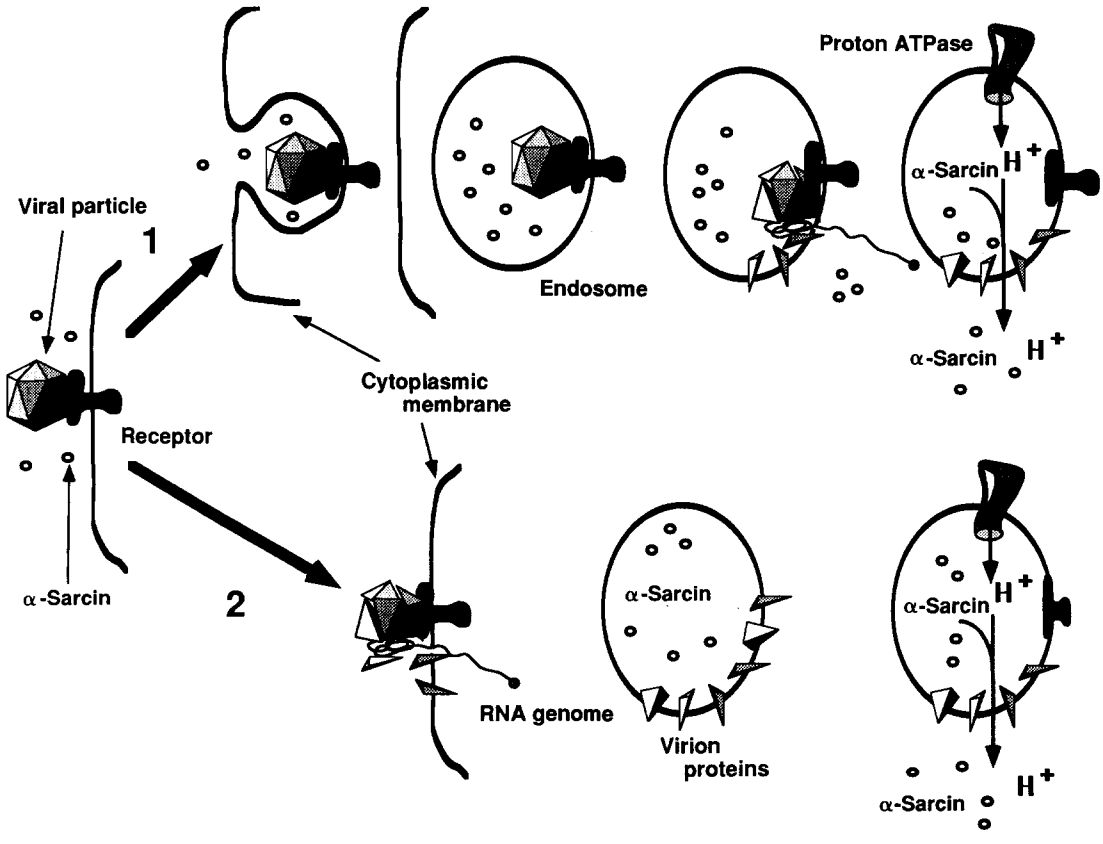


Fig. 7. Two potential routes for picornavirus entry into cells and the co-entry of the toxin α-sarcin. 1: the viral particle binds to its receptor and follows the endosomal route. 2: direct entry through the plasma membrane. The proton gradient generated by the vacuolar proton-ATPase is used by the virion proteins to translocate α-sarcin through the vacuolar membrane.

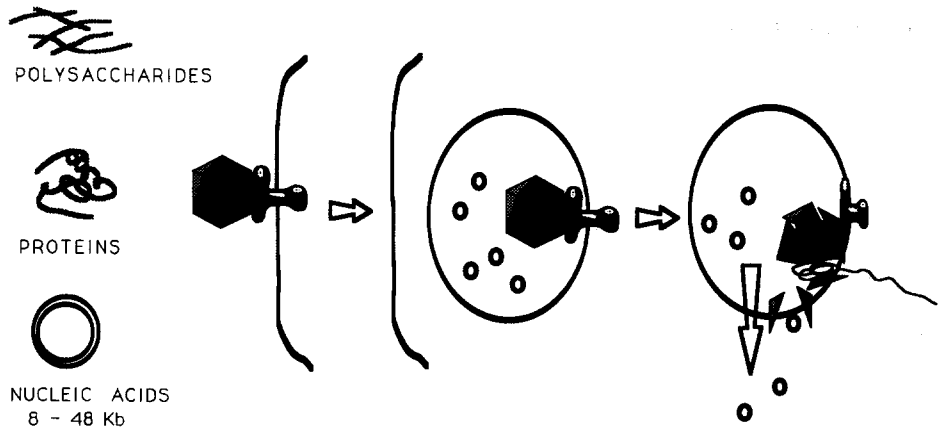


Fig. 8. Mechanism of the co-entry of different macromolecules (polysaccharides, proteins or nucleic acids) into cells as promoted by virus particles.

2.4. Early Membrane Permeabilization Promoted by Virus Particles

One approach towards an understanding of the mechanisms by which animal viruses traverse the cellular membrane is to elucidate, in molecular terms, how the co-entry of protein toxins, or other macromolecules, and viral particles is achieved (Yamaizumi *et al.*, 1979; Fernández-Puentes and Carrasco, 1980; FitzGerald *et al.*, 1983; Carrasco *et al.*, 1989). The infection of susceptible cells by picornaviruses leads to profound changes in membrane permeability at two well-defined periods of the infection cycle: (1) early during infection when the virus enters the cells or (2) at the beginning of the late phase, before the onset of viral macromolecular synthesis takes place (for a review, see Carrasco *et al.*, 1989). This late modification of membrane permeability is also known as "late membrane leakiness" (Carrasco, 1978; Carrasco *et al.*, 1989). The molecular basis underlying both phenomena differs. Thus, only virion particles are necessary to accomplish the increase in membrane permeability early during infection (Fernández-Puentes and Carrasco, 1980; Carrasco, 1981), whereas viral gene expression is necessary for the development of the late membrane leakiness (Carrasco, 1978; Contreras and Carrasco, 1979). Selective compounds have been developed that block the replication of animal viruses during early and late modifications of the membrane (Carrasco and Vazquez, 1983).

Fernández-Puentes and Carrasco (1980) first observed that viral particles permeabilized cells to protein toxins that are otherwise unable to cross the membrane, because there are no receptors for them. The proteins are efficiently delivered into the cytoplasm shortly after addition of picornavirus to the medium. Indeed, almost 100% of the cells become permeabilized in a few minutes (Otero and Carrasco, 1987b; Lee *et al.*, 1990), suggesting that the viral particle contains a component that not only promotes the entry of the viral nucleocapsid into the cell, but also directs other macromolecules that are not physically bound to the particles towards the cytoplasm (Figs 7 and 8).

We now know that different protein toxins can cross the membrane in the presence of animal virus particles, and that other proteins, such as luciferase or horse-radish peroxidase (Otero and Carrasco, 1987b), and polysaccharides (González *et al.*, 1987) are also delivered into cells by these particles (Fig. 8). No infectious virions are required for early membrane permeabilization to occur, because this phenomenon takes place with UV-inactivated (but not heat-inactivated) virions (Carrasco, 1981; Otero and Carrasco, 1987b; Carrasco *et al.*, 1989). More recently, the permeabilization of cells by adenovirus particles has been used to introduce DNA into cells. In a series of elegant experiments, Birnsteil and colleagues have shown that plasmids can bind to cells when they are complexed with transferrin-polylysine molecules (Zenke *et al.*, 1990; Wagner *et al.*, 1991). The entry of these complexes is enhanced by more than 2000-fold when adenovirus particles are present in the culture medium. In this fashion, more than 90% of the cells express the transfected gene (Cotten *et al.*, 1992; Wagner *et al.*, 1992a, b; Zatloukal *et al.*, 1992).

2.4.1. *Mechanism of the Early Membrane Permeabilization*

We are starting to understand the exact mechanism by which the early membrane permeabilization of membranes, induced by viral particles, occurs. Certainly, receptor binding of poliovirus particles and virion uncoating are necessary for this process to take place (Almela *et al.*, 1991). It has been speculated that the delivery of protein toxins by animal viruses involves the disruption of endosomes (FitzGerald *et al.*, 1983; Carrasco *et al.*, 1989), but other more selective mechanisms are possible. α-Sarcin has no effect on protein synthesis when added to uninfected HeLa cells, whereas the toxin efficiently blocks translation when added together with virion particles (Fernández-Puentes and Carrasco, 1980; Otero and Carrasco, 1987b). This co-internalization of the protein toxin by poliovirus is potently blocked by bafilomycin A1 (BFLA1) (see Section 3.3.2), at concentrations that prevent infection with SFV or VSV (Pérez and Carrasco, 1993).

These results indicate that an active vacuolar proton-ATPase is necessary for efficient delivery of α-sarcin into the cytoplasm mediated by poliovirus particles. We may conclude that most, if not all, of the toxin delivered into the cytoplasm comes from endosomal entry, because the inhibition of HeLa cells by α-sarcin was totally prevented by inhibiting the vacuolar proton-ATPase with BFLA1 (Pérez and Carrasco, 1993). A model of poliovirus entry illustrating the co-entry of α-sarcin and the site of action of different inhibitors is discussed in Section 3.4. Poliovirus enters from endosomes into the cytoplasm. The endosomal membrane remains intact because its rupture would lead to the eruption of the endosomal content, including α-sarcin, into the cytoplasm. Although poliovirus permeabilizes cells to α-sarcin and this event occurs in endosomes, it is conceivable, although unlikely, that the virus has already released its genome into the cytoplasm after binding to the cell surface receptor, and that the virion proteins present in the membrane and internalized in endosomes are the ones that control permeabilization to α-sarcin (Fig. 7).

Thus, the simple idea that enveloped animal viruses enter cells only by fusion between two membranes (Marsh and Helenius, 1989) is perhaps untenable, because such a model does not explain how the co-internalization of other macromolecules takes place (Yamaizumi *et al.*, 1979; FitzGerald *et al.*, 1983; Carrasco *et al.*, 1989; Lee *et al.*, 1990). Perhaps the proton-motive force can be used to drive the viral nucleocapsid and other macromolecules through the lipid barrier of the membrane. Virion proteins may couple the energy liberated by ATP hydrolysis or the movement of protons and other ions into the cytoplasm in favour of its gradient to the translocation of the genome or protein toxins in the same direction (Carrasco *et al.*, 1993).

3. COMPOUNDS THAT BLOCK EARLY STEPS OF INFECTION

3.1. Compounds that Bind to Virus Particles

As indicated in Section 2.1, on the surface of virion particles of rhinoviruses and poliovirus, there is a canyon crossing each protomer (Rossman, 1989b; Rossmann and Johnson, 1989; Harber and Wimmer, 1993). Below this canyon there is a hydrophobic pocket within the VP1 β-barrel, where a number of compounds that block virion uncoating bind (see reviews by Diana *et al.*, 1985c; Rossman, 1989a; Rossmann and Johnson, 1989; McKinlay *et al.*, 1992) (Fig. 5). This binding increases protein rigidity and stabilizes the particle in such a manner that uncoating cannot proceed (Rossmann, 1988; Chapman *et al.*, 1991; Gruenberger *et al.*, 1991; McKinlay *et al.*, 1992) (Figs 5 and 9). Thus, these uncoating inhibitors act as "staples" on virion particles, stabilize them and block the disassembly of viruses.

3.1.1. *WIN Compounds*

The most refined studies on uncoating inhibitors have been carried out on a series of compounds developed by Sterling-Winthrop that are collectively referred to as WIN compounds (Fig. 10) (Rossmann, 1989a; McKinlay *et al.*, 1992). Current knowledge on the interaction of these antiviral agents with rhinovirus particles has laid the groundwork for computer-assisted drug design (Diana *et al.*, 1993).

One of the earliest steps during picornavirus uncoating is the formation of "A particles", which

have a sedimentation coefficient of 125 S and have lost VP4 (Lonberg Holm *et al.*, 1975; Guttman and Baltimore, 1977). These particles are formed after infection of cells by picornaviruses or *in vitro* by exposure of virions to various treatments, e.g. altering the ionic conditions or the pH (Giranda *et al.*, 1992). The formation of these particles *in vivo* and *in vitro* is blocked by WIN compounds (Fox *et al.*, 1986; Zeichhardt *et al.*, 1987; Gruenberger *et al.*, 1991).

Studies with human rhinovirus particles at atomic resolution (Rossmann *et al.*, 1985) have aided the elucidation of the mode of interaction of a number of WIN compounds with these particles (Smith *et al.*, 1986; Badger *et al.*, 1988, 1989; Rossmann, 1988, 1989a; Kim *et al.*, 1989, 1993; Diana *et al.*, 1990; McKinlay *et al.*, 1992). The binding site of these antiviral agents is in a hydrophobic pocket located beneath the canyon floor (Smith *et al.*, 1986; Rossmann, 1989a; Diana *et al.*, 1990, 1993; Chapman *et al.*, 1991; McKinlay *et al.*, 1992; Kim *et al.*, 1993; Diana and Mallamo, 1993) (see Figs 5 and 9). Binding of the drug to this pocket induces conformational changes in the floor of the canyon, causing it to be pushed upwards (Pevear *et al.*, 1989). This deformation can interfere with the binding of some rhinovirus serotypes with receptors (Pevear *et al.*, 1989), although other rhinovirus serotypes and poliovirus are not inhibited by WIN compounds with respect to their interaction with cell surface receptors (Fox *et al.*, 1986; Zeichhardt *et al.*, 1987; Almela *et al.*, 1991). Thus, some rhinovirus serotypes would be blocked at both the binding and uncoating steps (Shepard *et al.*, 1993), whereas inhibition of other picornaviruses by these drugs would be at the uncoating step only (Fox *et al.*, 1986; Zeichhardt *et al.*, 1987; Pevear *et al.*, 1989; Almela *et al.*, 1991).

Rhinovirus 1A and polioviruses, in contrast with other rhinoviruses, bind a cofactor of lipidic nature in the pocket underneath the canyon floor (Filman *et al.*, 1989; Yeates *et al.*, 1991; Kim *et al.*, 1993). This cofactor is displaced after binding of WIN compounds, without alteration to the floor of the canyon (Kim *et al.*, 1993). This observation may explain why attachment of virion particles in the case of rhinovirus 1A and poliovirus is not blocked by WIN compounds (Kim *et al.*, 1989).

A number of clinical trials against common colds, using compounds that bind to rhinovirus particles and block uncoating, have been carried out (Tyrrell, 1984; Barrow *et al.*, 1990; McKinlay *et al.*, 1992; Diana *et al.*, 1993). Although results with dichloroflavan, Ro 09-0410 and 44,081 RP have been disappointing, recent trials with WIN 54954, R 77975 and R 61837 are more promising (see below). One of the major problems in the therapeutic treatment of common colds is to reach drug levels in the nasal epithelium that are sufficiently high to block viral growth (Reed, 1980; Tyrrell, 1984). WIN 51711 (disoxaril) was one of the first WIN compounds to be evaluated therapeutically (see below). The development of new derivatives with a broader spectrum of action and increased potency against rhinovirus prompted their clinical evaluation. WIN 54954 was not effective against rhinovirus when administered orally to a group of volunteers (Turner *et al.*, 1993). However, a prophylactic trial against the common cold caused by coxsackie A21 virus demonstrated the oral efficacy of this compound (McKinlay *et al.*, 1992; Schiff *et al.*, 1992).

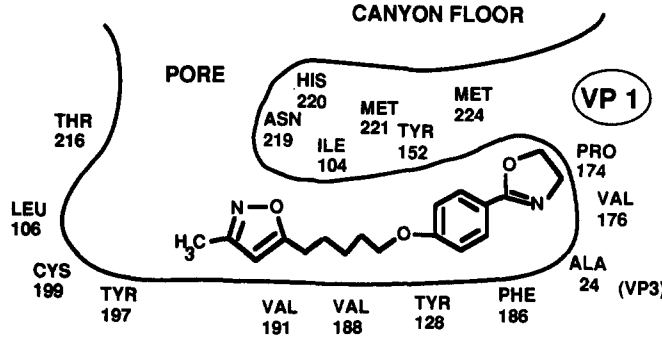


Fig. 9. Representation of the interaction of a WIN compound into the hydrophobic pocket underneath the canyon floor in a rhinovirus particle (modified from Badger *et al.* (1988)).

3.1.2. *Arildone and Disoxaril*

Arildone (WIN 38020) and disoxaril (WIN 51711) (Fig. 10) also belong to the group of WIN compounds. Arildone (4-6-(2-chloro-4-methoxyphenoxy)hexyl-3,5-heptanedione) is a selective inhibitor of poliovirus uncoating (McSharry *et al.*, 1979; Caliguiri *et al.*, 1980; McSharry and Pancic, 1982; Eggers and Rosenwirth, 1988; Everaert *et al.*, 1989). Arildone and some related derivatives block the replication of several RNA and DNA viruses, including herpes simplex virus types 1 and 2 (Diana *et al.*, 1977a, b; Kuhrt *et al.*, 1979), cytomegalovirus, SFV and VSV (Kim *et al.*, 1980). Administration of radioactive arildone to mice, dogs and monkeys by various routes indicates that it is extensively metabolized (Benziger and Edelson, 1982). Arildone and the 3,5-dimethoxy isoxazole are active when administered orally in mice inoculated intracerebrally with poliovirus type 2 (Diana *et al.*, 1985a). The 3,5-dimethoxy isoxazole is more active against rhinovirus type 2 in cultured cells than is 4,6-dichloroflavan (Diana *et al.*, 1985a).

Disoxaril, 5-7-4-(4,5-dihydro-2-oxazolyl)phenoxy heptyl-3-methylisoxazole is a broad spectrum antipicornaviral agent (Diana *et al.*, 1985a, b; McKinlay, 1985; Otto *et al.*, 1985). Oral administration of WIN 51711 was effective against echovirus and prevented poliovirus-induced paralysis and death in mice (McKinlay, 1985; McKinlay and Steinberg, 1986; McKinlay and Rossmann, 1989). The W-2 strains of poliovirus type 2 can cause persistent infections in the CNS of immunosuppressed mice. Disoxazil treatment of these mice significantly decreased the incidence of disease and induced a rapid clearance of the virus from the CNS (Jubelt *et al.*, 1989). Unfortunately, side effects appear in patients when high oral doses of this compound are given (Diana *et al.*, 1993).

3.1.3. *R 77975 (Pirodavir) and R 61837*

Both of these compounds belong to a series of pyridazinamine analogues synthesized by Janssen Research Foundation (Fig. 10). They block the replication of different rhinovirus serotypes by stabilizing the viral particle and inhibiting uncoating (Andries *et al.*, 1988; Rombaut *et al.*, 1991). Internalization of poliovirus particles into cells is not affected by R 78206, whereas uncoating and release of these particles into the cytosol are blocked (Rombaut *et al.*, 1991; Ofori-Anyinam *et al.*, 1993). Assuming that these agents directly inactivate viral particles (Andries *et al.*, 1988) and that mutants resistant to R 61837 are cross-resistant to other antiviral agents, including WIN 51711 (Andries *et al.*, 1989), it is likely that these agents can bind into the same hydrophobic pocket, located beneath the canyon of human rhinoviruses, that is used by WIN compounds (Chapman *et al.*, 1991). Direct evidence for this interaction has been obtained by X-ray crystallographic analysis of human rhinovirus 14 complexed with R 61837 (Chapman *et al.*, 1991). This agent binds towards the pocket entrance, but fails to occupy the end of the pocket (Chapman *et al.*, 1991). It has been suggested that adding a tail to R 61837 to fit into the end of the pocket would improve the drug's activity against rhinovirus 14 (Chapman *et al.*, 1991). Clinical efficacy against the common cold was first demonstrated for R 77975 (pirodavir) (Hayden *et al.*, 1992). Administration of R 61837, applied as a nasal spray with hydroxy-β-cyclodextrin as a vehicle, to human volunteers infected with rhinovirus 9 shows efficacy when given prophylactically, but not when used therapeutically (Al-Nakib *et al.*, 1989; Barrow *et al.*, 1990).

3.1.4. *SCH 38057*

SCH 38057 (1[6-(2-chloro-4-methoxy)-hexyl]imidazole hydrochloride) (Fig. 10) is a water-soluble antiviral agent that binds irreversibly to enterovirus and rhinovirus particles (Zhang *et al.*, 1993). The structure of rhinovirus 14 complexed with SCH 38057 shows that this compound binds at the innermost end of the hydrophobic pocket of VP1, leaving the entrance unoccupied. This interaction induces conformational changes involving a stretch of at least 36 amino acids of VP1 and VP3 (Zhang *et al.*, 1993).

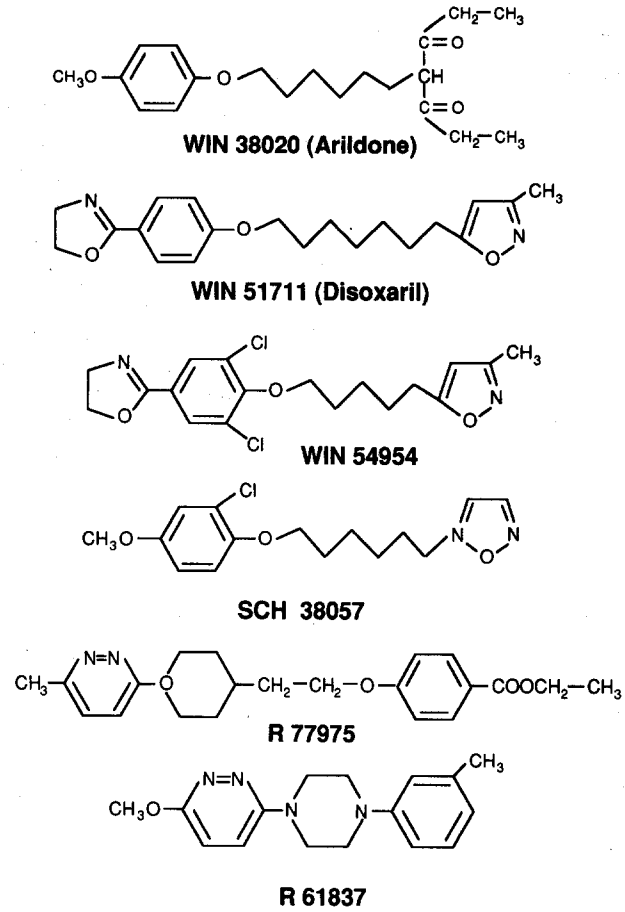


Fig. 10. Chemical structures of different compounds: WIN 38020, WIN 51711, WIN 54954, SCH 38057, R77975 and R61837.

3.1.5. *Phenoxypyridinecarbonitriles*

2-(3,4-Dichlorophenoxy)-5-nitrobenzonitrile (MDL-860) (Fig. 11) exhibited antipicornavirus activity *in vitro* (Powers *et al.*, 1982; Torney *et al.*, 1982). A number of phenoxybenzenes and phenoxypyridines were synthesized and evaluated for antiviral activity (Kenny *et al.*, 1985). Among these compounds, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile was found to have a broad-spectrum antiviral activity (Kenny *et al.*, 1985). The best antipicornavirus activity was found with 6-(3,4-dichlorophenoxy)-3-(ethylthio)-2-pyridine carbonitrile (DEPC) (Kenny *et al.*, 1985). The uncoating of photosensitized coxsackie virus A21 was reduced by this compound (Kenny *et al.*, 1985). Furthermore, DEPC has recently been shown to block the uncoating step in poliovirus (González *et al.*, 1990a; Almela *et al.*, 1991).

3.1.6. *MDL 20,610*

A series of 2-phenyl-2H-pyrano[2,3-b]pyridines has been synthesized (Kenny *et al.*, 1986), and some of them (MDL 20,610, MDL 20,646 and MDL 20,957) (Fig. 11) showed potent antirhinovirus activity (Kenny *et al.*, 1986). The 3′,4′-dichloro substitution, coupled with a 6-methylsulfonyl, 6-bromo or 6-chloro, is required for strong inhibition, and these compounds inhibit rhinovirus growth by 99% at concentrations of 4 ng/mL (Kenny *et al.*, 1986). Studies of their mode of action indicated that MDL 20,610 binds directly to rhinovirus particles and blocks uncoating (Kenny *et al.*, 1988).

3.1.7. *4,6-Dichloroflavan (BW683C and BW4294)*

A number of flavan derivatives (Fig. 11) were synthesized and assayed against rhinovirus type 1B (Bauer *et al.*, 1981; Tisdale and Selway, 1983). At a concentration of 7 nM, 4,6-dichloroflavan (BW683C) blocked the replication of this rhinovirus in cultured cells, making this compound, at that time, the most active inhibitor of rhinovirus in culture systems (Tisdale and Selway, 1983). More recently, 3,5-dimethoxy isoxazole was shown to be more active than BW683C (Diana *et al.*, 1985a). Clinical trials with BW683C, given orally or intranasally, failed to protect from rhinovirus infections, despite the fact that the drug achieved good blood levels when given orally. However, it could not be found in nasal secretions (Phillpotts *et al.*, 1983a; Al-Nakib *et al.*, 1987). Radioactive BW683C binds to rhinovirus particles and acts on an early step during infection (Bauer *et al.*, 1981; Tisdale and Selway, 1983). Several 4,6-dicyanoflavans based on BW683C have now been synthesized and give good inhibition not only of rhinoviruses, but also of other picornaviruses (Conti *et al.*, 1990; Desideri *et al.*, 1990). Finally, 1-anilino-9-benzyl-2-chloropurines, containing a lipophilic *para* substituent, are good inhibitors of rhinovirus 1B (BW4294) (Kelley *et al.*, 1990).

3.1.8. *Ro 09-0410 and Ro 09-0696*

Ro 09-0410 (4′-ethoxy-2′-hydroxy-4,6′-dimethoxychalcone) (Fig. 11) is an active compound against rhinoviruses and binds to the viral capsid (Ishitsuka *et al.*, 1982a, b; Ninomiya *et al.*, 1984,

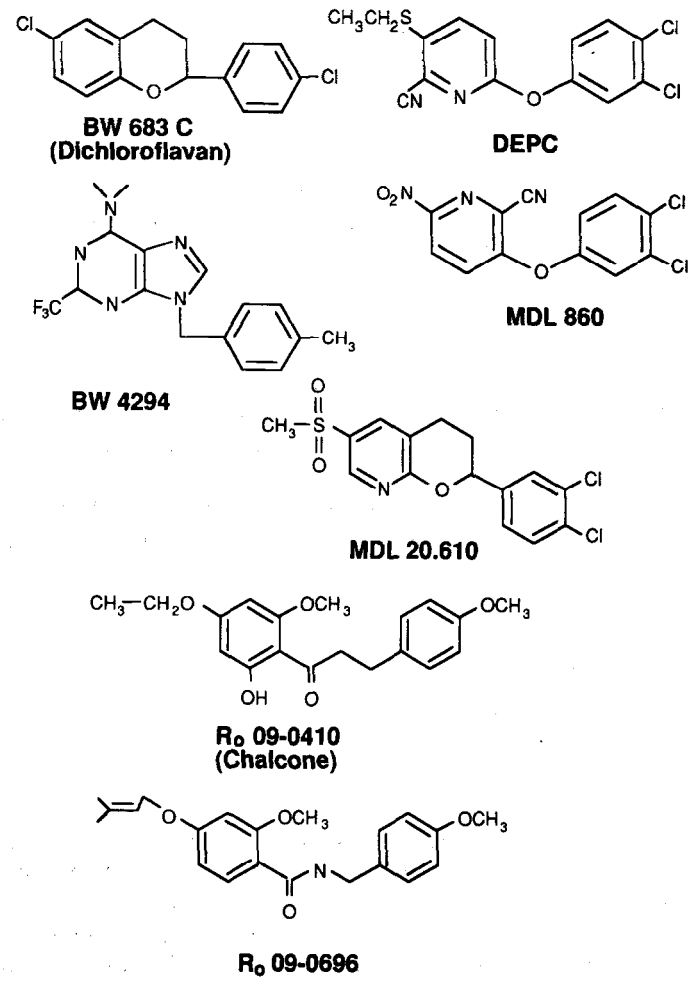


Fig. 11. Chemical structures of different compounds: BW 683C, DEPC, BW4294, MDL 860, MDL 20,610, Ro 09-0410 and Ro 09-0696.

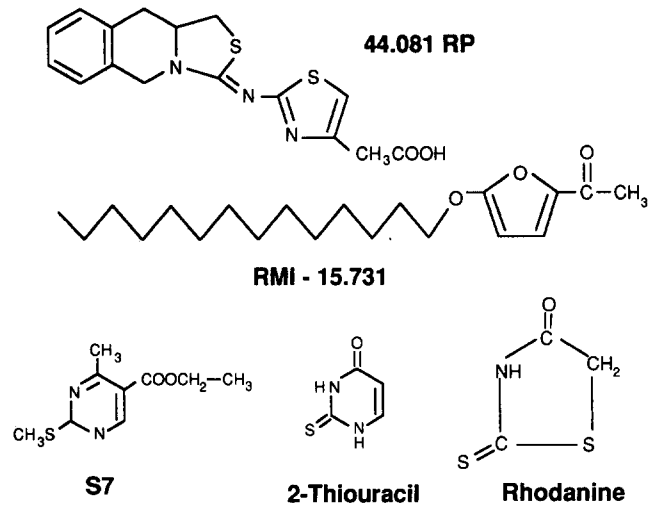


Fig. 12. Chemical structures of different compounds: 44,081 RP, RMI-15,731, S7, 2-thiouracil and rhodanine.

1985, 1990). Radioactive chalcone (Ro 09-0410) binds specifically to rhinovirus particles and this binding is inhibited by BW683C or RMI-15,731 (Ninomiya *et al.*, 1985; Ishitsuka *et al.*, 1986). Of 53 rhinovirus serotypes tested, 46 were inhibited by Ro 09-0410 (Ishitsuka *et al.*, 1982a). Mutant rhinoviruses resistant to chalcone were cross-resistant to BW683C or RMI-15,731 (Ishitsuka *et al.*, 1986). These results indicate that the three compounds had a similar mode of action based on their binding to virion particles. Several analogues of Ro 09-0410 have been synthesized, some of them being 10 times more active than the parent compound (Ninomiya *et al.*, 1990). Administration of 1–2 ng/mL Ro 09-0696 is sufficient to significantly reduce rhinovirus replication in cultured cells (Ninomiya *et al.*, 1990). These analogues bind to the same site on the virus particles as Ro 09-0410 and have a similar mode of action (Ninomiya *et al.*, 1990).

3.1.9. *44081 RP*

44081 RP, 2-(1,5,10,10a-tetrahydro-3H-thiaxolo 3,4-β-isoquinolin-3-ylidine) amino-4-thiazole acetic acid (Fig. 12), shows selective antirhinovirus activity (Zerial *et al.*, 1985) and is active at concentrations lower than those that cause cytotoxic effects (125 μg/mL). The compound was not virucidal and acted on an early step in infection (Zerial *et al.*, 1985) by blocking the uncoating of virion particles (Alarcón *et al.*, 1986).

3.1.10. *Rhodanine*

2-Thio-4-oxothiazolidine (rhodanine) (Fig. 12) selectively inhibits the uncoating of echovirus type 12 (Eggers, 1977). The adsorption of virions to cells, and their subsequent entry into the cytoplasm, are not affected by this compound. Other picornaviruses and RNA- or DNA-containing viruses are not susceptible to inhibition by rhodanine. The compound has no deleterious effects on cell morphology or cellular macromolecular synthesis (Eggers, 1977; Eggers *et al.*, 1979; Rosenwirth and Eggers, 1979) and protects the virion from both alkaline degradation (Eggers, 1977) and heat-inactivation (Rosenwirth and Eggers, 1979). Rhodanine apparently blocks uncoating, since the compound prevents the shut-off of host protein synthesis induced by echovirus (Rosenwirth and Eggers, 1977). It is well established that inhibition of host translation by picornaviruses requires translation of the input genomic RNA (Carrasco and Castrillo, 1987), a step that does not take place in the presence of uncoating inhibitors.

3.1.11. *RMI-15,731*

RMI 15,731 (1-[5-tetradecyloxy-2-furanyl]-ethanone) (Fig. 12) directly inactivates rhinovirus particles (Ash *et al.*, 1979). The mode of action of this agent has still not been investigated in detail. It is possible that the compound blocks uncoating (Ninomiya *et al.*, 1985), but it may have other effects because it was active if cells were pretreated and then washed, or if the agent was added 6 hr after infection (Ash *et al.*, 1979). The chemical structure of RMI 15,731 is similar to that of 5-(tetradecyloxy)-1-furoic acid (TOFA), an inhibitor of poliovirus genome replication (see Section 6.11).

3.1.12. *2-Thiouracil and Methylthiopyrimidine (S-7)*

There are several agents that interact with picornavirus particles and stabilize them against heat-inactivation and/or decapsidation. This is the case for L-cystine, but not for other amino acids (Pohjanpelto, 1958), glutathione (Fenwick and Cooper, 1962), 2-thiouracil (Steele and Black, 1967) and the methylthiopyrimidine derivative S-7 (Fig. 12) (Yamazi *et al.*, 1970; La Colla *et al.*, 1972; Lonberg Holm *et al.*, 1975). Treatment of poliovirus with 1 mM 2-thiouracil resulted in direct inactivation of infectivity. Perhaps oxidized forms of this compound react with sulfhydryl groups of capsid proteins, inactivating them (Steele and Black, 1967). S-7 inhibits the thermal inactivation of poliovirus particles and their decapsidation in HeLa cells (Lonberg Holm *et al.*, 1975).

3.2. Receptor Blockade and Soluble Receptors

Inhibition of virus attachment to its receptor results in a block of viral growth (Lentz, 1990). This inhibition can be achieved by several means. One of them is to use antibodies against viral particles that block their interaction with receptors. Another approach is to antagonize the receptor by antibodies directed against the receptor itself (Minor *et al.*, 1984; Colonno *et al.*, 1986; Minor, 1990, 1992). Application of this latter approach in human volunteers infected with rhinovirus delayed the onset of symptoms of the disease, but not the infection (Hayden *et al.*, 1988). Finally, anti-idiotypic antibodies directed against virions or viral receptor antibodies also inhibit viral infection (Lentz, 1990).

Another approach to blocking poliovirus or rhinovirus infections uses soluble receptors, i.e. moieties of receptor molecules devoid of the carboxy terminus responsible for anchoring the molecule to the membrane (Kaplan *et al.*, 1990a, b; Marlin *et al.*, 1990; Hoover-Litty and Greve, 1993). Incubation of virus particles with their receptors or truncated (soluble) receptors leads to the formation of complexes and the selective inhibition of infection (Kaplan *et al.*, 1990a, b; Marlin *et al.*, 1990; Hoover-Litty and Greve, 1993). Chimeric molecules of ICAM-1 and immunoglobulins are more effective in inhibiting rhinovirus binding to cells (Martin *et al.*, 1993). The utility of this approach for attacking viral infections in the organism is limited since, for example, mutant viruses that are resistant to neutralization by the receptor readily arise (Kaplan *et al.*, 1990b).

3.3. Inhibitors of Endosome Function

3.3.1. *Amines and Ionophores*

Amines are thought to accumulate in endosomes and other organelles of the vesicular system, where they become protonated. Accordingly, they permeate less readily and, thus, cause a rise in the endosomal pH (Fletcher *et al.*, 1965; Seglen, 1983). In contrast, monensin and nigericin (Fig. 13) are ionophores that exchange Na^+ or K^+/H^+ in favour of an H^+ gradient, thus dissipating the pH gradient created by the proton-ATPase pump (Seglen, 1983). As indicated above, there are, in principle, two possible mechanisms of poliovirus entry, i.e. direct entry through the plasma membrane (Dunnebacke *et al.*, 1969) or internalization in endosomes (Zeichhardt *et al.*, 1985). Poliovirus might use both of these mechanisms in different cell types or even in the same kind of cell. Once the viral particles are in endosomes, they must exit from this organelle in order to initiate translation of the genomic RNA in the cytoplasm. It is still a matter of debate as to whether

poliovirus requires acidification of endosomes to allow the passage of the genome through the endosomal membrane (Madshus *et al.*, 1984b, c; Zeichhardt *et al.*, 1985), or if poliovirus accomplishes this task in a pH-independent fashion (Gromeier and Wetz, 1990; Pérez and Carrasco, 1993). Indirect experiments involving complexing of poliovirus particles with neutral red prior to incubation for several minutes with weak amines or monensin, followed by very long incubation (18–40 hr), have led to the suggestion that poliovirus requires a low-pH step for infectivity (Madshus *et al.*, 1984b, c). Virus entry was evaluated by measuring the cytopathic effect caused by poliovirus complexed with neutral red after several rounds of viral replication (Madshus *et al.*, 1984b, c). High concentrations of amines have also been used, e.g. 0.3 mM chloroquine, or 70 mM NH_4Cl (Zeichhardt *et al.*, 1985). Poliovirus polymerase production was strongly inhibited, even when the compounds were added 1 or 2 hr after virus entry (Zeichhardt *et al.*, 1985). Certainly, the lysosomotropic agents employed have a number of side effects that influence a variety of enzymes and functions apart from raising the endosomal pH (Seglen, 1983; Hiebsch *et al.*, 1991). Hence, it is not surprising that during the long incubation times, where several rounds of viral replication take place, there was inhibition of the virus (Madshus *et al.*, 1984b, c). Shorter incubation times testing the inhibition of poliovirus by chloroquine or monensin during the uncoating period lead to the idea that poliovirus entry is pH-independent (Gromeier and Wetz, 1990; Pérez and Carrasco, 1993). The presence of lysosomotropic agents, such as chloroquine, amantadine, dansylcadaverine or monensin, during poliovirus entry did not inhibit infection (Pérez and Carrasco, 1993).

Studies on the requirement of a low-pH step for the entry of other members of the *Picornaviridae* family indicate that replication of FMDV is inhibited by lysosomotropic compounds (Carrillo *et al.*, 1984, 1985; Baxt, 1987). A direct action of these agents in the initial steps of virus infection is still uncertain; however, chloroquine was still active in the inhibition of FMDV replication, even when added 2.5 hr after infection (Baxt, 1987), whereas monensin (Neubauer *et al.*, 1987), as well as BFLA1 (Pérez and Carrasco, 1993), blocked the internalization of labelled rhinovirus particles into HeLa cells. It has been claimed that monensin might even increase infection of cells by EMC virus when low-pH medium is used during virus penetration (Madshus *et al.*, 1984a). Entry of EMC virus into HeLa cells is not inhibited by increasing the pH in endosomes (Pérez and Carrasco, 1993).

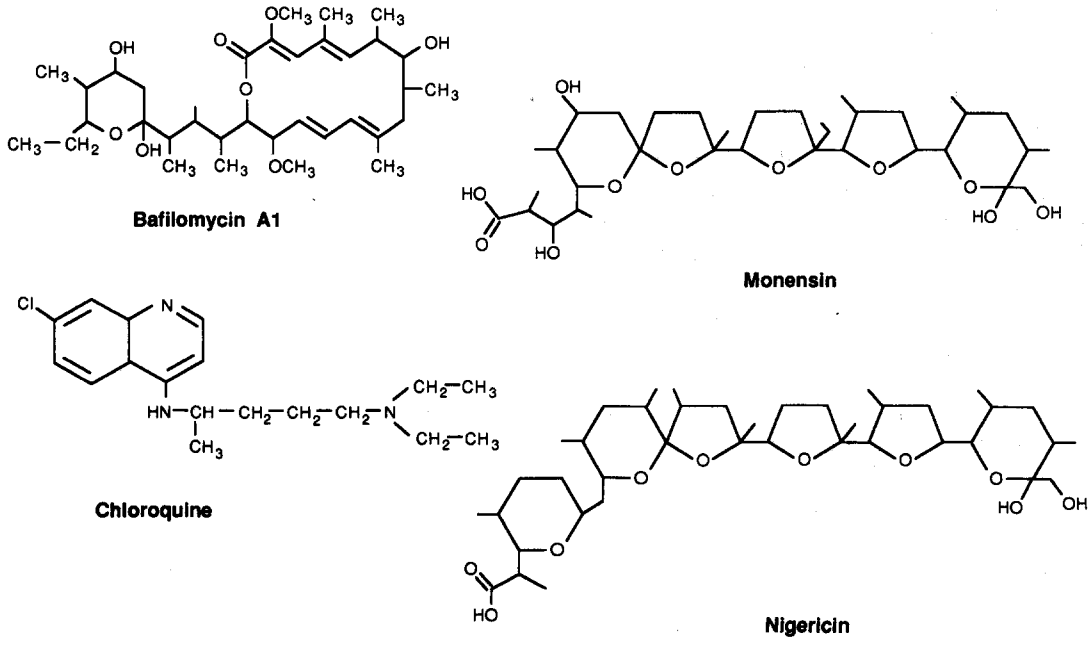


Fig. 13. Chemical structures of different compounds: bafilomycin A1, monensin, chloroquine and nigericin.

Table 1. *Characteristics of Some Toxins that Especifically Enter Together with Virus Particles*

Toxin	Producer organism	MW AA	Mode of action
Diphtheria toxin	*Corynebacterium diphteriae*	58,000 (535 AA)	ADP-ribosylation elongation factor EF-2
Exotoxin A	*Pseudomonas aeruginosa*	54,000 (613 AA)	ADP-ribosylation elongation factor EF-2
Ricin	*Ricinus communis*	(A + B chains) 64,000 (262 AA + 240 AA)	Adenine removal from 28S ribosomal RNA
Abrin	*Abrus precatorius*	(A + B chains) 65,000 (251 AA + 267 AA)	Adenine removal from 28S ribosomal RNA
PAP	*Phytolacca americana*	31,000	Adenine removal from 28S ribosomal RNA
Alpha-sarcin	*Aspergillus giganteus*	16,000 (150 AA)	28S ribosomal RNA cleavage
Mitogillin	*Aspergillus restrictus*	16,900 (149 AA)	28S ribosomal RNA cleavage
Restrictocin	*Aspergillus restrictus*	16,000 (149 AA)	28S ribosomal RNA cleavage

The producer organism, the molecular mass (MW), number of amino acids (AA) and the mode of action are indicated.

3.3.2. *Bafilomycin A1*

Three major classes of proton-ATPases exist in mammalian cells: (1) the mitochondrial F-class ATPases, (2) the plasma membrane proton ATPases of the P-class and (3) the V-class of vacuolar proton-ATPases (Schneider, 1987; Nelson and Taiz, 1989). Each class is distinguished by several parameters, including their sensitivity to inhibitors (Nelson and Taiz, 1989). Endosomes, along with other organelles of the vesicular system, become acidified by the action of the vacuolar proton-ATPases (Schneider, 1987; Nelson and Taiz, 1989). These enzymes pump protons into the organelles at the expense of ATP hydrolysis (Schneider, 1987; Nelson and Taiz, 1989). The two major classes of inhibitors, hitherto used to assess the requirement for a low-pH step during virus infection, are either weak amines (NH_4Cl, chloroquine, amantadine, etc.) that raise the endosomal pH by their accumulation of ionophores (monensin, nigericin) that dissipate the proton gradient (Seglen, 1983) (see Section 3.3.1). In some studies, the inhibitor dicyclohexylcarbodiimide (DCCD), which blocks all types of proton-ATPases, has been assayed (Seglen, 1983; Hiebsch *et al.*, 1991). BFLA1 (Fig. 13), a recently discovered antibiotic, is a powerful and selective inhibitor of the vacuolar proton-ATPases (Bowman *et al.*, 1988; Nelson and Taiz, 1989; Zeuzem *et al.*, 1992; Dröse *et al.*, 1993). BFLA1, in contrast to DCCD, which nonspecifically blocks all types of proton-ATPases (Nelson and Taiz, 1989), is a unique compound for assaying the roles that acidification of endosomes and the action of the vacuolar proton-ATPase play in the entry of animal viruses. Viruses such as SFV or VSV, which enter cells through endosomes and need their acidification, are potently inhibited by BFLA1, whereas poliovirus infection is not affected by the antibiotic (Pérez and Carrasco, 1993). Therefore, the entry of poliovirus is not restricted by the three different techniques used to increase the endosomal pH, i.e. (1) the blockade of the vacuolar proton-ATPase with BFLA1, (2) the raising of the endosomal pH by weak amines and (3) the dissipation of the pH gradient with monensin. The effect of BFLA1 on other members of the *Picornaviridae* family indicates that entry of EMC virus into HeLa cells was not affected by this macrolide antibiotic, whereas rhinovirus was sensitive to its action (Pérez and Carrasco, 1993). The co-entry of toxins, such as α-sarcin, together with poliovirus particles is potently inhibited by BFLA1, indicating that an active vacuolar proton-ATPase is necessary for the early membrane permeabilization induced by poliovirus (see Section 2.4).

3.4. Toxins that Enter Cells Together with Virus Particles

A number of protein toxins are effective inhibitors of protein synthesis (Vázquez, 1979; Jimenez and Vázquez, 1985) (Table 1). The mode of action of these toxins in intact cells involves two steps, namely attachment of the toxin to cell membrane receptors and entry of the toxin, or its effector moiety, into the cytoplasm where enzymatic inactivation of the components of the protein synthesizing machinery occurs. Some toxins, such as diphtheria or *Pseudomonas* exotoxin A,

inactivate the elongation factor EF-2 by ADP-ribosylation (Aktories, 1992), whereas ricin, abrin, α-sarcin, mitogillin, etc., inactivate ribosomes by acting on the 28S rRNA (Endo *et al.*, 1987; Endo and Tsurugi, 1987; Hartley *et al.*, 1991; Better *et al.*, 1992; Irvin and Uckun, 1992). Some plant toxins, such as mitogillin, restrictocin or α-sarcin, do not bind to receptors, but are potent inhibitors of translation in cell-free systems or in permeabilized cells (Jimenez and Vázquez, 1985; Carrasco and Vázquez, 1983).

Poliovirus is able to co-internalize, and, thus, deliver, protein toxins efficiently into cells (Carrasco, 1981; Carrasco *et al.*, 1989; Lee *et al.*, 1990; Almela *et al.*, 1991). The antiviral potency of these toxins is high because after the toxin has entered along with the infecting virus, the translation of viral proteins does not occur and the formation of the progeny virus is strongly suppressed (Ussery *et al.*, 1977; Yamaizumi *et al.*, 1979; Fernandez-Puentes and Carrasco, 1980; Carrasco, 1981; Carrasco and Esteban, 1982; Irvin and Uckun, 1992). In addition to protein toxins, low molecular weight antibiotics also enter cells early during infection (Carrasco, 1981; Carrasco and Vázquez, 1983). In general, hydrophilic antibiotics are selective inhibitors of viral translation (Carrasco and Vázquez, 1983), thus blocking viral replication at concentrations that do not affect uninfected cells (Lacal and Carrasco, 1983b) (see also Section 4.3.1). The mode of action of different inhibitors on the entry of poliovirus and the co-entry of toxins is illustrated in Fig. 14.

4. TRANSLATION OF PICORNAVIRUS RNA

Each picornavirus possesses a single species of mRNA that is identical to its genome (Harber and Wimmer, 1993). This long RNA (7.2–8.5 Kb) has a single initiator AUG (Meerovitch and Sonenberg, 1993) except for FMDV, where initiation can take place on two different AUGs that are in phase and direct the synthesis of two different L proteins (Belsham, 1992). The translation

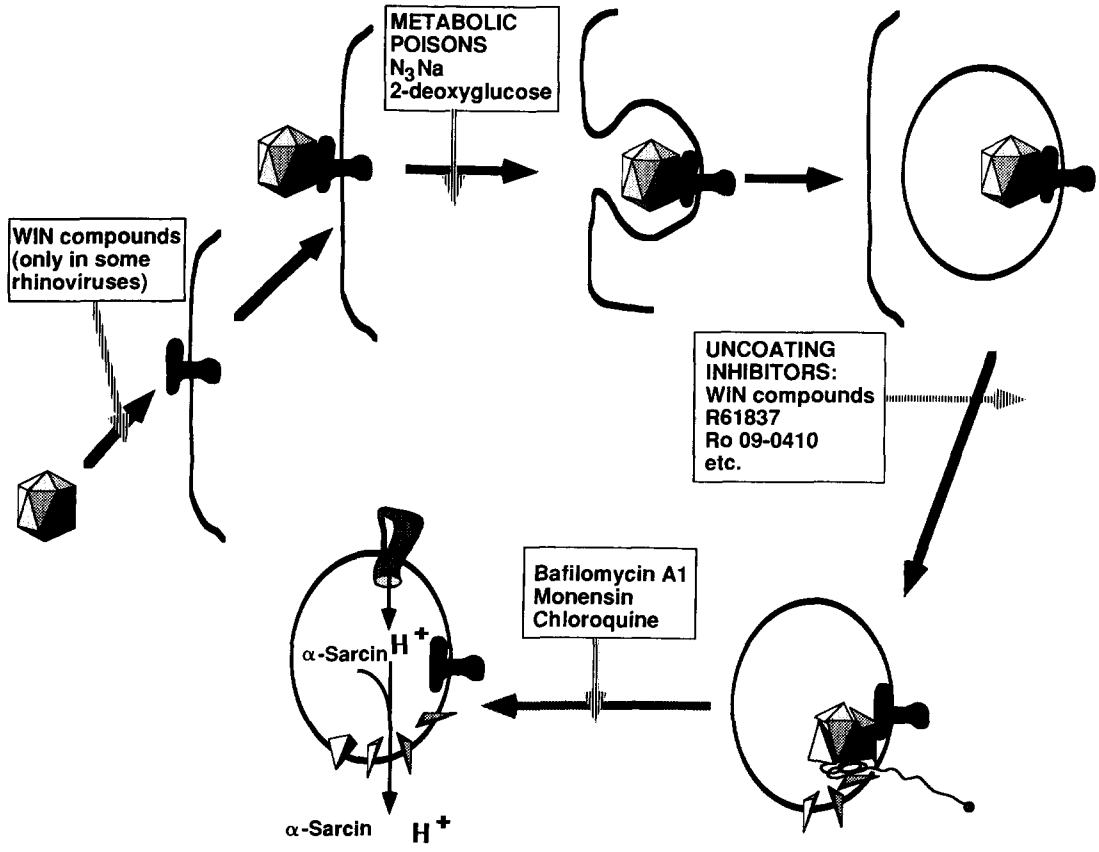


Fig. 14. Site of antiviral action of several inhibitors during the early steps of picornavirus entry into cells. The cellular components are as illustrated in Fig. 7.

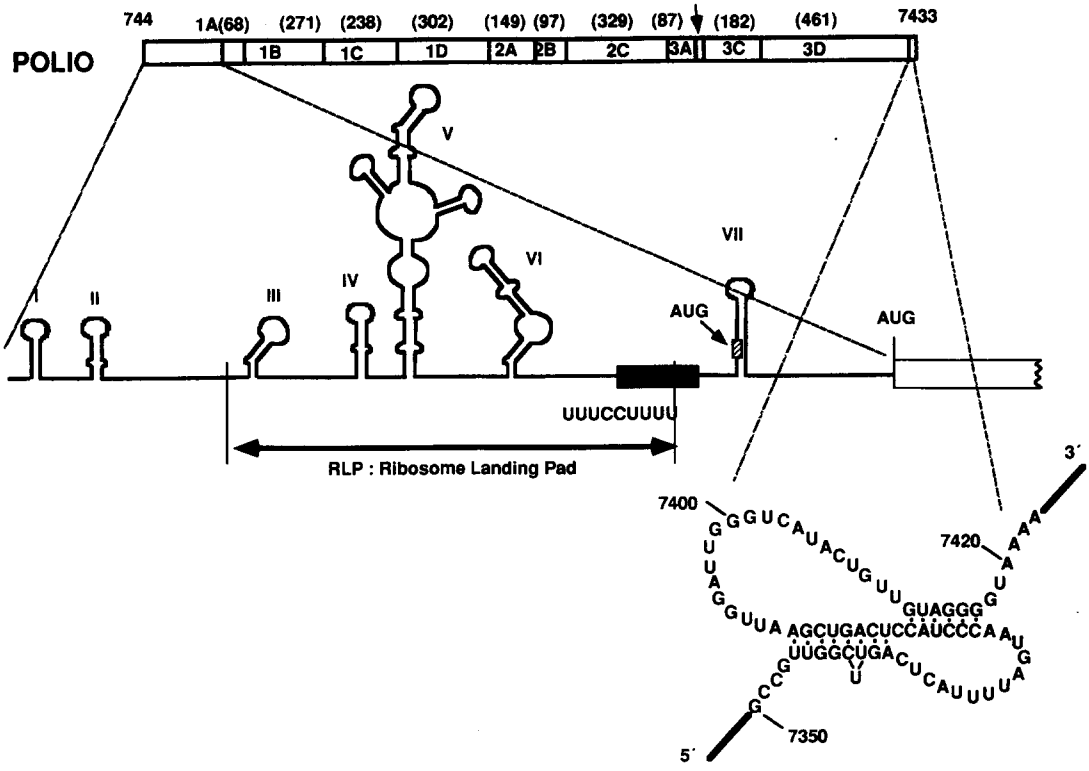


Fig. 15. Representation of the non-coding regions of the poliovirus genome: RLP is also known as the IRES region, responsible for the internal initiation of translation. The pseudoknot present in the 3′ region is depicted. For further explanation see text.

of picornavirus RNA is accomplished by the host protein synthesizing machinery using, in general, the same mechanism as that used to translate any cellular mRNA, particularly with respect to the elongation and termination phases. However, the initiation of translation of picornavirus RNA shows some unusual features that will now be considered.

All known cellular mRNAs possess a cap structure at their 5′-end that is recognized by the translation initiation complex (Rhoads, 1988, 1993). Once the ribosomal 40S subunit plus a number of initiation factors are bound to this capped 5′-end, they scan the mRNA leader sequence until an AUG is found in the correct context to start translation (Sonenberg, 1990; Kozak, 1992). This mechanism is not used for the initiation of picornavirus translation, because the initiation complex binds to a unique internal sequence within the long leader sequence (Sonenberg, 1990). In poliovirus, this region is located approximately between nucleotides 134 to 585–612 of the leader sequence (Sonenberg, 1990; Kozak, 1992). The secondary structure of the 5′ leader region of different picornaviruses reveals two different patterns, one for entero- and rhinoviruses and another for cardio- and aphtoviruses (Jang *et al.*, 1988; Pelletier and Sonenberg, 1988; Belsham, 1992; Harber and Wimmer, 1993) (Fig. 15). It is even possible that the requirements and mechanism of initiation on the RNAs of these two classes of viruses differ (Sonenberg, 1991; Meerovitch and Sonenberg, 1993). Thus, the initiation of translation on the poliovirus mRNA can start by the attachment of the initiation complex to this internal region of the leader sequence, which has been christened as the ribosome landing pad (RLP) or internal ribosome entry site (IRES). This event could be followed by a scanning phase for selection at the initiator AUG (Sonenberg, 1990; Kozak, 1992). In contrast, initiation on EMC virus RNA commences by the direct binding of the initiator complex to the IRES (or RLP) and to the initiator AUG. A scanning phase does not appear to be involved (Kaminski *et al.*, 1990; Meerovitch and Sonenberg, 1993).

Since picornavirus RNA has a high binding affinity for ribosomes, it exhibits different requirements for translation with respect to initiation factors. Picornavirus RNA was the first mammalian mRNA to be translated in cell-free systems (Kerr and Martin, 1971), and these studies showed that its translation required different concentrations of some initiation factors when

compared with translation of cellular mRNAs (Wigle and Smith, 1973). More recent studies have analyzed the effects of exogenously added individual initiation factors to rabbit reticulocyte lysates programmed with capped or uncapped mRNAs. Internal initiation on synthetic bicistronic mRNAs that contain the poliovirus 5′-end in the second cistron is increased by addition of eIF-4B and eIF-4F (Anthony and Merrick, 1991), indicating that eIF-4F participates in the translation of naturally uncapped mRNAs (Anthony and Merrick, 1991; Thomas *et al.*, 1991).

Apart from the initiation factor requirement for efficient translation, a number of cellular proteins with binding affinity for picornavirus RNA have been identified, but the roles that these proteins could play during internal initiation of translation, or as "initiation correction factors", deserve further investigation (Meerovitch and Sonenberg, 1993; Meerovitch *et al.*, 1993). Viral proteins can also bind to specific regions on the 5′-end of mRNA and, perhaps, might affect translation. This is the case for poliovirus protein 3CD that binds to the 5′-end (Andino *et al.*, 1990a), or for protein 2A, which enhances translation of poliovirus RNA by an as yet unidentified mechanism (Hambidge and Sarnow, 1992).

One of the most interesting aspects of the translation of picornavirus RNAs is that, in comparison with cellular mRNAs, they require a higher concentration of monovalent cations for optimal translation (Carrasco and Smith, 1976). In fact, under the ionic conditions required to initiate translation efficiently on picornavirus RNA, the initiation on cellular mRNAs is restricted (Saborio *et al.*, 1974; Carrasco and Smith, 1976; Carrasco *et al.*, 1979). The discrimination between viral and cellular mRNAs shown by monovalent cations in cell-free systems is such that at higher cation concentrations, the resulting effects perfectly mimic the shut-off of host translation observed in intact cells after infection (Carrasco and Smith, 1980). The physiological significance of these requirements is related to the fact that viral protein synthesis takes place mainly in a cytoplasm where monovalent ion concentrations have drastically changed (see review by Carrasco *et al.*, 1989) as a consequence of membrane leakiness produced during picornavirus infection. This observation explains the shut-off of host translation during infection that is observed in some members of the *Picornaviridae* family (Carrasco, 1977, 1987; Carrasco and Castrillo, 1987).

4.1. Picornavirus Proteases

Picornaviruses encode for the proteases responsible for the processing of the polyprotein to produce mature viral proteins (Krausslich and Wimmer, 1988; Hellen *et al.*, 1989; Lawson and Semler, 1990; Palmenberg, 1990). The picornavirus proteases have been classified as cysteine peptidases (Rawlings and Barrett, 1993). The two poliovirus proteases, proteins 2A and 3C, are active in the precursor form and are able to cleave substrates in *cis*. Indeed, after their proteolytic modification, they can recognize their substrates in *trans* even in the nascent polypeptide chain. Viral proteases are highly specific and cannot be interchanged between different picornavirus species (Krausslich and Wimmer, 1988; Hellen *et al.*, 1989; Lawson and Semler, 1990; Palmenberg, 1990). Apart from cleaving the viral polyprotein and the polypeptide precursors, they can also cleave a limited number of cellular proteins (Grigera and Tisminetzky, 1984; Urzáinqui and Carrasco, 1989; Falk *et al.*, 1990; Clark *et al.*, 1991; Joachims and Etchison, 1992). The best illustrated example is the cleavage of polypeptide p220 that forms part of the translation initiation factor eIF-4F induced by poliovirus protease $2A^{pro}$ (Etchison *et al.*, 1982; Lloyd *et al.*, 1988). Cleavage of p220 is not directly accomplished by $2A^{pro}$, but by another cellular protease that is activated by $2A^{pro}$ (Wyckoff *et al.*, 1990, 1992). It was suggested that cleavage of p220 was a crucial step in the inhibition of host translation by poliovirus infection (Etchison *et al.*, 1982). However, recent evidence indicates that this may not be so. Thus, there is no correlation in time between p220 cleavage and the shut-off of host translation (Etchison *et al.*, 1982; Bonneau and Sonenberg, 1987; Pérez and Carrasco, 1992); the presence of some inhibitors of poliovirus RNA synthesis allows complete cleavage of p220, while for hours there is still a substantial amount of host protein synthesis (Bonneau and Sonenberg, 1987; Pérez and Carrasco, 1992); translation of several capped viral mRNAs can take place in poliovirus-infected cells (Alonso and Carrasco, 1982b; Muñoz *et al.*, 1985a; Castrillo and Carrasco, 1987b; Schrader and Westaway, 1990); eIF-4E, an initiation factor that, together with p220 forms eIF-4F, is localized in the nucleus and not in the cytoplasm (Lejbkowicz *et al.*, 1992); monocytic cell lines persistently infected with poliovirus synthesize

proteins even though p220 has been extensively, but not completely, degraded (Lloyd and Bovee, 1993); a poliovirus mutant in 2A that is unable to cleave p220 still shuts down host translation (Bernstein *et al.*, 1985); eIF-4F stimulates the translation of capped or naturally uncapped viral mRNAs (Anthony and Merrick, 1991; Thomas *et al.*, 1991); transient expression of poliovirus protein 2A, the protease that induces p220 cleavage, has a major impact on transcription of a reporter gene, compared with its translation (Davies *et al.*, 1991); finally, disruption of the yeast p220 gene homologue does not block cellular growth (Lanker *et al.*, 1992).

4.2. Proteases as Targets of Antipicornavirus Agents

Precise action of picornavirus proteases is vital for generating the mature viral proteins that are, in turn, required for a number of viral processes, including replication of the picornavirus genome (Krausslich and Wimmer, 1988; Hellen *et al.*, 1989; Lawson and Semler, 1990; Palmenberg, 1990). Thus, it seems feasible that selective interference with protease function would lead to the specific inhibition of viral growth (Korant *et al.*, 1986; Hellen and Wimmer, 1992a). Indeed, a number of agents have been described as inhibitors of protease function (Korant *et al.*, 1986; Korant, 1987, 1990, 1991) and can be broadly classified in two categories: (1) compounds that modify the conformation of the viral polyprotein and render it refractory to protease action and (2) inhibitors of the proteases themselves.

With respect to the first category, a number of amino acid analogues (Jacobson *et al.*, 1970; Jacobson and Baltimore, 1968), zinc ions (Korant *et al.*, 1974) or high temperature lead to conformational changes of the poliovirus polyprotein, which is then no longer recognized by viral proteases (Korant, 1990, 1991). However, this approach is not selective for inhibition of viral proteases since cellular proteins are also affected.

A number of amino acid analogues have been used to inhibit the proteolytic degradation of the picornavirus polyprotein. In fact, these agents were employed to identify picornavirus precursors and to block the formation of mature proteins. Compounds such as DL-ethionine (Brown and Ackermann, 1951), TPCK (tolylsulfonylphenylalanyl chloromethyl ketone), TLCK (tolylsulfonyl lysyl chloromethyl ketone) (Korant, 1972), ZPCK (carbobenzyloxyphenylalanyl chloromethyl ketone) (Summers *et al.*, 1972), ZLCK (carbobenzoxyleucyl chloromethyl ketone) (Korant *et al.*, 1979), canavanine or fluorophenylalanine (Brown *et al.*, 1961; Levintow *et al.*, 1962; Scharff and Levintow, 1963) (Fig. 16) inhibit the proteolytic processing of the picornavirus polyprotein.

4.2.1. *Cystatin C*

The activities of cysteine proteases are controlled by a set of protein inhibitors that constitute the cystatin superfamily of proteins. Cystatin C is a human cysteine proteinase inhibitor present in extracellular fluids. Cystatins are small proteins (110–120 amino acids) that bind and selectively inhibit cysteine proteases. Human cystatin C, obtained from human serum, and human stefin B, obtained from spleen, also bind and inhibit poliovirus protease 3C and block the replication of poliovirus in cultured cells (Korant *et al.*, 1985, 1986). Not only cystatin C, but also peptides that mimic the proteinase-binding center of cystatin C, such as Z-LVG-$CHNH_2$ (*N*-benzyloxycarbonyl-leucyl-valyl-glycine diazomethylketone), show antiviral activity against poliovirus and herpes simplex virus (see Section 4.2.2) (Bjorck *et al.*, 1990).

4.2.2. *Peptidyl Diazomethyl and Chloromethyl Ketones*

The use of peptides that mimic the viral cleavage site provides a potential for the development of new protease inhibitors, because they inhibit protease function (Green and Shaw, 1981). Therefore, analogues of the recognition site of viral proteases have a modified C-terminal or the N-terminal blocks viral replication (Bjorck *et al.*, 1990; Korant, 1990). Tri-, tetra- or pentapeptides having, in the C-terminal, chloromethyl ketone or methyl ketone moieties, were active against poliovirus and rhinovirus (Kettner and Korant, 1987).

4.2.3. *E-64 (L-Trans-Epoxysuccinyl-Leucylamido(4-Guanidine) Butane)*

The thiol protease inhibitor E-64 (Fig. 16) is a natural product isolated from *Aspergillus japonicus*. It has also been chemically synthesized. E-64 and leupeptin are inhibitors of the cysteine protease calpain (Mehdi, 1991). E-64 derivatives lacking charged groups inhibit intracellular proteases (Mehdi, 1991). E-64 specifically blocks the L-protease of FMDV, leading to a reduction in virus yields (Kleina and Grubman, 1992). Addition of a permeable analogue to cultured cells infected with FMDV decreases viral RNA synthesis, cleavage of p220 and retardation of the virus-induced shut-off of host translation (Kleina and Grubman, 1992). It is not yet known whether this last effect is due to inhibition of the L-protease itself or to the general retardation of viral replication. E-64 and its permeable derivatives are nontoxic in animals, and doses of 400 mg/kg of body weight are well tolerated by various administration routes (Komatsu *et al.*, 1986; Kleina and Grubman, 1992).

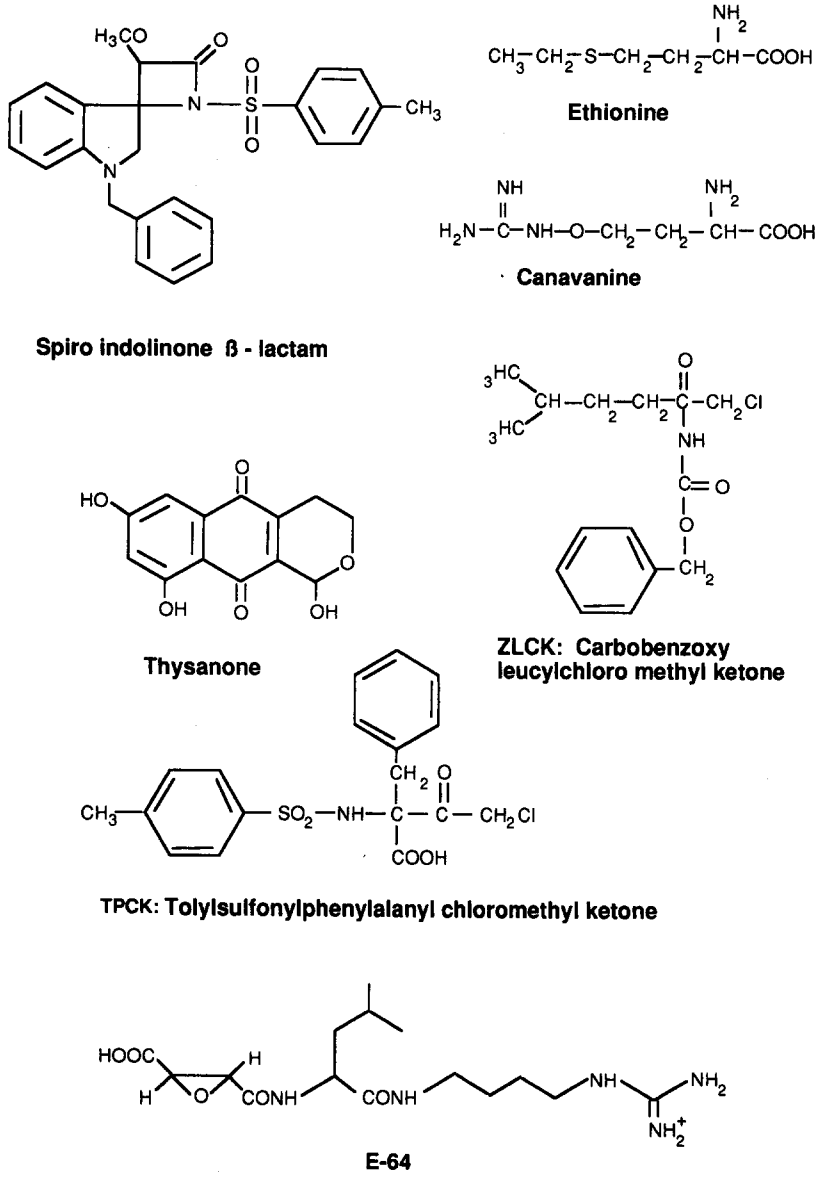


Fig. 16. Chemical structures of different compounds: spiro indolinone β-lactam, ethionine, canavanine, thysanone, ZLCK, TPCK and E-64.

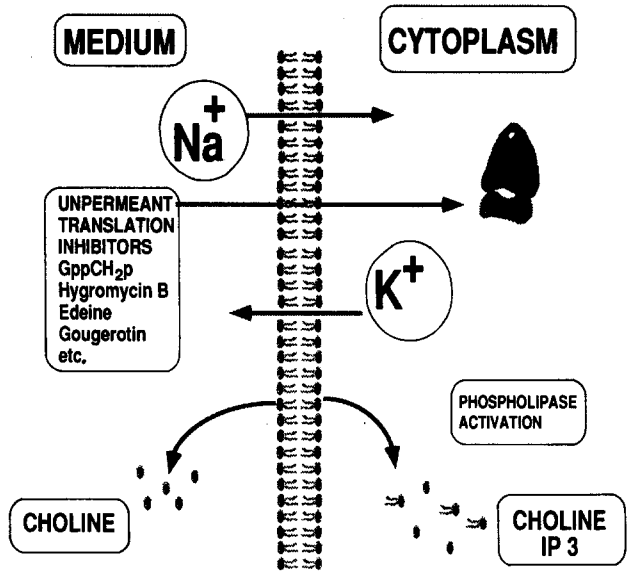


Fig. 17. Modification of membrane permeability and phospholipase action late after poliovirus infection. The modification of the membrane late during viral infection leads to a disbalance of the ionic gradients and the increased entry of unpermeant inhibitors. The activation of phospholipase C results in the release of IP3, choline and phosphorylcholine from phospholipids.

4.2.4. *Spiro Indolinone β-Lactams and Thysanone*

A series of spiro indolinone β-lactams have been synthesized and their activity assayed against poliovirus and rhinovirus 3C proteases (Skiles and McNeil, 1990). The sulfonamide derivative shown in Fig. 16 was active not only against these picornavirus proteases, but against other cellular proteases such as human leukocyte elastase and cathepsin G (Skiles and McNeil, 1990).

Thysanone (Fig. 16) is a novel naphthoquinone isolated from the fungus *Thysanophora penicilloides*, which is active against rhinovirus 3C protease (Singh *et al.*, 1991). The selectivity of these compounds in cell culture has yet to be investigated.

Although viral proteases have repeatedly been suggested as potentially effective targets for the inhibition of viral growth (Korant, 1987; Hellen and Wimmer, 1992a), no clinical results have been reported to support this premise.

4.3. Modification of Membrane Permeability by Picornaviruses

The most drastic modifications of membrane permeability occur in the late phase of the replication cycle of many cytolytic viruses, and a detailed review on this subject has already been published (Carrasco *et al.*, 1989) (Fig. 17). The major salient feature is that a viral gene product is required for the development of late modifications in membrane permeability. This modification starts in HeLa cells at about the third hour after infection with poliovirus (Nair *et al.*, 1979; Lacal and Carrasco, 1982; Muñoz and Carrasco, 1983; Nair, 1984; López-Rivas *et al.*, 1987; Garry, 1989), or at the second hour p.i. in cells infected with SFV (Garry *et al.*, 1979; Ulug *et al.*, 1987). Thus, the majority of viral macromolecules are synthesized in cells with an altered plasma membrane in a cytoplasm where the concentrations of ions, and perhaps pH and divalent cations, are gradually changing (Carrasco and Smith, 1976; Muñoz *et al.*, 1985a, b; López-Rivas *et al.*, 1987; Holsey *et al.*, 1990). The mechanism of membrane permeabilization by poliovirus is fairly well understood. There is an overall increase in permeability to a variety of molecules (Carrasco *et al.*, 1989, 1993), and the modification of the membrane is such that the gradients of monovalent cations are destroyed. In addition, other metabolites and compounds that are excluded by the membrane leak out from cells and can readily pass to the cytoplasm (Egberts *et al.*, 1977; Benedetto *et al.*, 1980; Nair, 1981).

An updated view of the modifications of membrane permeability by animal viruses is shown in

Fig. 17. The putative viral protein responsible for membrane modification associates with membranes once it is synthesized and travels through the vesicular system to the plasma membrane. As we have already indicated, these viral proteins may possess an ionophore-like activity (Carrasco, 1977, 1987; Carrasco *et al.*, 1993), such that the pH and ionic content of vesicles of the vesicular system will be modified. Once these proteins reach the plasma membrane, they increase the permeability to ions, leading to the disruption of the membrane potential (Lacal and Carrasco, 1982, 1983a). We have suggested that these changes of ions and pH in the cytoplasm influence an array of cellular functions, including the translation of host mRNAs (Carrasco, 1977, 1987; Carrasco *et al.*, 1993).

With respect to the mechanism of late membrane permeabilization, we have found that phospholipase A2 activity is inhibited early following poliovirus infection, whereas the phospholipase C that hydrolyses phosphatidylinositol is stimulated from the third hour p.i. (Guinea *et al.*, 1989). Thus, in poliovirus-infected cells, the amount of inositol triphosphate (IP3) present in the cytoplasm constantly increases (Guinea *et al.*, 1989). This increase would lead to an augmentation of free calcium. However, we are not aware of any analysis of free calcium levels in cells infected by picornaviruses. Recently, we have analyzed in detail the activation of the phospholipase C that hydrolyses phosphatidylcholine, and found that both choline and phosphorylcholine drastically increase in the medium of poliovirus-infected cells from the third hour of infection (Irurzun *et al.*, 1993).

It is clear that a correlation in time exists between the increase in membrane permeability and the activation of phospholipase C in poliovirus-infected cells (Guinea *et al.*, 1989; Irurzun *et al.*, 1993). However, we do not know yet if there is a cause–effect relationship between them. A number of protein toxins, such as melittin, that modify membrane permeability also activate phospholipase activity (Durkin and Shier, 1981; Pellkofer *et al.*, 1982). It is tempting to speculate that poliovirus encodes a protein responsible for modifying the membrane (Lama and Carrasco, 1992a, b; Lama *et al.*, 1992). This protein could be poliovirus protein 3A, a membrane protein that contains an amphipathic helix. A similar motif is found in other viral membrane proteins that modify membrane permeability (Pinto *et al.*, 1992; Carrasco *et al.*, 1993). Such viral proteins that are responsible for increasing membrane permeability have been collectively referred to as *viroporins* (Carrasco *et al.*, 1993). The accumulation of a sufficient number of copies of such a viroporin (Carrasco *et al.*, 1993) would form pores in the membrane that may lead *per se* to the permeabilization of the membrane. A secondary effect could be the activation of phospholipase C, to generate diacylglycerol, which, in turn, also increases membrane permeability (Felix, 1982; Otero and Carrasco, 1988).

4.3.1. *Nonpermeant Translation Inhibitors*

In principle, translation inhibitors that have their target on components of the protein synthesizing machinery would block viral and cellular translation nonspecifically. The action of compounds such as puromycin (Levintow *et al.*, 1962) or pactamycin (Summers and Maizel, 1971; Taber *et al.*, 1971) have been used to study the dependence of viral RNA synthesis on viral translation (Levintow *et al.*, 1962) or to map the viral proteins on the picornavirus genome (Lawson and Semler, 1990). However, there is no selectivity in this inhibition compared with the blockade of host protein synthesis in uninfected cells. A selective way to block protein synthesis in animal virus-infected cells was devised based on modification of membrane permeability during virus infection. This approach employs the use of hydrophilic translation inhibitors that do not easily cross the membrane barrier of normal uninfected cells, but readily enter into a number of animal virus-infected cells (Carrasco, 1978; Contreras and Carrasco, 1979; Vázquez, 1979; Carrasco *et al.*, 1989). These studies showed that the bulk of picornavirus protein synthesis occurs in cells where the membrane has been drastically modified (Carrasco *et al.*, 1989). The presence of these translation inhibitors not only blocked selectively the synthesis of viral proteins, but also blocked the formation of new infectious virus, indicating that virus assembly takes place largely after the membrane has been modified (Benedetto *et al.*, 1980). Hence, inhibitors of translation that are hydrophilic and do not inhibit protein synthesis efficiently in intact cells are able to selectively block translation in a variety of animal virus-infected cells (Carrasco, 1978; Contreras and Carrasco,

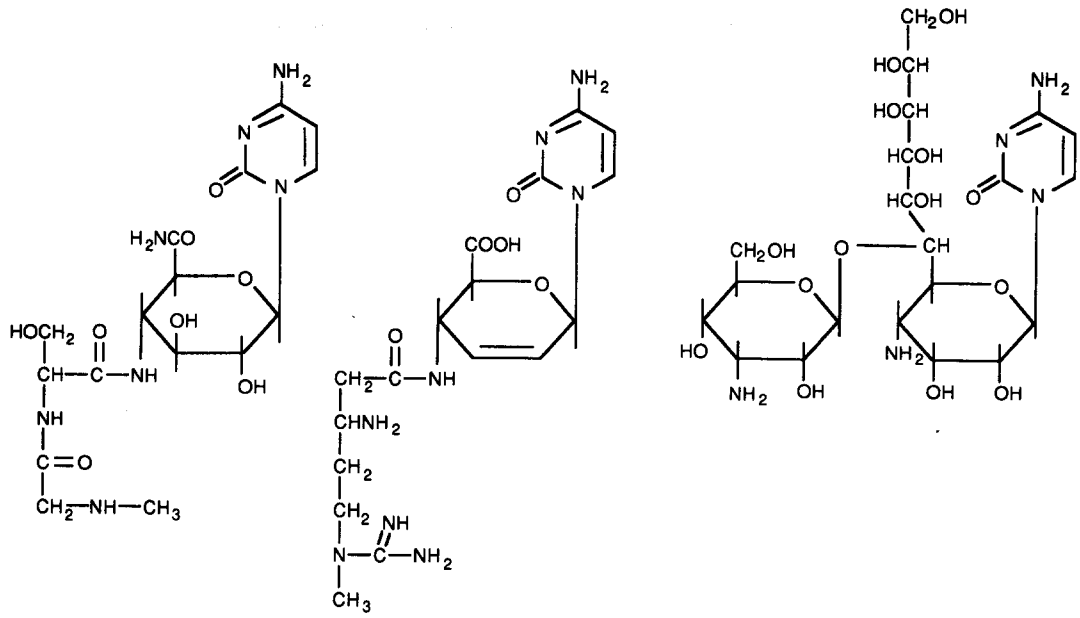


Fig. 18. Chemical structures of different compounds: gougerotin, blasticidin S and anthelmycin.

1979; Lacal *et al.*, 1980; Carrasco *et al.*, 1981; Carrasco and Vázquez, 1983; Lacal and Carrasco, 1983b). The structural formulae of some of these "non-permeant translation inhibitors" is shown in Figs 18 and 19. Perhaps the most widely used of these agents has been hygromycin B (Carrasco and Vázquez, 1983). The inhibition of protein synthesis by this aminoglycoside antibiotic can be

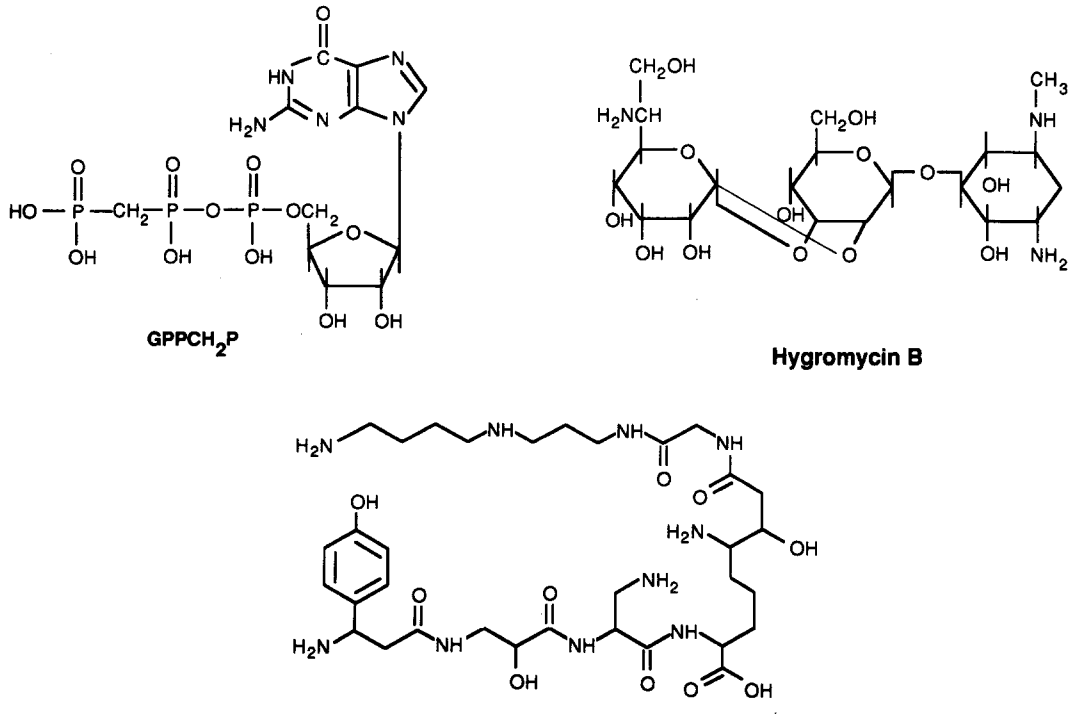


Fig. 19. Chemical structures of different compounds: GppCH$_2$p, hygromycin B and edeine A1.

used as a test to measure changes in membrane permeability not only in cells infected with animal viruses, but also in any cell type (eukaryotic or prokaryotic) where membrane permeability has been altered (Alonso and Carrasco, 1981a, b, 1982a; Otero and Carrasco, 1987a; Lama and Carrasco, 1992b).

The formation of infectious virus by these agents is strongly diminished at drug concentrations that do not affect translation in uninfected cells (Benedetto *et al.*, 1980; Lacal *et al.*, 1980; Lacal and Carrasco, 1983b). Therefore, cell killing by low multiplicities of poliovirus is prevented by treatment of cell monolayers with hygromycin B (Lacal and Carrasco, 1983b). This selectivity is not achieved by other inhibitors of translation that enter equally well in virus-infected or uninfected cells (Contreras and Carrasco, 1979; Lacal and Carrasco, 1983b). This approach has not been exploited in animals because some of the nonpermeant antibiotics that block translation are not very potent, as occurs with guanylyl methylene diphosphate (Dawson *et al.*, 1979), or showed toxicity (unpublished results), perhaps due to different cellular susceptibilities in the animal after prolonged treatment. However, the approach of using inhibitors of any viral or cellular function that selectively enter into virus-infected cells early or late during infection, as a result of modification of membrane permeability, remains open. In other words, selectivity can be conferred on antiviral compounds by adding charged residues to make them less permeable in uninfected cells, whereas these charged molecules will readily enter virus-infected cells. In fact, we have observed that highly charged compounds, such as sulphated polysaccharides, suramin or the dye trypan blue, show good antiviral activity (Alarcón *et al.*, 1984c), and the entry of sulphated polysaccharides into cells is enhanced by poliovirus particles (González and Carrasco, 1987) (see Section 2.4). These agents have been used by a number of laboratories in tests for antiviral effects against several animal viruses, including human immunodeficiency virus (HIV) (Mitsuya *et al.*, 1984, 1988; Balzarini *et al.*, 1986; González *et al.*, 1987; Zuckerman, 1987; Schols *et al.*, 1990; Callahan *et al.*, 1991; McClure *et al.*, 1991).

5. PICORNAVIRUS GENOME REPLICATION

Once the viral genome is liberated in the cytoplasm after poliovirus entry, it is recognized by the ribosomes and the translation machinery as mRNA (Sonenberg, 1990). Translation of the input viral RNA gives rise to a number of virus-encoded proteins that are necessary to replicate the genome (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). Although many details of the steps involved in the replication of poliovirus RNA have been unravelled, this is perhaps one of the least understood processes in molecular terms. This is particularly so for the initiation of new RNA chains, the possible role played by some cellular proteins and the requirement of membranes in genome replication (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). The replication of an RNA involving only RNA, and not DNA, intermediates, as occurs with retroviruses or hepadnaviruses, is perhaps one of the most primitive processes in the multiplication of biological molecules. An understanding of how picornaviruses accomplish their replication can shed light on the essential requirements of one of the most primitive forms of life and elucidate how other more complex RNA-containing viruses are able to replicate their genomes in infected cells. In addition, once the requirements for the replication of an RNA genome have been defined, they can be used for the amplification of any kind of RNA molecule, either in intact cells or in cell-free systems, as already achieved with phage RNA (Wu *et al.*, 1992).

One of the essential steps required for the amplification of a positive-stranded RNA molecule is the copying of this RNA to its complementary chain that, in turn, will be used as a template for synthesis of more positive-strand RNA molecules (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990) (Fig. 20). Thus, in picornavirus-infected cells, the only two classes of RNA molecules present are: the genomic positive-strand RNA or an exact copy of this RNA, the negative-strand RNA (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). There are approximately 100 times more (+)RNA than (−)RNA molecules in the infected cells (Hewlett *et al.*, 1977). This relation varies, depending upon the cell line infected (Lopez Guerrero *et al.*, 1991). The (−)RNA is found associated with (+)RNA, either as a replicative form (RF)

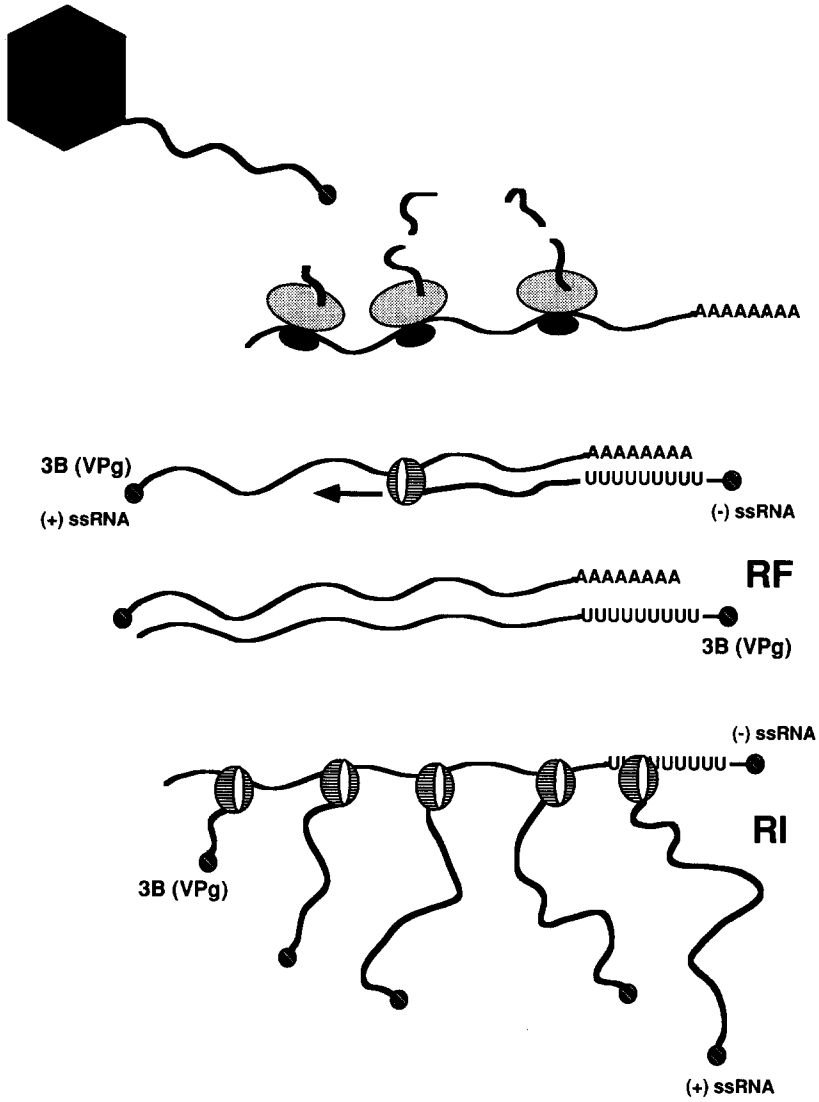


Fig. 20. Schematic representation of picornavirus RNA replication. The polymerase (protein 3D) elongates both (+) and (−)RNA. The terminal protein 3B (VPg) is covalently bound to the 5′-end of any kind of viral RNA (ssRNA, single-stranded RNA).

of double-stranded RNA (ds-RNA) or as a replicative intermediate (RI) of partially double-stranded and partially single-stranded RNA (Baltimore, 1966; Baltimore and Girard, 1966; Hewlett *et al.*, 1977). The single-stranded RNA found in cells as such is of positive polarity and it can be encapsidated in virions or serve as mRNA for the synthesis of viral proteins. In the encapsidated form, the virion RNA has a covalently bound protein (VPg) at the 5′-end of the molecule (Wu *et al.*, 1978). The RI found in cells consists of a complete (−)RNA molecule that is used as template to make copies of (+)RNA. These are present as nascent RNA molecules (4–8 nascent (+)RNA chains per RI), all of which contain a VPg molecule bound to the 5′-end. In fact, this molecule is present in all forms of RNA, positive or negative, that form part of RI or RF (Wimmer *et al.*, 1987).

It is well established that the enzyme required to elongate both (−) or (+)RNA is $3D^{pol}$ (Flanegan and Baltimore, 1977; Flanegan and Van Dyke, 1979). This enzyme, however, is unable to initiate the synthesis of new RNA chains (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). $3D^{pol}$ is a polymerase that is able to copy RNA, using a primer and another RNA molecule as template (Richards and Ehrenfeld, 1990). In fact, the first enzyme of this kind found in mammalian cells was described by Baltimore *et al.* (1963) in picornavirus-infected cells.

Apart from $3D^{pol}$, the total number of proteins required for replication of the picornavirus genome has not yet been established. Virus-encoded proteins are necessary and cellular proteins may also be involved (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). In addition, viral (or cellular) proteins need to be synthesized continuously for participation in the synthesis of viral RNAs, because inhibition of protein synthesis in picornavirus-infected cells leads to almost immediate arrest of viral RNA synthesis (Fig. 21).

Of the viral proteins implicated in the replication of poliovirus genomes, certainly $3D^{pol}$ participates in the elongation process and VPg is also necessary (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). It remains unclear if VPg acts as a primer to initiate the synthesis of new RNA chains or if VPg is added later after initiation has been accomplished (Takegami *et al.*, 1983; Tobin *et al.*, 1989), and these two alternative possibilities are illustrated in Fig. 22. According to model A, VPg, either as such or as the precursor 3AB, will, in the presence of UTP and a still unidentified protein, be uridylated to VPg-UMP and then further elongated to VPg-pUpUp (Takeda *et al.*, 1986). This substrate will be used as a primer to elongate the RNA chain (Takeda *et al.*, 1986). Both (−) and (+)RNA start with pUpUp at the 5′-end, and it is possible that VPg alone does not participate as such in the formation process, but as a precursor molecule 3AB (Wimmer *et al.*, 1987). Thus, 3A would serve to anchor 3AB to the membrane in such a way that 3B would be "hanging" from the membrane and ready to be uridylated (Takegami *et al.*, 1983; Wimmer *et al.*, 1987). Once 3AB-Up-Up is synthesized, or after the elongation process has commenced, $3C^{pro}$ will hydrolyze 3AB, releasing 3B from the membrane, while 3A would remain imbedded in the lipid bilayer (Wimmer *et al.*, 1987). We have indicated that 3A is a firm candidate to modify membrane permeability and to lyse the infected cells (Lama and Carrasco, 1992a; Carrasco *et al.*, 1993). In model B of Fig. 22, 3B (VPg) does not participate in a real initiation event, since the 3′-OH group present in the hairpin can be used directly by $3D^{pol}$ to elongate the RNA chain. 3B (VPg) will bind somewhere during elongation in a reaction catalyzed by 3B itself with no requirement for nucleotide hydrolysis (Tobin *et al.*, 1989). Thus far, this model has not been confirmed by other laboratories.

Apart from the roles that $3D^{pol}$, 3B (VPg), 3A and, indirectly, $3C^{pro}$ play in genome replication, other viral proteins are also implicated in this process although their functions are more obscure (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). Genetic studies indicate that the functions of 2C, 2B and perhaps $2A^{pro}$ are also necessary for replication of viral RNA

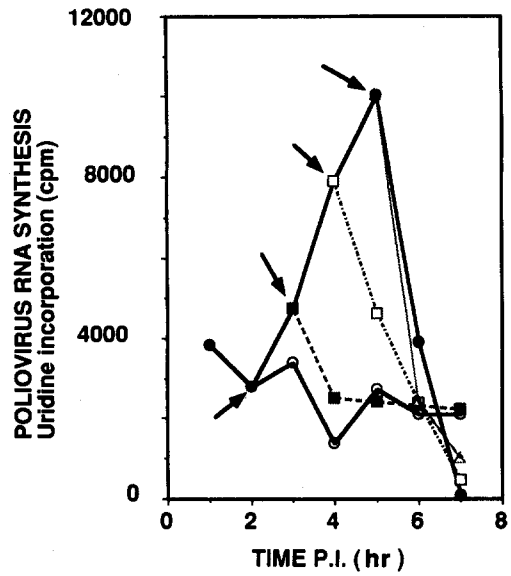


Fig. 21. Effect of cycloheximide on viral RNA synthesis in poliovirus-infected HeLa cells. Cycloheximide (50 μM) was added at the times indicated by the arrows and RNA synthesis was measured during 1-hr intervals in the presence of actinomycin D (Almela and Carrasco, unpublished observations).

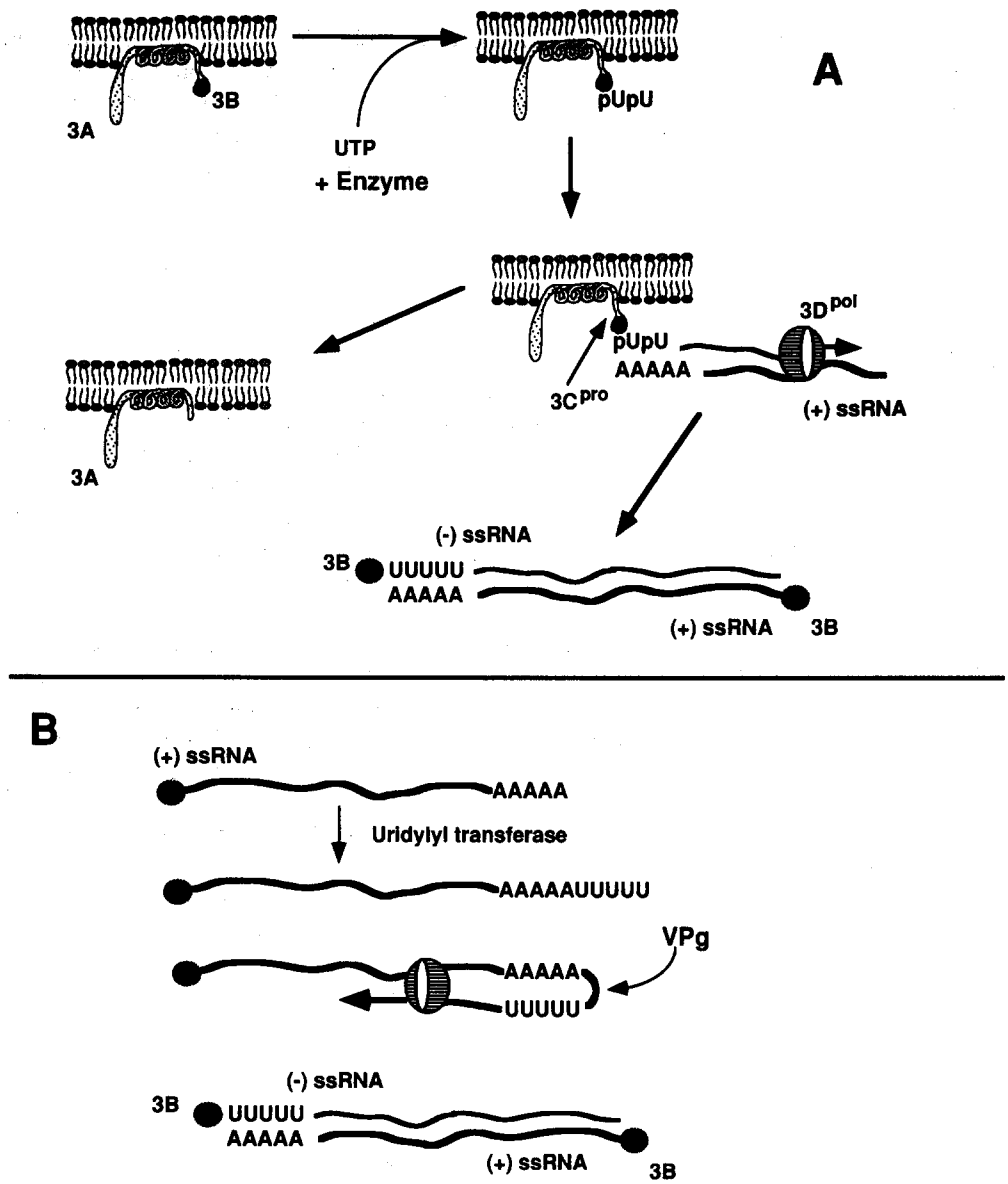


Fig. 22. Two alternative models for the initiation of poliovirus RNA synthesis. Model A: initiation occurs by priming with VPg. Model B: VPg does not participate in a real initiation step and is bound to the 5′-end of viral RNA by an autocatalytic event. For more details see text (ssRNA, single-stranded RNA).

(Sarnow *et al.*, 1990; Kirkegaard, 1992). The most compelling evidence implicating 2C in RNA synthesis came from studies with the viral inhibitor guanidine (Caliguiri and Tamm, 1973). This compound is a selective inhibitor of RNA synthesis in some picornaviruses (see Section 6.1), and elegant studies have proved that it acts on poliovirus protein 2C (Pincus *et al.*, 1986). In addition, poliovirus and mutations in 2C do not replicate the viral RNA (Li and Baltimore, 1988). These mutants also have a defect in virion assembly, suggesting that 2C may also be involved in this late viral step (Li and Baltimore, 1990). Sequence analysis of 2C suggested that it could be a GTPase because it possessed a Gly-rich region followed by the sequence GKS, a sequence present in some GTPases. Indeed, *in vitro* studies with the purified protein indicate that 2C has GTPase activity (Rodríguez and Carrasco, 1993). A homology between picornavirus 2C and the potyvirus protein CI (Lain *et al.*, 1989) has been suggested (Gorbalenya *et al.*, 1990). On this basis, it was claimed that 2C belongs to the RNA-helicase superfamily. However, no helicase activity has yet been demonstrated with isolated 2C (Rodríguez and Carrasco, 1993), although this activity is present

in $3D^{pol}$ (Cho *et al.*, 1993). In fact, we have suggested that 2C may represent a new class of small GTP-binding proteins and its role could be to direct the trafficking of RNA replicative complexes through the vesicular system (Rodríguez and Carrasco, 1993). Clearly, more experiments are needed to clarify the activity of 2C in the replication of the poliovirus genomes.

Protein 2B, either as such or as the precursor 2BC, is also required to replicate the picornavirus genome (Johnson and Sarnow, 1991). Poliovirus with mutations in 2B is impaired in its ability to synthesize viral RNA, but there is no clue as to the exact role that this protein has in this process (Bernstein *et al.*, 1986; Sarnow *et al.*, 1990; Kirkegaard, 1992). Mutations in protein 2B cannot be complemented in *trans*, suggesting that only the actual 2B proteins made on a particular RNA are functional in the replication of that RNA (Johnson and Sarnow, 1991). Indeed, this phenomenon very much complicates the possibility of using the picornavirus system as a model for the replication of any kind of RNA in cells or in cell-free systems (Kirkegaard, 1992). Finally, $2A^{pro}$ may also participate in RNA replication. Apart from cleaving itself from P1, while the nascent polypeptide chain is being synthesized (Krausslich and Wimmer, 1988), $2A^{pro}$ must have another role in viral growth. This idea is strongly supported by the fact that a poliovirus monster recently constructed, containing the P1 region separated from P2/P3 by the EMC 5′ untranslated region, requires the presence of $2A^{pro}$, even though P2 proteins are now initiated on an internal AUG (Molla *et al.*, 1992).

5.1. Cellular Proteins Involved in Picornavirus Genome Replication

The major evidence that host-factors are required for the replication of the picornavirus genome comes from cell-free systems (Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). These systems are extremely useful for clarifying this process in molecular detail, but they are also prone to artifacts. Thus, a 67 kDa protein with uridylation activity has been isolated from infected cells that can replace the oligo(U) primer in the initiation of new poliovirus RNA chains (Dasgupta *et al.*, 1980; Baron and Baltimore, 1982; Dasgupta, 1983). This host factor was identified as the protein kinase that phosphorylates eIF-2 (Morrow *et al.*, 1985). The role that such an activity may have in poliovirus-RNA replication has not been elucidated (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990), and the requirement of host factors for picornavirus RNA replication also requires further investigation.

5.2. Cellular Membranes and Picornavirus RNA Synthesis

The replication of poliovirus genomes takes place in close association with newly made membrane vesicles (Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). During poliovirus infection, the synthesis of membranes is profoundly modified, such that the cytoplasm is loaded with vesicles of various sizes that form a distinct perinuclear structure (Dales *et al.*, 1965; Caliguiri and Tamm, 1970a, b; Mosser *et al.*, 1972a). This distinctive structure was termed *viroplasm* (Dales *et al.*, 1965). The synthesis of phospholipids increases after poliovirus infection to provide components for membrane generation (Mosser *et al.*, 1972b). The poliovirus replicative complexes are tightly associated with these newly made membranes (Girard and Baltimore, 1967; Caliguiri and Tamm, 1969, 1970b; Bienz *et al.*, 1990, 1992; Troxler *et al.*, 1992), although little is known about the origin of the membranous vesicles that appear in poliovirus-infected cells or the connection between these vesicles and other membrane compartments. The *in vitro* synthesis of poliovirus minus- or plus-stranded RNA has only been achieved using a crude membranous replication complex (Girard, 1969; Etchison and Ehrenfeld, 1981; Takeda *et al.*, 1986). Prevention of lipid synthesis results in an abrupt inhibition of viral RNA synthesis (Guinea and Carrasco, 1990, 1991), suggesting that the replication of viral genomes requires continuous membrane formation (see Sections 6.10 and 6.12).

Elucidating the mechanisms used by poliovirus to replicate its genome is of interest for a number of reasons. For example, an understanding of the steps and proteins involved in this process sheds light on the relationship between the virus and the host cell (Koch and Koch, 1985; Wimmer *et al.*, 1987; Semler *et al.*, 1988; Richards and Ehrenfeld, 1990). The complete synthesis of poliovirus in cell-free systems has already been achieved (Molla *et al.*, 1991; Barton and Flanegan, 1993), and

this system should help to clarify the steps and components involved in poliovirus RNA replication. In addition, there is an interplay between the knowledge gained on poliovirus RNA synthesis and the inhibitors of this process, since a detailed understanding of the steps followed by poliovirus and other animal viruses in replicating their genomes is important for exploiting new approaches that interfere with the replication of these pathogens. Moreover, a better understanding of the mode of action of inhibitors of poliovirus RNA replication may help to elucidate this process.

6. COMPOUNDS THAT BLOCK PICORNAVIRUS RNA SYNTHESIS

6.1. Guanidine

Guanidine (Fig. 23) is a basic compound, positively charged at neutral pH, and is a natural constituent of animal serum. It is active against some members of the *Picornaviridae* and *Togaviridae* families (Friedman, 1970) and is, in many ways, a model inhibitor of poliovirus, despite the fact that it is one of the least potent agents against viral RNA synthesis (0.2–3 mM) (Tershak, 1974, 1982). Several guanidine derivatives have nevertheless been synthesized with more potent antiviral activity (Bucknall *et al.*, 1973). Soon after the discovery of guanidine as an antiviral agent (Rightsel *et al.*, 1961), poliovirus mutants resistant to or dependent on guanidine were described (Loddo *et al.*, 1963; Nakano *et al.*, 1963). The picornaviruses inhibited by guanidine are poliovirus, rhinovirus and FMDV, whereas EMC virus is resistant to the compound (Caliguiri and Tamm, 1973; Tershak *et al.*, 1982). Guanidine has no adverse effects on cell growth at concentrations that block virus growth and has no direct effect on isolated virions, nor on virus attachment. When the inhibitor is added 3 hr after infection, it does not block translation, whereas the incorporation of [^{3}H]uridine into poliovirus RNA is suppressed. If the compound is added after virus entry, the synthesis of viral proteins and of RNA are both prevented (Caliguiri and Tamm, 1973; Tershak *et al.*, 1982). Moreover, under low or moderate multiplicities of infection, guanidine largely prevents the shut-off of host protein synthesis induced by poliovirus, whereas at high multiplicity of infection, the kinetics of the early shut-off are similar in the absence or in the presence of inhibitory concentrations of guanidine (Lacal and Carrasco, 1983a). For details on the initial studies on guanidine, the reader is referred to comprehensive reviews by Caliguiri and Tamm (1973) and Tershak *et al.* (1982).

Early studies on the mode of action of guanidine indicated that this compound blocked the

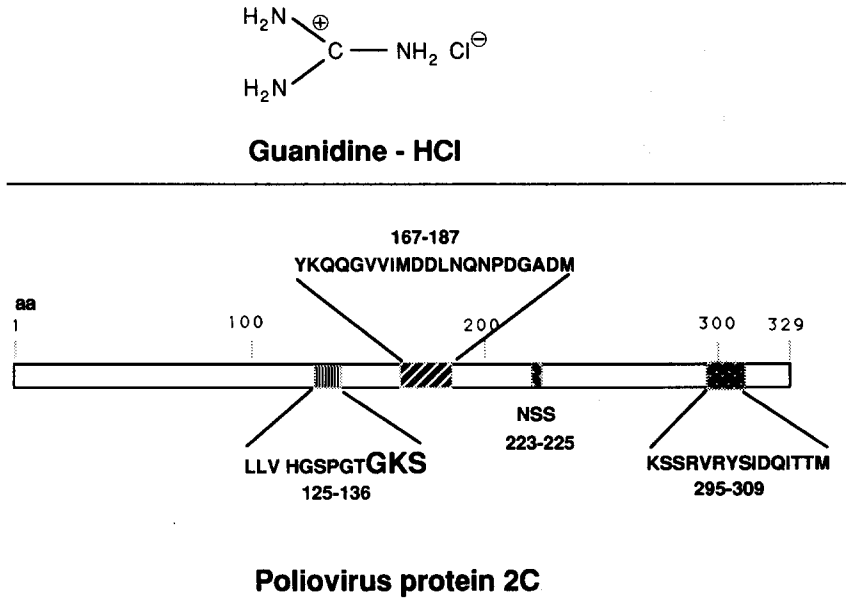


Fig. 23. Structure of guanidine and poliovirus protein 2C. The sequences on 2C containing the GKS motif and the region controlling guanidine-resistant or -dependent mutants are shown.

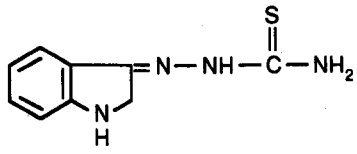
initiation of new rounds of viral RNA synthesis, but allowed completion of already initiated RNA strands (Caliguiri and Tamm, 1968a, b, 1973; Tershak *et al.*, 1972). It is not known with certainty whether guanidine is a selective inhibitor of the synthesis of plus-strand RNA, or blocks equally well the synthesis of plus- or minus-strand RNA. This indecision is due to the fact that it is difficult to block the synthesis of one strand of RNA without affecting the other. Careful kinetic analyses, using various concentrations of guanidine added at different times after infection, have not, to our knowledge, been carried out. Recently, the complete synthesis of infectious poliovirus has been accomplished in a cell-free system. In this system, poliovirus genomic RNA is added to a HeLa cell extract and after several hours of incubation, infectious poliovirus particles are made (Molla *et al.*, 1991). Guanidine blocks the formation of these particles, indicating that at least under some conditions, the synthesis of plus-strand RNA and the initiation of RNA takes place *in vitro* (Molla *et al.*, 1991).

The inhibition by guanidine of poliovirus or FMDV can be reversed by several amino acids, such as methionine, valine, leucine or threonine (Dinter and Bengston, 1964; Lwoff, 1965), and by other agents, including methylpropanolamine, ethanolamine or choline (Loddo *et al.*, 1966; Philipson *et al.*, 1966). This reversal very much depends on the host cell type analyzed. Transport of guanidine into HeLa cells involves at least two possible mechanisms (Nair, 1987b) that are regulated by the concentrations of cations in the medium (Nair, 1987a). Some antiguanidine agents can block this transport, but this may not constitute the basis of their antiguanidine action; rather they could antagonize guanidine intracellularly by an unknown mechanism (Nair, 1987b).

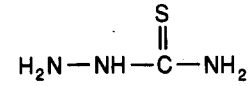
As indicated earlier in this section, the feasibility of generating mutants allowed genetic mapping, which provided information on the viral protein that was the target of guanidine action (Anderson Sillman *et al.*, 1984; Pincus *et al.*, 1987). Initial studies suggested that capsid proteins, proteases or even VPg were the targets of guanidine. Very detailed analyses of both guanidine-resistant and guanidine-dependent mutants of poliovirus indicate that they map in protein 2C (Pincus and Wimmer, 1986; Pincus *et al.*, 1986; Baltera and Tershak, 1989), and similar conclusions were reached for FMDV guanidine mutants (Saunders and King, 1982). Analyses of the polypeptides of FMDV or poliovirus guanidine-resistant mutants suggested that protein 2C was responsible for this trait. Transfection of COS cells, with plasmids containing cDNA fragments corresponding to poliovirus protein 2C from guanidine-resistant or guanidine-dependent mutants, and with plasmids containing infectious clones of wild-type poliovirus, gave rise to both types of mutant virus (Pincus and Wimmer, 1986). Sequence analyses indicated that mutations in amino acid residue 179 of protein 2C conferred resistance to high guanidine concentrations, whereas changes in residue 187, combined with mutations at position 142, 225 or 227, generate guanidine-dependence (Pincus *et al.*, 1987). Modifications in residue 164 or 187 of poliovirus protein 2C, combined with mutations at residues 223 or 295 and 309, could also confer guanidine resistance (Baltera and Tershak, 1989). Finally, the frequency of reversion of guanidine-dependent mutants of poliovirus to the resistance phenotype has established a mutation frequency of about 6×10^{-4}, a number consistent with the genetic variability of RNA-containing viruses (Domingo, 1989, 1992; Holland *et al.*, 1992; Drake, 1993).

An updated view of the mode of action of guanidine could be as follows: guanidine acts on protein 2C, perhaps inhibiting the interaction of this protein with membranes (Bienz *et al.*, 1992). Protein 2C is a GTPase that may be involved in trafficking the viral RNA replication complexes through the vesicular system (Rodríguez and Carrasco, 1993). This traffic is necessary for poliovirus, but not EMC virus, genome replication (Irurzun *et al.*, 1992). Thus, guanidine blocks the activity of 2C, the traffic of replication complexes and, hence, the initiation of viral RNA synthesis. This mode of action may also explain why guanidine affects other steps, such as the assembly of new virions that also takes place in close association with the newly-formed membrane vesicles (Koch and Koch, 1985).

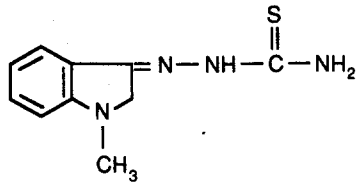
Guanidine and some of its derivatives have been used in several pharmacological studies (Davidoff, 1973). This compound provokes an enhanced nerve excitability after injection into frogs and may play a role in the pathogenesis of uremic poisoning. Although the serum levels of guanidine are similar in control and uremic patients, the excretion of methylguanidine is higher in patients with renal failure (Stein and Micklus, 1973). These findings, together with the fact that high concentrations (~ 1 mM) of guanidine are required for efficient blockage of poliovirus *in vitro*,



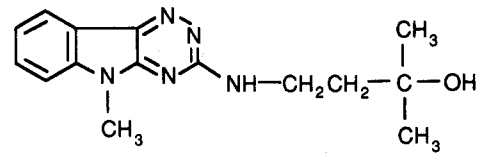
Isatin - ß - thiosemicarbazone



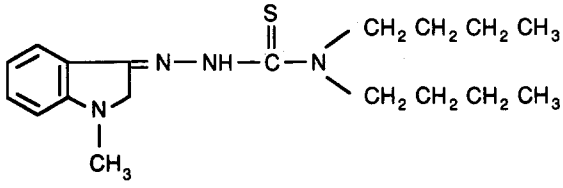
thiosemicarbazide



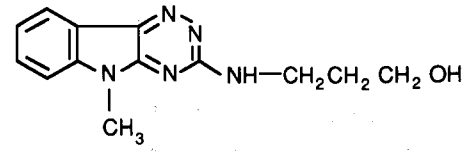
1 - methylisatin - ß -thiosemicarbazone (methisazone)



SKF 30097



1 - methyl - 4´- 4´- dibutyl - isatin - ß - thiosemicarbazone (busatin)



SKF 21687

Fig. 24. Chemical structures of different compounds: isatin-β-thiosemicarbazone, thiosemicarbazide, methisazone, SKF 30097, busatin and SKF 21687.

indicate that guanidine cannot be used therapeutically. Guanidine is ineffective for the treatment of poliovirus infection in animals, because of the rapid secretion of this compound and the rapid development of guanidine-resistant viruses in animals (Caliguiri and Tamm, 1973). In fact, administration of guanidine alone to mice infected with echovirus 9 or coxsackie virus A9 did not protect them. A partial response was observed after combination of 2-(α-hydroxybenzyl)-benzimidazole (HBB) and guanidine, as indicated by several days' delay, before death (Eggers, 1976).

6.2. Thiosemicarbazones

Thiosemicarbazones (Fig. 24) have been used as therapeutic agents for the treatment of tuberculosis and as antiviral agents against several animal viruses (Levinson, 1975; Pfau, 1982). Isatin-β-thiosemicarbazone inhibits vaccinia virus and rhinoviruses (Katz and Feliz, 1977; Pennington, 1977), but has no effect against poliovirus (Pearson and Zimmerman, 1969). The activity of methisazone (*N*-methyl-isatin-β-thiosemicarbazone) against several picornaviruses was reported (Bauer *et al.*, 1970), and various species of viruses, including poliovirus, were directly inactivated upon contact with methisazone in the presence of $CuSO_4$ (Fox *et al.*, 1977). 1-Methyl-4′,4′-dibutyl-isatin-β-thiosemicarbazone (busatin) blocks poliovirus (O'Sullivan and Sadler, 1961; Pearson and Zimmerman, 1969), interfering with the synthesis of viral RNA when added at any time during infection (Pearson and Zimmerman, 1969). In contrast with the action of HBB (see Section 6.3) or guanidine, busatin blocks poliovirus RNA synthesis in cell-free systems (Pearson and Zimmerman, 1969), and in this respect shows similarity to gliotoxin (Rodriguez and Carrasco, 1992).

Some derivatives of thiosemicarbazones, the 3-substituted triazinoindoles (SKF 21687, SKF 30097, SKF 36800, SKF 40491) (Fig. 24), are broad-spectrum antipicornavirus agents that block several rhinovirus strains (Matsumoto *et al.*, 1972; Pfau, 1982). SKF 40491 has antirhinovirus activity when administered intranasally to gibbons, but not when used orally (Pinto *et al.*, 1972). SKF 21687 and SKF 30097 had no effect on rhinovirus infection in humans (Togo *et al.*, 1973b).

6.3. Benzimidazoles

Several benzimidazole derivatives (Fig. 25) show activity against a number of animal viruses (Caliguiri and Tamm, 1973; Tamm and Sehgal, 1978; Eggers, 1982, 1985). For example, HBB is a selective inhibitor of picornavirus replication that does not inactivate virion particles, but blocks the replication of viral RNA without affecting the synthesis of cellular RNA (Eggers and Tamm, 1962; Singh *et al.*, 1963). The structure–activity relationships in HBB derivatives are fairly well documented (Caliguiri and Tamm, 1973; Tamm and Sehgal, 1978; Eggers, 1982, 1985), showing that the benzo group and the hydroxybenzyl residue at position 2 are critical for activity. Some HBB derivatives have been reported to be more active than HBB itself (Caliguiri and Tamm, 1973; Tamm and Sehgal, 1978; Eggers, 1982, 1985).

HBB is not a broad-spectrum antipicornavirus agent, being active against poliovirus and some coxsackie viruses, but devoid of activity against rhinoviruses, FMDV or hepatitis A virus (Siegl and Eggers, 1982). The concentration of HBB required for activity against poliovirus is similar to that of guanidine, whereas HBB is about 10-fold more potent than guanidine against coxsackie virus. The action of HBB on poliovirus, as found for that of guanidine and flavones, is reversible. Upon withdrawal of these compounds, virus growth resumes (Caliguiri and Tamm, 1973; Tamm and Sehgal, 1978; Eggers, 1982, 1985).

Studies on the mode of action of HBB indicate that it has no effect on early steps of infection, such as virus entry and uncoating (Eggers and Tamm, 1962). Addition of the compound after poliovirus entry prevents the appearance of viral proteins, because viral RNA replication is blocked. Thus, the incorporation of labelled uridine into viral RNA and the formation of poliovirus-specific RNA, as analyzed by dot-blot hybridization, are inhibited by HBB (Eggers and Tamm, 1963a; González *et al.*, 1990b). This compound has no effect on *in vitro* synthesis of viral RNA, and it is still not known if the initiation or elongation steps are selectively blocked by this agent *in vivo*.

A number of picornavirus mutants that are resistant to or dependent on HBB have been isolated, but they are not cross-resistant to guanidine (Eggers and Tamm, 1963b). The dependent mutants require HBB for the replication of viral RNA, and HBB can be replaced by guanidine in this respect (Eggers and Tamm, 1963b). Mutant HBB-dependent viruses back-mutate to the sensitive phenotype with high frequency ($\sim 10^{-4}$) (Eggers and Tamm, 1965; Caliguiri and Tamm, 1973), this figure being similar to the mutation rate of picornaviruses for other traits investigated (de la Torre *et al.*, 1990; Domingo, 1992).

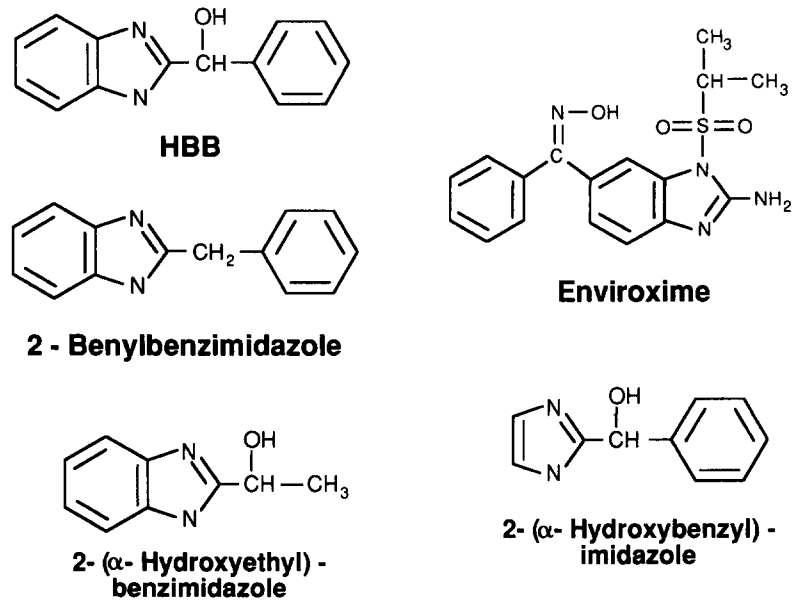


Fig. 25. Chemical structures of different compounds: HBB, enviroxime, 2-benzylbenzimidazole, hydroxyethyl-benzimidazole and hydroxybenzyl-imidazole.

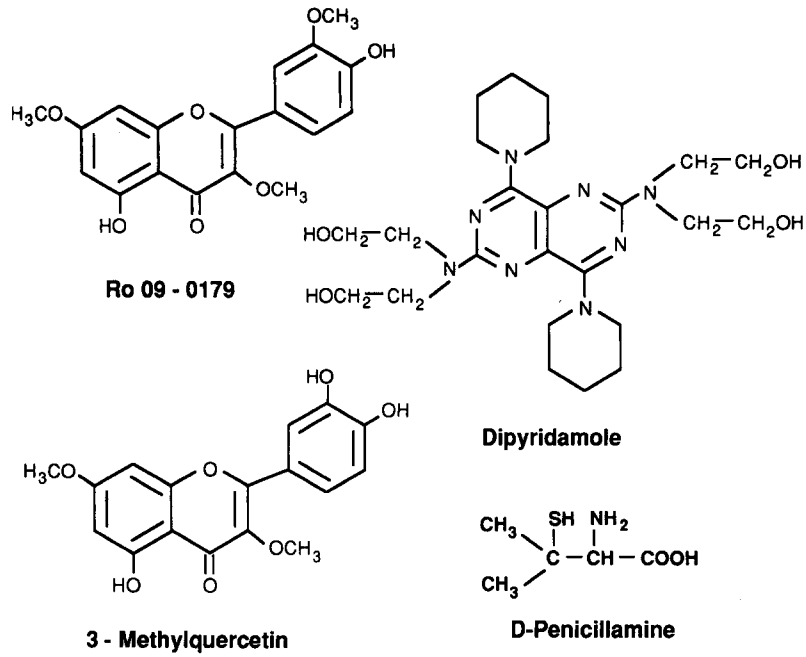


Fig. 26. Chemical structures of different compounds: Ro 09-0179, dipyridamole, 3-methyl quercetin and D-penicillamine.

HBB and guanidine act synergistically (Singh *et al.*, 1963), suggesting that the exact target inhibited by each compound is different, although they could act on the same viral protein. Investigations of the antiviral effects of HBB in mice and monkeys have been carried out (Caliguiri and Tamm, 1973; Eggers, 1982). Although early experiments yielded only marginal effects of HBB on virus-infected animals, newborn mice inoculated with echovirus 9 or coxsackie virus A9 were protected by combined treatment with HBB and guanidine (Eggers, 1976). HBB alone protected coxsackie virus-infected animals, but not those infected with echovirus (Eggers, 1976).

6.4. Enviroxime (LY 122772)

Enviroxime (2-amino-1-(isopropylsulfonyl)-6-benzimidazole phenyl ketone oxime) (Fig. 25) is a benzimidazole derivative with high activity against rhinoviruses (Delong and Reed, 1980), and several human trials using enviroxime as a prophylactic agent against the common cold produced by rhinoviruses have been reported (Hayden and Gwaltney, 1982; Levandowski *et al.*, 1982; Phillpots *et al.*, 1983b). No clear benefit was observed (Levandowski *et al.*, 1982), although incorporation of enviroxime into liposomes results in improved drug delivery to the lungs of mice (Wyde *et al.*, 1988). Studies on the mode of action of this drug have been retarded because the compound is not freely available from Eli Lilly Research Laboratories for research studies.

6.5. Flavones

Flavonoids are ubiquitous compounds in the plant kingdom (Harborne, 1988). The search for antiviral agents in plant extracts led to the discovery of 3-methyl quercetin (3-MQ) from the African medicinal plant *Euphorbia grantii* (Van Hoof *et al.*, 1984; Vlietinck *et al.*, 1986) and Ro 09-0179 (Fig. 26) from the Chinese medicinal herb *Agastache rugosa* (Ishitsuka *et al.*, 1982b). Analysis of the mode of action of 3-MQ indicated that it was a selective inhibitor of poliovirus RNA synthesis. At a concentration of 5 μg/mL, 3-MQ inhibited [^{3}H]uridine incorporation in poliovirus-infected cells by 90%, whereas at 20 μg/mL, 3-MQ had no effect on cellular transcription (Castrillo *et al.*, 1986). Analysis of the viral RNAs synthesized in the presence of this flavone showed that production of single-stranded RNA was totally blocked, whereas the synthesis of dsRNA was still detectable (Castrillo and Carrasco, 1987a). Thus, 3-MQ behaves like Ro 09-0179 in blocking the

synthesis of both plus- and minus-RNA when added from the inception of infection, because there is not enough genomic RNA to serve as a template to give rise to significant amounts of negative-stranded RNA that can be detected by hybridization (Lopez Pila *et al.*, 1989; González *et al.*, 1990b). Therefore, in order to test if these flavones are selective, they must be added later during infection, when sufficient amounts of plus- and minus-stranded RNA are being made. Addition of Ro 09-0179 late in infection affects viral RNA synthesis in a way that suggests a preferential inhibition of poliovirus genomic RNA (González *et al.*, 1990b). However, a detailed understanding of the molecular mode of action of both 3-MQ and Ro 09-0179 is still lacking, because we do not know yet which viral protein is their target. It is also not known if the initiation, elongation or both these steps of viral RNA synthesis are blocked by these compounds. Moreover, we have recently found that some flavones may block early steps of poliovirus growth, suggesting that some flavones could directly interact with viral particles and block uncoating (A. Irurzun and L. Carrasco, unpublished results).

3-MQ and Ro 09-0179 inhibit poliovirus replication at concentrations 100-fold, or 1000-fold, lower than those shown for HBB or guanidine, respectively (González *et al.*, 1990b). Ro 09-0179 selectively blocks viral RNA synthesis in poliovirus-infected HeLa cells more strongly than 3-MQ, and these findings make Ro 09-0179 a valuable compound for obtaining insight into the molecular mechanisms of poliovirus RNA replication.

Quercetin and morin are effective against mengovirus-induced encephalitis in mice when given orally (Veckenstedt *et al.*, 1978), but these two flavonoids do not show selectivity for inhibition of poliovirus in cultured cells (Castrillo *et al.*, 1986; González *et al.*, 1990b). The diacyl derivative of Ro 09-0179 is absorbed better than the parent compound in the intestinal tract, making it effective against lethal coxsackie B1 infections in mice (Ishitsuka *et al.*, 1982b).

6.6. Gliotoxin

Gliotoxin (Fig. 27) is a fungal metabolite produced by various species of *Trichoderma*, *Aspergillus* and *Penicillium*, and it possesses different biological activities, including antiviral effects (Suhadolnik, 1967a; Miller *et al.*, 1968; McDougall, 1969). Gliotoxin and a number of related compounds, derived either from natural sources or by chemical synthesis, are the most potent agents that interfere with poliovirus RNA synthesis (Miller *et al.*, 1968; Trown and Bilello, 1972). The presence of a reducing agent such as dithiothreitol prevents the inhibition of poliovirus RNA synthesis in intact cells (Trown and Bilello, 1972), suggesting that blockade by gliotoxin involves the formation of disulfide bridges between gliotoxin and essential sulfhydryl groups on the viral polymerase. The presence of dithiothreitol in cell-free systems partially lowered the gliotoxin inhibitory potential, in agreement with the idea that reducing the sulfhydryl groups abolishes the activity of gliotoxin (Trown and Bilello, 1972; Rodriguez and Carrasco, 1992).

The mode of action of gliotoxin against poliovirus has been analyzed in detail (Rodriguez and Carrasco, 1992). This fungal metabolite irreversibly inhibits the appearance of poliovirus proteins when present from the beginning of infection, but has no effect on viral translation when added later. This toxin potently inhibited the incorporation of [^{3}H]uridine into poliovirus RNA soon after its addition to the culture medium (Miller *et al.*, 1968; Rodriguez and Carrasco, 1992). Analysis of the synthesis of poliovirus plus- or minus-stranded RNA in the presence of gliotoxin indicated that this compound effectively hampered both processes (Rodriguez and Carrasco, 1992), a result which contrasts with the mode of action of other inhibitors of poliovirus RNA synthesis, such as flavones, that selectively block plus-strand RNA synthesis and suggests that the target of gliotoxin may differ from the target of flavones. Gliotoxin was a potent inhibitor of poliovirus RNA synthesis in cell-free systems using membranous crude replication complexes, a reaction that is not blocked by guanidine, HBB or Ro 09-0179. Moreover, an *in vitro* assay, using the purified poliovirus polymerase 3D^{pol}, was efficiently inhibited by gliotoxin, the first description of an inhibitor of this viral enzyme (Rodriguez and Carrasco, 1992).

Gliotoxin is rather toxic for animals, the LD_{50} for mice being 7.8 mg/mL intravenously and 32 mg/mL intraperitoneally (Larin *et al.*, 1965). Despite this toxicity, gliotoxin exerts antiviral effects in monkeys inoculated with large doses of poliovirus (Larin *et al.*, 1965).

Antipicornavirus activities have also been described for other compounds related to gliotoxin.

These include aranotin, acetylaranotin and apoaranotin (Fig. 27), all isolated from the culture medium of the fungi *Arachniotus aureus* and *Aspergillus terreus* (Trown, 1968), and sirodesmins (Fig. 27), isolated from the fungus *Sirodesmium diversum*. Sirodesmins A, B and C differ in the number of sulfur atoms in the bridge, being two, three or four, respectively. Several hyalodendrins (Fig. 27), which also differ in the number of sulfur atoms in the bridge, were isolated from the fungi *Hyalodendrin* species and *Penicillium turbatum* (Michel *et al.*, 1974). These compounds inhibited poliovirus, rhinovirus and coxsackie virus multiplication (Trown, 1968; Michel *et al.*, 1974). The synthetic compound *N,N'*-dimethylepidithiapiperazinedione (Fig. 27) was also a very potent inhibitor of coxsackie virus RNA synthesis (Trown, 1968).

6.7. Aldgamycin E

A natural compound, aldgamycin E (Fig. 28), a macrolide antibiotic produced by *Streptomyces lavandulae*, inhibits the growth of poliovirus, but not of other animal viruses tested (González and Carrasco, unpublished results). A blockade of viral translation takes place when the antibiotic is present from the inception of infection, but no effect on poliovirus protein synthesis was observed when this agent was added later, suggesting that protein synthesis was not the primary target for antibiotic action. Indeed, poliovirus RNA synthesis was profoundly depressed soon after the addition of aldgamycin E. Other macrolide antibiotics, such as chalcomycin, josamycin and venturicidin, also blocked poliovirus RNA synthesis, indicating that macrolide antibiotics constitute a promising source of new antiviral agents (González and Carrasco, unpublished results).

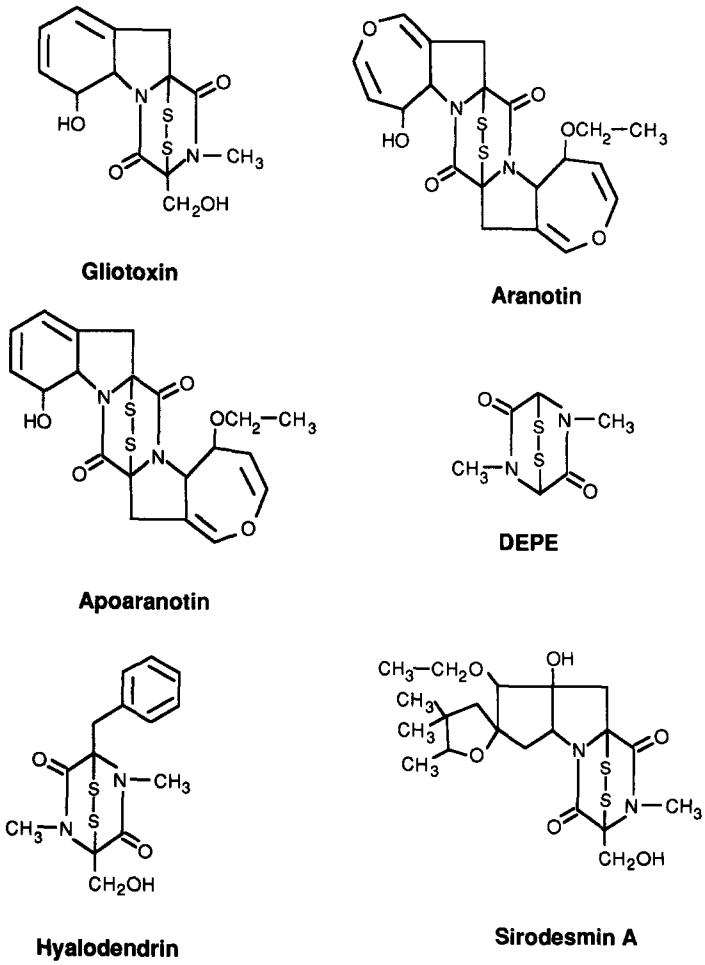
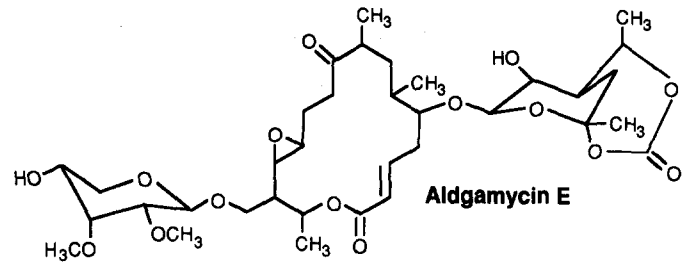
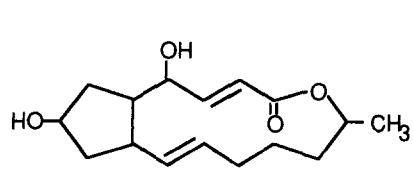
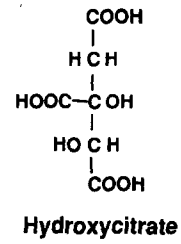
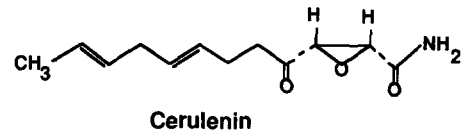


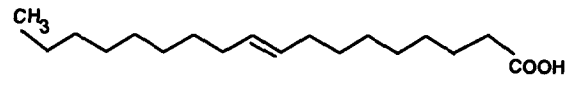
Fig. 27. Chemical structures of different compounds: gliotoxin, aranotin, apoaranotin, DEPE, hyalodendrin and sirodesmin A.











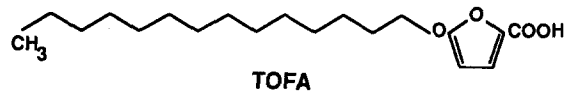


Fig. 28. Chemical structures of different compounds: aldgamycin E, brefeldin A, hydroxycitrate, cerulenin, oleic acid and TOFA.

6.8. Dipyridamole

Dipyridamole (Fig. 26) is a synthetic compound which is a coronary vasodilator. Dipyridamole and several of its derivatives are active against certain DNA and RNA viruses in cell culture, including mengovirus (Kuwata *et al.*, 1977; Tonew *et al.*, 1982). The synthesis of mengovirus RNA was completely blocked by dipyridamole under conditions where the transport of uridine was not affected, i.e. by precharging the cells with labelled uridine (Kuwata *et al.*, 1977; Tonew *et al.*, 1982), but more detailed analyses, using hybridization with specific probes, are necessary because dipyridamole also affects nucleoside transport.

6.9. D-Penicillamine

As indicated in Section 6.1, the action of guanidine can be antagonized by a number of amino acids, and on this basis, it was suggested that certain analogues of these amino acids might also show antipoliovirus activity (Gessa *et al.*, 1966). D-Penicillamine (Fig. 26), a valine analogue, inhibits poliovirus growth reversibly and blocks the synthesis of poliovirus RNA at drug concentrations of 0.1–0.5 mg/mL (Gessa *et al.*, 1966). The relative concentrations of single-stranded and double-stranded poliovirus RNA did not vary in the presence of this compound. Certainly, D-penicillamine is not a potent poliovirus inhibitor, because at a drug concentration of 1 mM, total blockade of viral DNA synthesis was not achieved (Merryman *et al.*, 1974). The inhibitory effect of D-penicillamine is reversible and can be antagonized by several amino acids.

6.10. Coupling Membrane Synthesis with Genome Replication: Cerulenin

One of the most potent inhibitors of lipid biosynthesis in prokaryotic and eukaryotic cells is cerulenin (Fig. 28), an antibiotic produced by *Cephalosporium caerulens* (Bachrach *et al.*, 1973). It blocks fatty-acid synthetase at the level of β-ketoacyl thioester synthetase (condensing enzyme) (Armstrong *et al.*, 1972; Butterworth and Rueckert, 1972; Bachrach *et al.*, 1973). If present from the initiation of poliovirus infection, there is a potent inhibition of poliovirus protein synthesis, but it has no effect on viral translation or on the proteolytic processing of the viral proteins, if added late (Guinea and Carrasco, 1990). Myristoylation of the viral protein VP4 or its precursors is not blocked by cerulenin. Cerulenin inhibits very quickly the replication of poliovirus RNA when added at any time during infection (Guinea and Carrasco, 1990), whereas it has no effect on cellular transcription. This antibiotic depresses the incorporation of labelled glycerol into phospholipids, and this inhibition parallels the blockade of viral RNA replication. The synthesis of poliovirus plus-stranded RNA synthesis is hampered (Guinea and Carrasco, 1990). Cerulenin does not have a direct inhibitory effect on poliovirus translation, indicating that the synthesis of phospholipids is continuously required for the synthesis of plus-stranded poliovirus RNA. Hence, there is some link between membrane traffic and poliovirus RNA replication.

This antibiotic is also an inhibitor of some enveloped RNA-containing viruses (Schlesinger and Malfer, 1982; Ikuta and Luftig, 1986; Pal *et al.*, 1988). Thus, the replication of retroviruses, including HIV type 1, is blocked by cerulenin (Pal *et al.*, 1988). It was proposed that the processing of structural proteins and the acylation of some glycoproteins could be targets for cerulenin in its inhibition of viral growth (Schlesinger and Malfer, 1982; Ikuta and Luftig, 1986; Pal *et al.*, 1988). The inhibition of phospholipid synthesis by cerulenin also impairs the synthesis of nucleic acids in other viruses, such as EMC virus, SFV and VSV (Guinea and Carrasco, 1990; Pérez and Carrasco, 1991; Pérez *et al.*, 1991). In fact, the synthesis of nucleic acids, not only in togaviruses, but in many other animal viruses, is closely connected to the synthesis of new membranous structures. If the synthesis of these membrane structures requires *de novo* synthesis of phospholipids, it seems plausible that inhibition of lipid synthesis would certainly lead to inhibition of viral nucleic acid synthesis (Pérez and Carrasco, 1991; Pérez *et al.*, 1991).

6.11. TOFA and Hydroxyacetic Acid

Other compounds that block phospholipid synthesis include TOFA and hydroxyacetic acid (Fig. 28), which act in a similar manner as cerulenin (Guinea and Carrasco, unpublished results). Clofibrate, a compound that potentiates the oxidation of fatty acids, pentenoic acid, an inhibitor of fatty acid oxidation, hemicholinium, an inhibitor of choline incorporation into phosphatidylcholine, and compactine, an inhibitor of cholesterol synthesis, all had no effect on poliovirus. On the other hand, agaric acid, an inhibitor of fatty acid synthesis, sodium palmoxirate, another agent that blocks fatty acid oxidation, and 3-morpholine-1-propanol, were all too toxic in control cells to allow assay of their effects on poliovirus-infected cells (Guinea and Carrasco, unpublished results). TOFA and hydroxycitrate, two known inhibitors of lipid synthesis, blocked the appearance of poliovirus proteins when present from early stages of infection. Further analyses on the effects of these two compounds on poliovirus replication indicated that they had no action on poliovirus protein synthesis if added late during infection, whereas they potently depressed poliovirus RNA synthesis (Guinea and Carrasco, unpublished results). Furthermore, both compounds also had an inhibitory effect on phospholipid synthesis in poliovirus-infected cells. These results indicate that agents that interfere with phospholipid biosynthesis, such as cerulenin, TOFA and hydroxycitrate, hamper the replication of poliovirus RNA. Thus, the mode of action of these agents differs from that of the well-known poliovirus RNA inhibitor guanidine, because guanidine has no effect on lipid synthesis.

6.12. Membrane Traffic and Genome Replication: Brefeldin A

Membranes are continuously in motion through several compartments in eukaryotic cells (Morré and Ovtracht, 1977; Rothman and Orci, 1992; Lippincott-Schwartz, 1993). Brefeldin A (BFA)

(Fig. 28), a fungal antibiotic isolated from *Penicillium brefeldianum*, has antiviral properties (Tamura *et al.*, 1968) and blocks protein secretion and vesicular traffic (Hunziker *et al.*, 1992; Klausner *et al.*, 1992). It induces a rapid disaggregation of the Golgi complex, causing redistribution into the endoplasmic reticulum (Lippincott-Schwartz *et al.*, 1989, 1991; Alcalde *et al.*, 1991). Within seconds of BFA treatment, a peripheral protein (110 kDa) of the Golgi apparatus is released in the cytoplasm and a Golgi membrane enzyme that catalyzes exchange of guanine nucleotide onto ARF is inhibited (Donaldson *et al.*, 1992; Helms and Rothman, 1992). Since transport of proteins into post-Golgi compartments in the cell is potently blocked by BFA (Klausner *et al.*, 1992), so too is the delivery of VSV G glycoprotein to the plasma membrane (Misumi *et al.*, 1986; Fujiwara *et al.*, 1988; Doms *et al.*, 1989; Oda *et al.*, 1990). In addition to VSV, an increasing number of animal viruses are reported to be affected by this inhibitor (Ulmer and Palade, 1990; Chen *et al.*, 1991; Cheung *et al.*, 1991; Whealy *et al.*, 1991). BFA inhibits the processing and transport of glycoproteins that are present in all these viruses. In this manner, enveloped viruses that mature from the plasma membrane or mature intracellularly are inhibited by this macrolide antibiotic (Ulmer and Palade, 1990; Chen *et al.*, 1991; Cheung *et al.*, 1991; Whealy *et al.*, 1991).

BFA strongly inhibits poliovirus replication (Irurzun *et al.*, 1992; Maynell *et al.*, 1992), even though this virus lacks a lipid envelope and does not encode any glycoproteins (Urzainqui and Carrasco, 1988). Addition of BFA from the beginning of poliovirus infection blocks the synthesis of late proteins, but has no effect on p220 cleavage, indicating that the input viral RNA is translated to produce active $2A^{pro}$ (Irurzun *et al.*, 1992). The presence of BFA at later times has no effect on poliovirus protein synthesis, so that this step is not a direct target for the antibiotic. Indeed, the target of BFA is viral RNA synthesis, because addition of the antibiotic at any time after poliovirus infection drastically reduces the incorporation of labelled uridine into poliovirus RNA (Irurzun *et al.*, 1992; Maynell *et al.*, 1992). Both plus- and minus-stranded RNA synthesis are diminished when BFA is present from the beginning of infection, but plus-stranded RNA synthesis is more affected when the inhibitor is added at later times (Irurzun *et al.*, 1992).

The effects of BFA on late protein synthesis by other animal viruses varies according to the virus species (Irurzun *et al.*, 1992). Among picornaviruses, rhinoviruses are sensitive to the antibiotic, whereas EMC virus is resistant. A negative-stranded RNA virus, such as vesicular stomatitis, is blocked by BFA, whereas vaccinia virus, a cytoplasmic DNA virus, is resistant (Irurzun *et al.*, 1992). It follows that even viruses that do not contain a lipid envelope and do not synthesize glycoproteins, may require newly made membranes to allow the synthesis of new genomes (Irurzun *et al.*, 1992; Maynell *et al.*, 1992). If so, the poliovirus proteins, once synthesized, will bind to membranes in the rough endoplasmic reticulum and then migrate through the Golgi complex, following the same route used by glycoproteins (Fig. 29). Once the poliovirus proteins reach the *trans*-Golgi network, they will be assigned to the specialized structures formed by the membranous vesicles and the RNA replication complexes (see Section 5.2). The finding that BFA immediately blocks poliovirus RNA synthesis without affecting translation suggests that RNA synthesis takes place in association with newly made vesicles and that preexisting vesicles do not support the formation of new genomes. These findings will help to clarify the action of a number of antiviral agents that interfere with the replication of genomes without affecting the viral polymerase itself. In addition, they can be exploited to devise more selective antiviral agents because, at least theoretically, we could interfere with the action of the viral proteins involved in the coupling between the membranous vesicles and the RNA replication complexes.

6.13. Oleic Acid

Addition of some fatty acids to cells in culture increases lipid synthesis (Cowen and Heydrick, 1972; Burkhardt, 1991). Conversely, unsaturated fatty acids, such as oleic acid, increase membrane fluidity and diminish the infectivity and hemolytic activity of enveloped animal virus particles. No such effects have been described for non-lipid-enveloped animal virus particles (Kohn *et al.*, 1980; Sola *et al.*, 1986a). Oleic acid (Fig. 28) blocks the synthesis of poliovirus proteins at concentrations that do not affect translation in uninfected cells (Guinea and Carrasco, 1991). This inhibition is due to a blockade of viral RNA synthesis, most probably as a consequence of modifications in the membranes connected with the RNA replication complexes. Addition of oleic acid, but not other

fatty acids, from the beginning of poliovirus infection, specifically inhibits the appearance of virus polypeptides at concentrations that do not affect translation in mock-infected HeLa cells. This inhibition is most probably due to the blockade of viral RNA synthesis, because oleic acid interferes with the synthesis of poliovirus RNA both *in vivo* and in cell-free systems (Guinea and Carrasco, 1991). Oleic acid increased the synthesis of phosphatidylcholine and most neutral lipids. Membranes made in poliovirus-infected cells in the presence of oleic acid differ in their buoyant density from control membranes. The incorporation of oleic acid into membranes leads to increased membrane fluidity and decreased buoyant density, making these membranes nonfunctional for RNA replication (Guinea and Carrasco, 1991). Therefore, not only is the synthesis of phospholipids required for viral RNA synthesis (Guinea and Carrasco, 1990), but the proper structure of the newly made membranes is also important to permit replication of poliovirus genomes.

7. INHIBITORS WITH UNKNOWN MODES OF ACTION

7.1. Interferons

Interferons are cellular proteins, synthesized in response to several inducers (Stringfellow, 1981; Sehgal *et al.*, 1982), that possess antiviral activities (De Maeyer *et al.*, 1981; Staeheli, 1990; Kerr and Stark, 1992). The interaction of the interferon molecule with the appropriate receptor on the cell surface induces the synthesis of a number of proteins, thus establishing the antiviral state (Kerr and Stark, 1992; McNair and Kerr, 1992). The precise action of these proteins against the replication of animal viruses remains largely unknown (Taylor and Grossberg, 1990).

In the case of picornaviruses, it is well established that treatment of susceptible cells with different interferon species profoundly blocks viral growth (see review by Muñoz and Carrasco, 1987). Treatment of cells with human interferon, followed by subsequent infection with poliovirus, does not prevent inhibition of host protein synthesis, indicating that translation of the input viral RNA takes place (Muñoz and Carrasco, 1983). Isolation of RNAs from poliovirus-infected cells treated with interferon indicated that the ribosomal RNAs remained intact and that the cellular mRNAs were translatable, so that degradation of cellular RNAs is not the basis of interferon action (Muñoz *et al.*, 1983). The precise step inhibited in picornavirus growth in cells treated with interferon remains largely unexplored, despite the large number of publications investigating this effect in

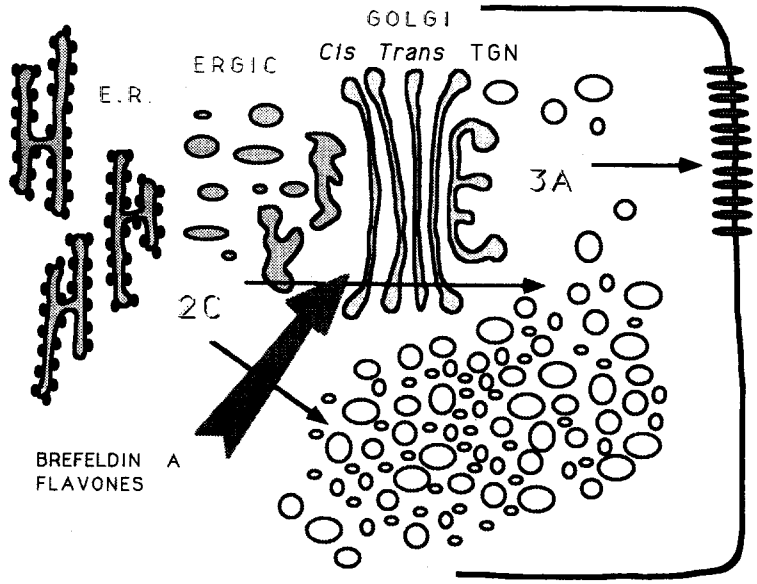


Fig. 29. Diagrammatic representation of membrane flow in mammalian cells. Hypothetical sites of action for protein 2C and the inhibitors brefeldin A and flavones. E.R., endoplasmic reticulum; ERGIC, endoplasmic reticulum–Golgi intermediate compartment; TGN, trans-Golgi network.

cell-free systems and a good knowledge of the particular enzymes induced by interferon in cells (Muñoz and Carrasco, 1987). It is quite probable that the synthesis of picornaviral RNA is the step blocked in interferon-treated cells (Muñoz and Carrasco, 1983; Muñoz *et al.*, 1983), but it is assumed by many groups that interferons primarily block viral translation with this effect being mediated by the PPP(A2′p5′A)nA (2′-5′ A) synthetase and/or the P1 protein kinase upon activation by dsRNA (Taylor and Grossberg, 1990; Kerr and Stark, 1992; Zhou *et al.*, 1993).

It is difficult to establish whether interferon primarily blocks transcription or translation, not only for picornaviruses, but also for other RNA viruses, because interference with viral RNA synthesis usually leads to a reduction in viral protein synthesis and *vice versa*. Nevertheless, in the case of poliovirus, translation of the input RNA is not impaired in interferon-treated cells (Muñoz and Carrasco, 1983). Moreover, human cells treated with interferon and doubly-infected with poliovirus and reovirus synthesize reovirus proteins, even though poliovirus replication is impaired (Muñoz *et al.*, 1985a; Muñoz and Carrasco, 1987). These findings indicate that there is no indiscriminate inhibition of viral proteins in interferon-teated cells and infected with poliovirus, because these cells make reovirus proteins at control levels, even though the antiviral state against poliovirus exists in the same cells.

The mechanism underlying the blockade of picornaviruses by interferon is also unclear. If interferon blocks viral RNA synthesis, how is this accomplished? The possibility that interferon blocks translation of EMC virus or mengovirus through activation of the 2′-5′ A synthetase and/or the P1 protein kinase has been investigated (Verhaegen-Lewalle *et al.*, 1982; Rice *et al.*, 1985; Kumar *et al.*, 1988; Lewis, 1988). Various reports show no correlation between the activation of these enzymes and the establishment of an antiviral state, not only in picornavirus-infected cells (Silverman *et al.*, 1982; Verhaegen-Lewalle *et al.*, 1982; Kumar *et al.*, 1988; Lewis, 1988), but also in other viral systems (Muñoz and Carrasco, 1987; Taylor and Grossberg, 1990). Even in the case of reoviruses that contain dsRNA as genome, there is no inhibition of viral protein synthesis in HeLa cells even though the 2′-5′ A synthetase and the P1 protein kinase have been induced (Feduchi *et al.*, 1988). Transfection of the 2′-5′ A synthetase or the P1 protein kinase inhibit virus growth (Chebath *et al.*, 1987; Coccia *et al.*, 1990; Meurs *et al.*, 1992; Lee and Esteban, 1993), but it remains to be determined if this inhibition is related to the mode of action of interferon, because cells transfected with the 2′-5′ A synthetase gene support the growth of VSV (Chebath *et al.*, 1987; Coccia *et al.*, 1990). Furthermore, vaccinia virus, which is resistant to interferon blockade, is inhibited in cells transfected by the P1 protein kinase gene (Meurs *et al.*, 1992; Lee and Esteban, 1993). The intracellular location of the 2′-5′ A synthetase and the role that this enzyme plays in cellular metabolism deserve further investigation.

7.2. 2′-5′ A Analogues

Cell-free systems from interferon-treated cells synthesize a series of oligo(A) compounds in the presence of ATP and dsRNA (Williams *et al.*, 1978, 1979; Kerr *et al.*, 1982; Kerr and Stark, 1992). These oligo(A)s characteristically have phosphodiester bonds that are 2′-5′ instead of the usual 3′-5′ bonds. They are collectively referred to as 2′-5′ A (Fig. 30) and are synthesized in minute amounts both in normal cells and in interferon-treated cells after virus infection (Silverman *et al.*, 1982; Laurence *et al.*, 1984; Kerr and Stark, 1992), but they can be synthesized in higher amounts in extracts or by using the purified 2′-5′ A synthetase, or even chemically (Haugh *et al.*, 1983; Kerr and Stark, 1992). The exact role that the 2′-5′ A plays in the antiviral action of interferon remains obscure, because there is no correlation between the antiviral action of interferon and the presence of 2′-5′ A synthetase in cultured cells (Jacobsen *et al.*, 1983; Lewis, 1988; Taylor and Grossberg, 1990). Apart from the role that oligo(A) could play in the action of interferon against animal viruses, direct addition of pppA2′p5′A to the cell medium prevents virus infection (Alarcón *et al.*, 1984a). pppA2′p5′A is active against several animal viruses, including EMC virus and poliovirus, at concentrations between 0.05 and 0.5 mM. This compound does not inhibit translation; rather it blocks an early step during infection of VSV. The mode of action against picornaviruses remains undetermined (Alarcón *et al.*, 1984a).

Since 2′-5′ A is a charged molecule that does not enter easily into cells, the 2′-5′ A core, or a number of derivatives, have been used to inhibit translation and to block virus infection in intact

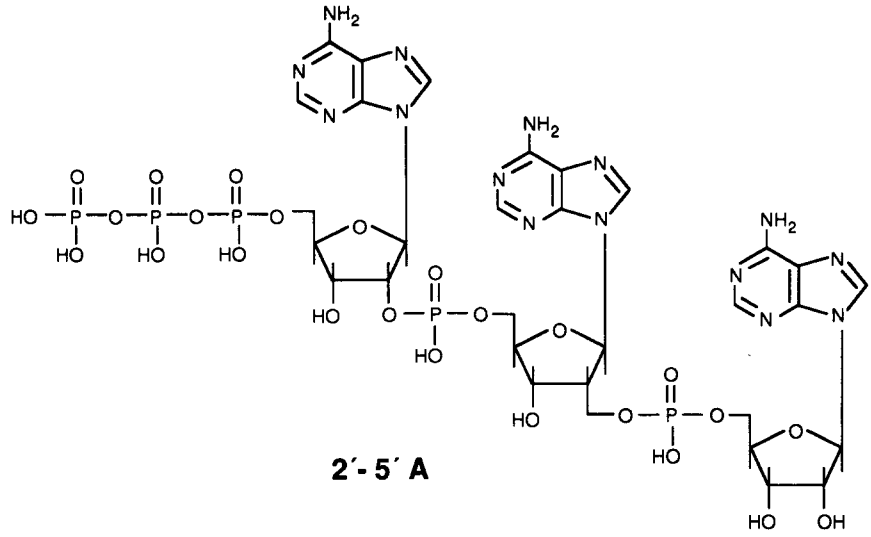


Fig. 30. Chemical structure of 2'-5'A.

cells (Imai *et al.*, 1982; Eppstein *et al.*, 1982; Haugh *et al.*, 1983; Sawai *et al.*, 1983; Goswami *et al.*, 1984; Eppstein *et al.*, 1986). The xyloadenosine analogue of 2'-5' A that is dephosphorylated at the 5'-terminus is much more stable and more active than the 2'-5' A core. Phosphorothioate analogues of 2'-5' A interfere with the activation of RNase L in HeLa cells, perhaps by competition with authentic 2'-5' A (Charachon *et al.*, 1990). Cordycepin or 3-methyl analogues of 2'-5' A have also been synthesized. However, although these analogues had antiviral effects against several DNA-containing viruses in cultured cells, their precise mode of action against picornaviruses is not known.

7.3. Polynucleotides and Oligonucleotides

Double-stranded polynucleotide complexes mediate interferon induction, thereby exerting antiviral effects (Shugar, 1974; Sehgal *et al.*, 1982; Shannon, 1984; Streissle *et al.*, 1985). Apart from this action, polynucleotides themselves may have antiviral activity (Shugar, 1974; Shannon, 1984; Stebbing, 1984; Streissle *et al.*, 1985). Thus, protection of mice against EMC virus has been observed with poly (I), tRNA or poly (C) (Stebbing *et al.*, 1976, 1977). However, the antiviral action of these polynucleotides does not seem to be mediated by interferon induction and subsequent stimulation of the immune system, because protection was also achieved in immunosuppressed mice or athymic mice (Stebbing *et al.*, 1976).

Other approaches used to block virus infection involve antisense RNA and ribozymes (James, 1991). Antisense oligonucleotides or oligonucleotide derivatives possess good activities against a variety of animal viruses (Stephenson and Zamecnik, 1978; Goodchild *et al.*, 1988; Leonetti *et al.*, 1990). Particularly effective are poly (L-lysine) conjugates (Degols *et al.*, 1989) and phosphorothioate oligodeoxynucleotides, because they more readily enter cells and are more stable (Leiter *et al.*, 1990; Agrawal *et al.*, 1992). Phosphorodithioate DNA oligomers are powerful inhibitors of HIV-1 reverse transcriptase (Marshall and Caruthers, 1993). To my knowledge, this approach has not been used to block picornaviruses.

7.4. Nucleoside Analogues

Until the 1980s the synthesis of nucleoside analogues was largely directed towards inhibition of members of the *Herpesviridae* family. This led to the development of a most effective antiviral agent, acycloguanosine (Schaeffer *et al.*, 1978; Elion, 1989). Research on nucleoside analogues in the last decade has concentrated on finding selective inhibitors of HIV. Some nucleotide analogues possess a broad spectrum of action and some of them also block members of the *Picornaviridae* family.

However, this is not a general rule because certain of these analogues, such as (S)DHPA or aristeromycin, have no effect against poliovirus (De Clercq *et al.*, 1978, 1984).

Although some nucleoside analogues may have a single viral enzyme as their site of action, some of them may affect a variety of cellular and viral enzymes (Carrasco and Vázquez, 1984). This is particularly true for adenosine (ATP) or guanosine (GTP) analogues, since these nucleosides and nucleotides participate in a variety of functions. Therefore, it can be anticipated that the action of some nucleoside analogues will be rather complex (Carrasco and Vázquez, 1984). To our knowledge, the mode of action of none of the nucleoside analogues active against picornaviruses has been analyzed in detail, although some of them, such as ribavirin (1,β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), show interesting activity.

7.4.1. *Pyrazofurin, Ribavirin (Virazole), Selenazofurin and Bredinin*

Pyrazofurin (3(β-D-ribofuranosyl)-4-hydroxyrazole-5-carboxamide) (Fig. 31), originally called pyrazomycin, was isolated from the fermentation broth of *Streptomyces candidus*. This antibiotic displayed wide-spectrum antiviral activity with a very good antiviral index. It is active against rhinovirus, and in this respect is more potent than ribavirin (De Clercq *et al.*, 1986). An excess of uridine in the culture medium abolished the antiviral effects, suggesting that the drug blocked steps in the metabolism of nucleotides (Gerzon *et al.*, 1971). Pyrazofurin-5′-phosphate is an antagonist of uridine metabolism and blocks orotidylic decarboxylase (Shugar, 1974; Gutowski *et al.*, 1975), but it also inhibits 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate (AICAR) formyltransferase, an enzyme involved in purine nucleotide biosynthesis. Inhibition of IMP-dehydrogenase activity by related compounds such as ribavirin has also been reported (Streeter *et al.*, 1973; Robins *et al.*, 1985; Yamada *et al.*, 1988).

Ribavirin (Fig. 31) was chemically synthesized and proved to be also a wide-spectrum antiviral agent (Witkowski *et al.*, 1972; Streeter *et al.*, 1973; Hahn, 1979) that shows activity against HIV (Fernandez-Larsson and Patterson, 1990). This compound has good antiviral activity in several

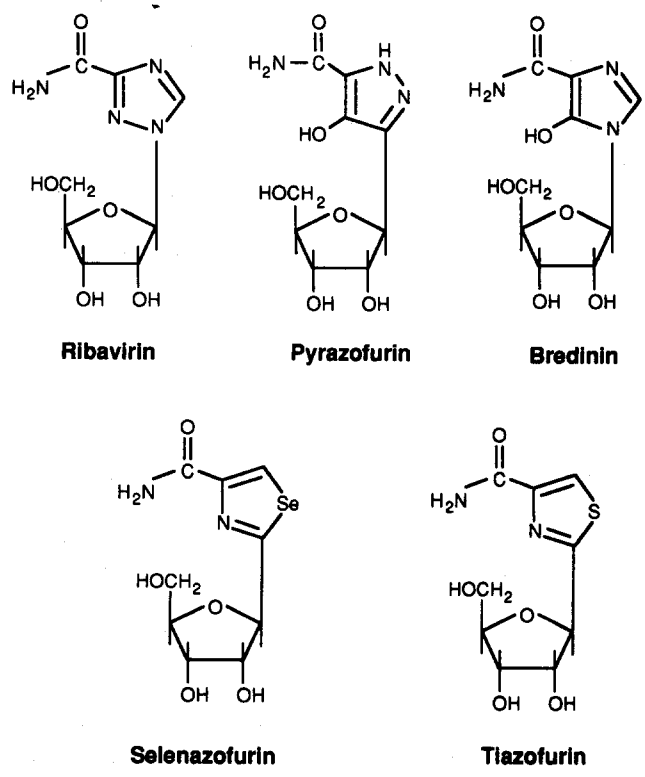


Fig. 31. Chemical structures of different compounds: ribavirin, pyrazofurin, bredinin, selenazofurin and tiazofurin.

animal models and in humans (Hahn, 1979; Hayden, 1985; Fernandez *et al.*, 1986; Patterson and Fernandez Larsson, 1990) and has been licensed in the USA for the treatment of respiratory syncytial virus infections. Ribavirin shows antiviral activity at concentrations well below those that cause cytotoxicity (Hahn, 1979; Sidwell *et al.*, 1979; Smith and Kirkpatrick, 1980), although the mode of action of this interesting antiviral agent remains obscure. The idea that ribavirin depletes GTP pools and/or interferes with cap mRNA formation (Goswami *et al.*, 1979) is not supported by several lines of evidence (Smith and Kirkpatrick, 1980; Patterson and Fernandez Larsson, 1990). Picornaviruses do not possess a cap structure in their mRNA, but ribavirin potently inhibits their growth. Indeed, ribavirin is active against several members of the *Picornaviridae* family, including poliovirus (De Clercq, 1985; Van Aerschot *et al.*, 1989; Andersen *et al.*, 1992), EMC virus, FMDV (de la Torre *et al.*, 1987), rhinoviruses and coxsackie virus (Smith and Kirkpatrick, 1980; Andersen *et al.*, 1992). Ribavirin is the only antipicornavirus agent shown to cure cells from persistent picornavirus infections (de la Torre *et al.*, 1987). Thus, BHK cells persistently infected with FMDV were cured after passages through medium containing low concentrations of ribavirin (de la Torre *et al.*, 1987). Inhibition of FMDV by ribavirin in lytic infections required higher doses of the antiviral agent (de la Torre *et al.*, 1987). Recently, the actions of ribavirin and selenazofurin against several picornaviruses have been investigated (Andersen *et al.*, 1992). Prevention of poliovirus protein synthesis by ribavirin occurs when several rounds of virus replication take place (Andersen *et al.*, 1992), but the drug does not inhibit poliovirus protein synthesis in a single growth cycle, even if present from the beginning of infection (Almela and Carrasco, unpublished observations).

Some compounds are structurally related to ribavirin, such as selenazofurin, that also has a broad spectrum of antiviral activity, and tiazofurin (2 β-D-ribofuranosylthiazole-4-carboxamide) that is mainly active against some RNA viruses (Srivastava *et al.*, 1977; Huggins *et al.*, 1984; Kirsi *et al.*, 1984). Bredinin, an antibiotic isolated from *Eupenicillium brefeldianum*, does not possess broad-spectrum antiviral activity.

7.4.2. *Tubercidin, Toyocamycin and Sangivamycin*

These three antibiotics (Fig. 32) are pyrrolopyrimidine nucleosides, which are adenosine analogues. They have been isolated from different *Streptomyces* species: Tubercidin from *Streptomyces tubercidius*, toyocamycin from *Streptomyces toyocaensis* and sangivamycin from *Streptomyces rimosus* (Suhadolnik, 1967b). All three were potent inhibitors of several rhinoviruses at drug concentrations below 1 μg/mL, but were toxic to cells at concentrations about 10-fold higher (Bergstrom *et al.*, 1984; De Clercq *et al.*, 1986). Several tubercidin analogues have been synthesized that are much more potent against coxsackievirus (Bergstrom *et al.*, 1984; De Clercq *et al.*, 1984). Although these compounds may have no therapeutic utility, some of them may be very useful as inhibitors of viral RNA replication, particularly for those picornaviruses, such as cardioviruses, against which no selective inhibitors of viral RNA synthesis have been described. Thus far, we do not know how these compounds affect EMC virus, nor do we know the exact step that they block in rhinovirus or coxsackie virus growth.

7.4.3. *Neplanocins and Carbocyclic Adenosine Analogues*

Neplanocins (Fig. 33) are carbocyclic analogues of purine nucleosides in which the ribose moiety has been replaced by a cyclopentene ring. They have been isolated from the actinomycete *Ampullaria regularis*. Neplanocins, as well as other carbocyclic analogues of adenosine, have been described as potent inhibitors of *S*-adenosyl-L-homocysteine hydrolase, resulting in the blockade of *S*-adenosylmethionine-dependent methylation reactions (De Clercq *et al.*, 1984). Neplanocins A, B and C are also broad-spectrum antiviral agents that show activity mainly against DNA and (−)RNA viruses (De Clercq, 1985). These agents are good inhibitors of the coxsackie virus in Vero cells. Concentrations of 40 ng/mL of neplanocin A inhibited coxsackie B4 (De Clercq, 1985), whereas higher concentrations of these agents are required to block poliovirus or coxsackie virus in HeLa cells (De Clercq, 1985) or rhinovirus in WI-38 cells (De Clercq *et al.*, 1991).

The carbocyclic analogues of adenosine, such as aristeromycin (C-Ado), 3-deazaadenosine (C-c^{3}Ado) and 7-deazaadenosine (C-c^{7}Ado) (Fig. 33), are also active against several animal viruses,

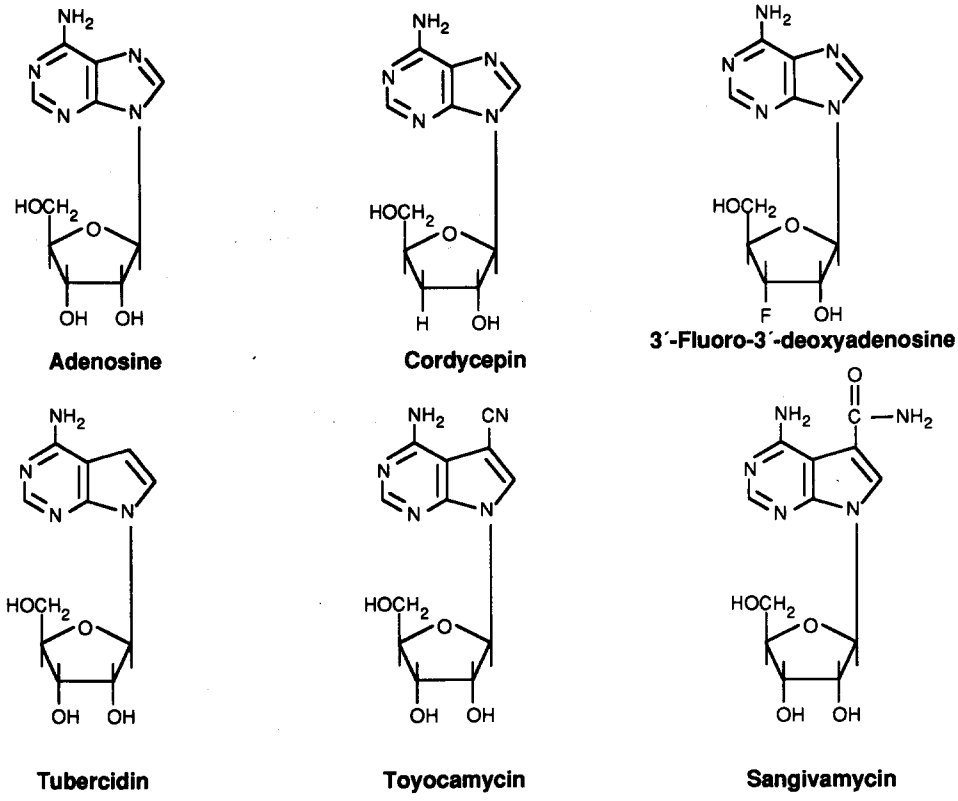


Fig. 32. Chemical structures of different compounds: adenosine, cordycepin, 3′-fluoro-3′-desoxyadenosine, tubercidin, toyocamycin and sangivamycin.

including picornaviruses. C-c^{3}Ado fails to protect HeLa cells from poliovirus or coxsackie virus B4 infection, whereas the compound was active against coxsackie in Vero cells (poliovirus was not tested) (De Clercq and Montgomery, 1983; De Clercq, 1985). C-c^{7}Ado was more active against both viruses in HeLa cells than C-c^{3}Ado (De Clercq *et al.*, 1984).

7.4.4. *Cyclopentenylcytosine*

Carbocyclic cytidine (C-Cyd) and cyclopentenylcytosine (Ce-Cyd) (Fig. 33) are broad-spectrum antiviral agents active against DNA- and RNA-containing animal viruses (De Clercq *et al.*, 1990, 1991). Poliovirus, coxsackie virus B4 and rhinovirus 1A were not inhibited by C-Cyd, whereas they were potently blocked by Ce-Cyd (De Clercq *et al.*, 1991).

7.4.5. *3-Deaza Guanine*

3-Deaza guanine (Fig. 33), a base analogue of guanine, has good antirhinovirus activity in WI-38 cells at drug concentrations well below those that show cytotoxicity (De Clercq *et al.*, 1986).

7.4.6. *3′-Fluoro-3′-Deoxyadenosine*

Several fluorinated nucleosides have been synthesized and evaluated for their antiviral potency (Van Aerschot *et al.*, 1989). 3′-fluoro-3′-deoxyadenosine (Fig. 32) was very active against poliovirus and coxsackie virus (Van Aerschot *et al.*, 1989). Although the action of some of these derivatives may be related to their inhibition of *S*-adenosyl-L-homocysteine hydrolase, the exact mode of action of 3′-fluoro-3′-deoxyadenosine remains undetermined.

7.4.7. *Cordycepin*

Cordycepin (3′-deoxyadenosine) (Fig. 32) was the first natural nucleoside antibiotic isolated. It is synthesized by *Cordiceps militaris* and *Aspergillus nidulans* and inhibits the formation of poly(A) tails in mRNAs. Cordycepin is incorporated into the 3′ terminus of several RNAs and acts as a chain terminator. The antibiotic inhibits the replication of poliovirus and rhinovirus, with inhibition of the latter being particularly sensitive (Nair and Panicali, 1976). The triphosphate inhibited incorporation of GMP by picornavirus-specific polymerase complexes in cell-free systems. RNA products synthesized *in vitro* lacked full-length viral RNA, indicating that RNA chain termination occurred in the presence of cordycepin (Panicali and Nair, 1978).

7.5. Other Picornavirus Inhibitors

7.5.1. *Amicetin and Actinobolin*

Amicetin (Fig. 34), an antibiotic produced by *Streptomyces fasciculatus* and *Streptomyces plicatus*, is an inhibitor of peptide bond formation (Vázquez, 1979). Amicetin is active in bacteria, but is less effective against mammalian cells. It inhibits both poliovirus and EMC virus at concentrations that do not affect cultured cells (Alarcón *et al.*, 1984c).

Actinobolin (Fig. 34), another translation inhibitor (Vázquez, 1979), is produced by *Streptomyces fasciculatus* and is also an effective antiviral agent in cell culture (Alarcón *et al.*, 1984c). At concentrations of 30 or 75 μg/mL, the drug protected HeLa cells from infection by either poliovirus or EMC virus (Alarcón and Carrasco, unpublished results).

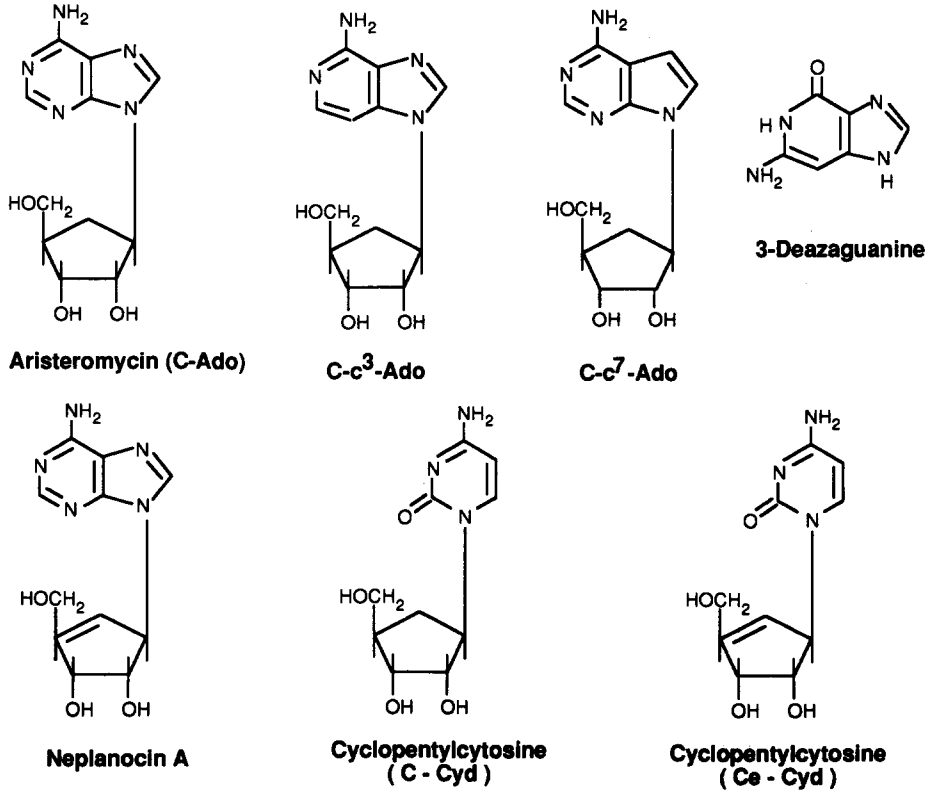
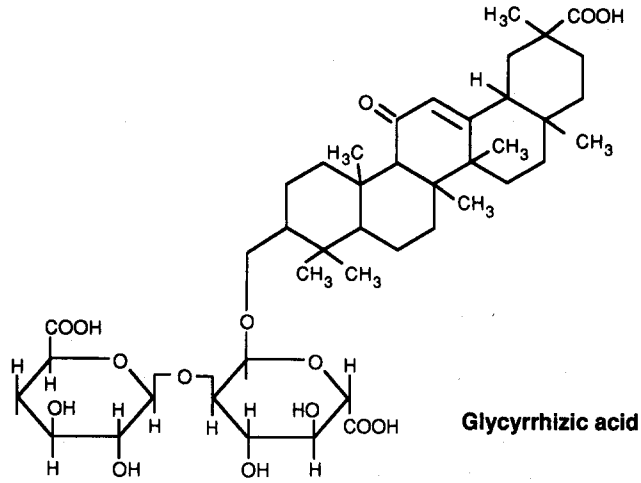
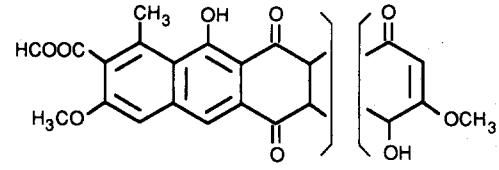


Fig. 33. Chemical structures of different compounds: aristeromycin, C-c^3-ado, C-c^7-ado, 3-deazaguanine, neplanocin A, C-Cyd and Ce-Cyd.





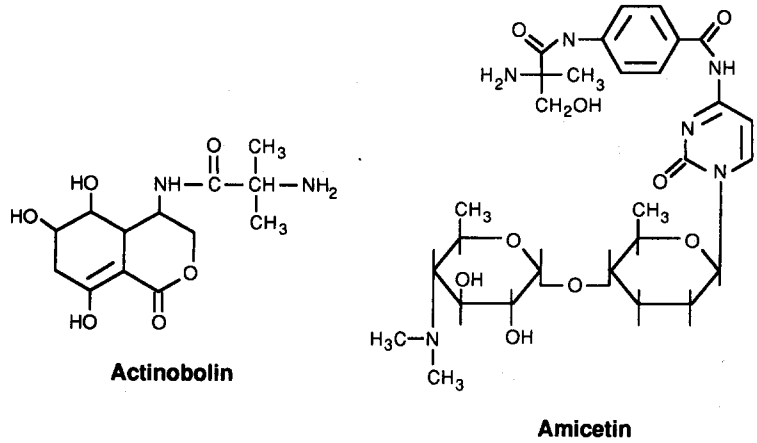


Fig. 34. Chemical structures of different compounds: glycyrrhizic acid, tetracenomycin C, actinobolin and amicetin.

7.5.2. *Tetracenomycin C*

Tetracenomycin C (Fig. 34) is an antibiotic produced by *Streptomyces glaucescens*. This agent blocks the growth of several animal viruses, including poliovirus and EMC virus (Alarcón *et al.*, 1984c). Concentrations of 15 μg/mL tetracenomycin C protected HaLa cell monolayers from infection by these two viruses (Alarcón *et al.*, 1984c).

7.5.3. *Sulfated Polysaccharides*

Sulfated polysaccharides and oligosaccharides inhibit viral replication at very low concentrations and show a wide spectrum of antiviral action (Alarcón *et al.*, 1984c; González *et al.*, 1987). These agents have no adverse effects on cultured cells even at high drug concentrations (Alarcón *et al.*, 1984c; González *et al.*, 1987). Some picornaviruses, such as poliovirus, are not affected by these compounds, whereas EMC virus growth is potently blocked by carrageenan or low-MW dextran sulfate (González *et al.*, 1987). Preliminary results from our laboratory indicate that carrageenan

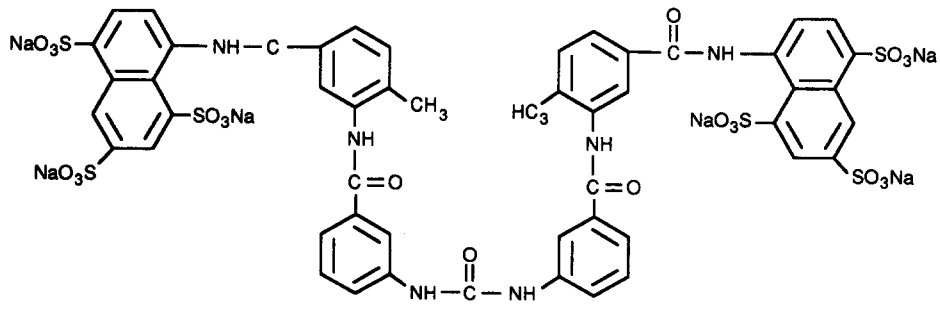
has no effect on protein synthesis in EMC virus-infected cells, implicating a late step of EMC virus replication as the target of these agents. This result contrasts with the action of sulfated polysaccharides on herpes virus or HIV, where there is interference with an early step of infection after interaction of the virus with the host cell receptor (González *et al.*, 1987; Callahan *et al.*, 1991).

7.5.4. *Suramin and Trypan Blue*

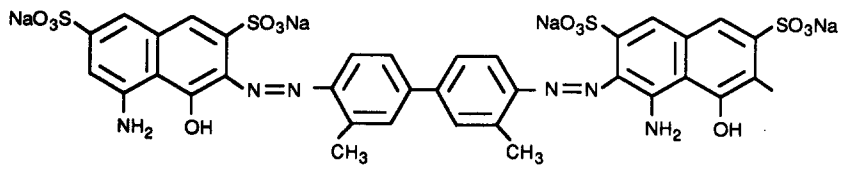
Suramin (Fig. 35) is a polyanionic synthetic compound that inhibits several viral and cellular polymerases (Basu and Modak, 1985), including the reverse transcriptase of RNA tumour viruses in cell-free systems (De Clercq, 1979; Jentsch *et al.*, 1987). It shows a potent antiviral effect in cultivated cells infected with different animal viruses, including EMC virus, but not poliovirus (Alarcón *et al.*, 1984c; Mitsuya *et al.*, 1984; Sola *et al.*, 1986b; Schols *et al.*, 1990). Suramin had no effect on EMC virus protein synthesis even when present at early stages of infection, suggesting that it blocks a late step in the replication cycle (Alarcón and Carrasco, unpublished results). Trypan blue, a very nonpermeant dye, also protected cell monolayers from viral infection (Alarcón *et al.*, 1984c), as occurs with other charged compounds (Balzarini *et al.*, 1986). In contrast with trypan blue, high concentrations of suramin had no adverse effects on either cell morphology or cell growth, even after prolonged incubation. Mice infected intravaginally with HSV-2 survived when treated with suramin (Alarcón and Carrasco, unpublished results).

7.5.5. *HPA 23*

The mineral condensed ion ammonium 5-tungsto-2-antimonate (HPA 23) is a heteropolyanion that has a broad spectrum of antiviral activity and blocks EMC virus (Werner *et al.*, 1976; Machamer *et al.*, 1992). A single intraperitoneal injection of HPA 23 protected mice from EMC virus (Werner *et al.*, 1976). Since this compound is a polyanion that also blocks reverse transcriptase (Chermann *et al.*, 1975; Ablashi *et al.*, 1977), it is possible that its mode of action may be similar to that of sulfated polysaccharides.



Suramin



Trypan Blue

Fig. 35. Chemical structures of different compounds: suramin and trypan blue.

7.5.6. *Glycyrrhizic Acid*

Glycyrrhizic acid (Fig. 34) is a natural compound extracted from the roots of *Glycyrrhiza glabra*. This compound has a broad spectrum of antiviral action (Pompei *et al.*, 1979; Alarcón *et al.*, 1984c) and blocks EMC virus, but not poliovirus, growth (Pompei *et al.*, 1979). Addition of this agent from the beginning of EMC virus infection allows the synthesis of viral proteins at control levels, whereas the formation of infectious and physical particles is severely diminished (Lacal and Carrasco, unpublished observations). It is possible that this agent blocks the assembly of new EMC virions, but such an action has not yet been demonstrated.

7.5.7. *Tenuazonic Acid*

The antibiotic tenuazonic acid (Fig. 36) was reported as an inhibitor of poliovirus growth (Miller *et al.*, 1963). It was later found that this compound blocks protein synthesis in mammalian cells, and it was, in fact, the first inhibitor of eukaryotic ribosomes to show selectivity in the blockade of human, as opposed to yeast ribosomes (Carrasco and Vázquez, 1973).

7.5.8. N-*Phenyl*-N′-*Arylthiourea*

Derivatives of *N*-phenyl-*N*′-arylthiourea (Fig. 36) are inhibitors of coxsackie virus, poliovirus, FMDV and rhinoviruses. Drug concentrations of approximately 2 μg/mL of *N*-phenyl-*N*′-3-hydroxyphenyl-thiourea (PTU-23) reduced rhinovirus growth by one-log (Galabov *et al.*, 1977). The potency and antiviral index of *N*-phenyl-*N*′-arylthiourea are rather low and probably these compounds block an early step of rhinovirus replication after adsorption (Galabov *et al.*, 1977; Galabov, 1979). PTU-23 had activity against coxsackie infection in mice, whereas another derivative (PTU-24) was active against FMDV in mice and guinea pigs (Galabov, 1979).

7.5.9. *Dichlorobenzoate (M12325)*

5-Aminosulfonyl-2,4-dichlorobenzoate (M12325) (Fig. 36) inhibits several species of RNA-containing viruses, including echovirus and rhinovirus, but has no effect against DNA viruses (Ohnishi *et al.*, 1982). It has a good antiviral index in cultured cells and is effective in mice against influenza virus (Ohnishi *et al.*, 1982).

7.5.10. *G413*

2-Amino-5-(2-sulfamoylphenyl)-1,3,4-thiadiazole (G413) (Fig. 36) belongs to a series of derivatives that block DNA and RNA viruses (Bonina *et al.*, 1982). Drug concentrations above 10 μg/mL inhibited poliovirus, echovirus and coxsackie virus. It has been suggested that G413 may bind to structural proteins and prevent virion assembly (Bonina *et al.*, 1982), although more profound studies on this agent are required to obtain a firm conclusion of its mode of action.

7.5.11. *Thiazolinone Derivatives*

A series of water-soluble thiazolinone derivatives (IMTO) (Fig. 36) exhibit antiviral properties. The *N*-(2-dimethylamino-2-methylpropoxycarbonyl) derivative was effective against influenza and coxsackie viruses in cultured cells (Harnden *et al.*, 1979).

7.5.12. *Mycophenolic Acid*

Various species of the genus *Penicillium* produce mycophenolic acid (Fig. 37). This antibiotic blocks the replication of several animal viruses, including some retroviruses (Williams *et al.*, 1968), EMC virus and coxsackie virus (Ando *et al.*, 1968; Cline *et al.*, 1969; Planterose, 1969). It is still uncertain if this blockade is due simply to cellular toxicity.

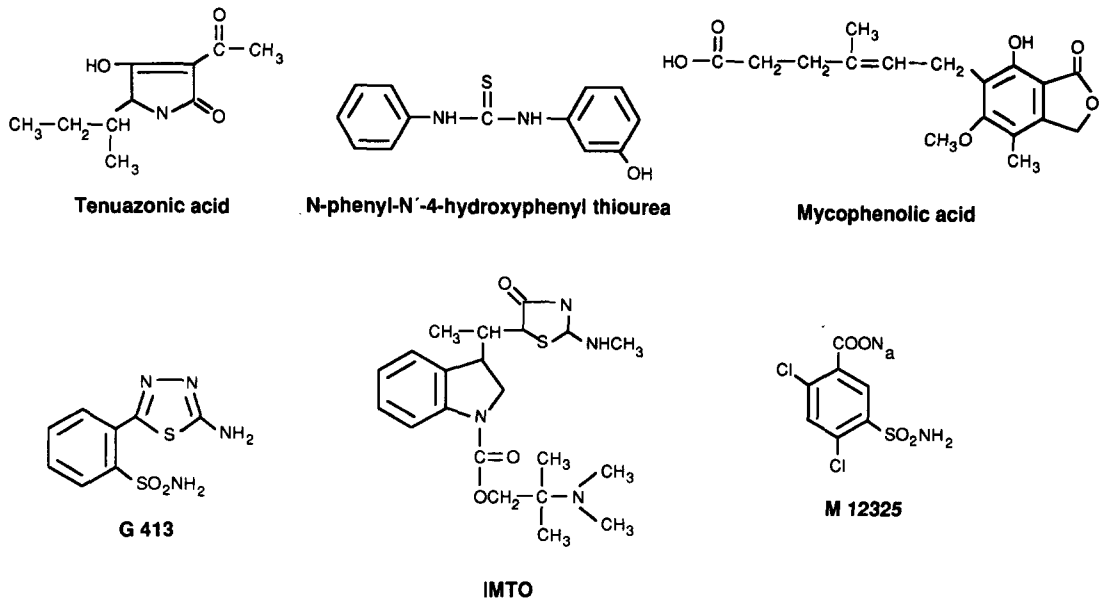


Fig. 36. Chemical structures of different compounds: tenuazonic acid, *N*-phenyl-*N*′-4-hydroxyphenyl thiourea, mycophenolic acid, G 413, IMTO and M 12325.

7.5.13. *Didemnins*

A new class of depsipeptides, didemnins (Fig. 37), were isolated from a Caribbean tunicate (sea squirt, family *Didemnidae*) that had effects against coxsackie virus (Rinehart and Gloer, 1981). Although some of these compounds, particularly didemnin B, had a potent antiviral effect in cell culture, their cytotoxicity was very high and, hence, the antiviral index was poor (Rinehart and Gloer, 1981).

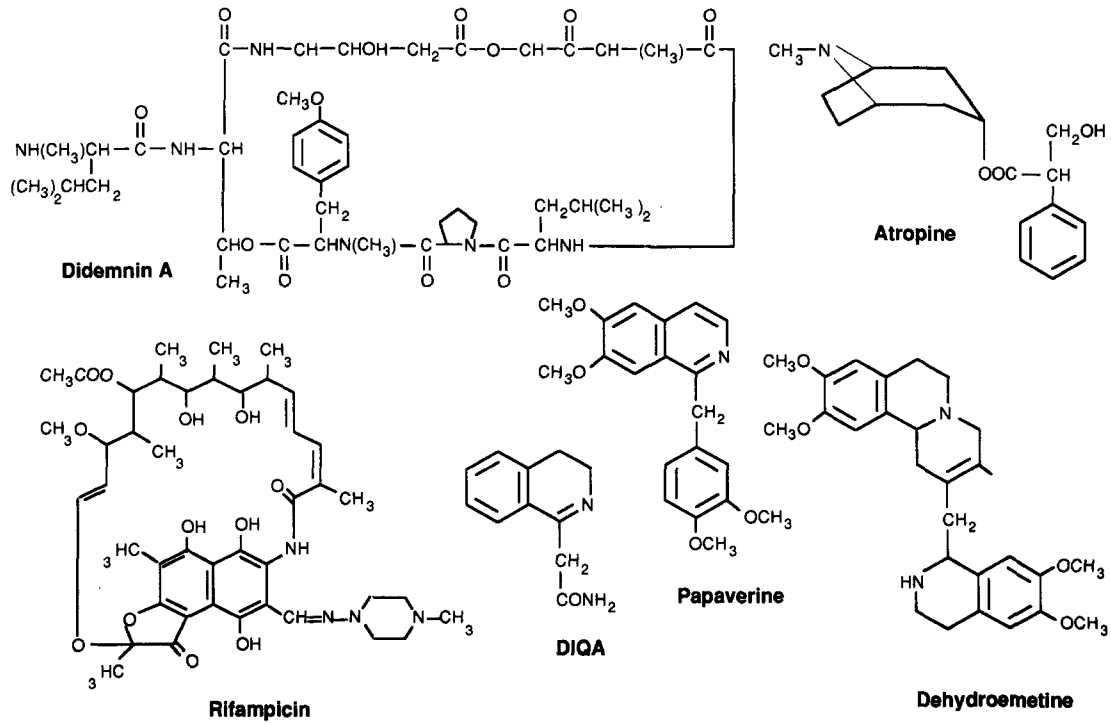


Fig. 37. Chemical structures of different compounds: didemnin A, atropine, rifampicin, DIQA, papaverine and dehydroemetine.

7.5.14. *Atropine*

Atropine (Fig. 37) is an antiviral substance extracted from *Atropa belladonna*. This agent inhibits the growth of several animal viruses (Alarcón *et al.*, 1984b), including poliovirus, but not EMC virus (Alarcón and Carrasco, unpublished results).

7.5.15. *DIQA*

3,4-Dihydro-1-isoquinolineacetamide hydrochloride (DIQA) (Fig. 37) and some related compounds, dehydroemetine and papaverine, show some antiviral effects against picornaviruses (Grunberg and Prince, 1968, 1970). Only DIQA (0.1 mg/mL) was active against coxsackie virus in tissue culture (Grunberg and Prince, 1970). Studies on the mode of action of this agent indicated that it blocked an early step of Columbia SK virus growth, different from adsorption of the virus to L cells (Murphy and Glasgow, 1970). Probably, this early step is the entry of virions into cells (Murphy and Glasgow, 1970). Clinical trials with DIQA have been conducted to prevent rhinovirus infection in volunteers (Togo *et al.*, 1973a). Oral administration did not prevent the development of colds, but the symptoms in the treated group were milder than in the placebo-treated group (Togo *et al.*, 1973a).

7.5.16. *Rifamycins*

Rifamycins A–E have been isolated from the broth of *Nocardia mediterranei*. Rifampicin is a chemical derivative, 3-(4-methylpiperazinoiminomethyl) rifamycin B (Fig. 37). These antibiotics and several derivatives have activity against several animal viruses (Gurgo *et al.*, 1982). FMDV and poliovirus growth is inhibited by rifampicin in suspension culture, but not in cell monolayers (Grado and Ohlbaum, 1973). Rifampicin was equally effective when added before or after virus entry. The specificity and reproducibility of this inhibition is obscure, because the same authors also found inhibition of these viruses by actinomycin D (Grado and Ohlbaum, 1973).

7.5.17. *Antibiotic K-582*

K-582 is a peptide antibiotic isolated from the culture filtrate of *Metarhizium anisopliae*, which exhibits antiviral activity against polio, influenza and Newcastle disease virus, perhaps by inducing an antiviral substance related to interferon (Sakai *et al.*, 1983).

7.6. Undefined Agents

Several undefined agents have been reported to block picornaviruses. Indeed, antiviral researchers are well acquainted with a numerous list of publications claiming antiviral effects with extracts, exudates, secretions, etc., from the most diverse organisms. In many instances, these crude preparations (or even purified components) owe their "antiviral activity" simply to toxicity. In some cases, however, some specific antiviral effects have been documented. Thus, helenine, perhaps a ribonucleoprotein substance, produced by *Penicillium funiculosum*, has effects against Columbia SK virus and SFV (Shope, 1953a, b, c).

Extracts from different plants and fungi can interfere with the growth of poliovirus, coxsackie virus and echoviruses in cultured cells (Goulet *et al.*, 1960). Treatment with neurotoxoids from cobra venom interferes with poliovirus infection in monkeys and cultured cells (Sanders *et al.*, 1953, 1958; Miller *et al.*, 1977). Abalone (*Haliotis refescens*) is a marine animal commonly used as food in China, and its juice has some inhibitory effects on mice infected with poliovirus (Li, 1960). Apple juice, grape juice and wines are virucidal for some viruses, including poliovirus (Konowalchuk and Speirs, 1976; Knowalchuk and Speirs, 1978). A lipid component of milk inhibits viral growth (Sabin and Fieldsteel, 1962; Matthews *et al.*, 1976). The same occurs with acidic substances from pine cones and pine seed shell extracts (Fukuchi *et al.*, 1989a; Mukoyama *et al.*, 1991b) and with tea extracts (Green, 1949; Mukoyama *et al.*, 1991a). These effects perhaps relate to tannin and

polyphenol content, substances that are known to be antiviral agents (Green, 1948; Kucera and Herrmann, 1967; Fukuchi *et al.*, 1989b).

Finally, a number of antiviral compounds have been reported to have a negligible or no effect against several picornaviruses. Such compounds include camptothecin (Horwitz *et al.*, 1972), gossypol (Dorsett *et al.*, 1975) and hypericin (Tang *et al.*, 1990).

8. CONCLUSIONS AND FUTURE PROSPECTS

Picornaviruses represent one of the best understood animal virus groups in molecular terms. They were the first animal viruses containing an RNA genome that was sequenced, the first animal virus particles to be solved at atomic resolution and the first animal viruses to be totally synthesized in the test tube (Harber and Wimmer, 1993). The analysis of the picornavirus replication cycle, in many instances, guides work with other virus groups, although picornavirus biology also shows peculiarities typical of this virus group, e.g. the internal initiation of translation on picornavirus mRNA (Meerovitch and Sonenberg, 1993).

Picornavirus inhibitors have very much aided in the elucidation of the different steps of the picornavirus replication cycle at the molecular level. The growing armamentum to block given steps of picornavirus growth provides useful tools to investigate details of the molecular biology of picornaviruses. In addition, these studies provide, in some instances, new approaches to develop antiviral agents. This is the case of the recently found connection between picornavirus genome replication and membrane traffic in mammalian cells (Guinea and Carrasco, 1990; Irurzun *et al.*, 1993), or the finding that picornaviruses encode for viroporins (Lama and Carrasco, 1992b), viral proteins that make pores in the membrane and modify membrane permeability (Carrasco *et al.*, 1993), allowing the passage of nonpermeant inhibitors into virus-infected cells (Carrasco, 1978).

The fact that some picornaviruses cause disease in humans and in animals prompted the investigation of more effective and nontoxic compounds to inhibit their replication. The elegant studies on agents that bind to virion particles and block their uncoating exemplifies not only one of the most refined analyses on the mode of action of antiviral agents, but also provides promising compounds to effectively attack the common cold in humans (Rossmann and Johnson, 1989; McKinlay *et al.*, 1992).

Finally, the growing interest in picornavirus inhibitors will expand the number of new antipicornavirus agents and will help to unravel the modes of action of many of them. I predict that future studies to broaden our knowledge of the exact mechanism of action of these inhibitors will provide more surprises to the molecular biology of this ever-interesting group of viruses.

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REFERENCES

Ablashi, D. V., Twardzik, D. R., Easton, J. M., Armstrong, G. R., Luetzeler, J., Jasmin, C. and Chermann, J. C. (1977) Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus-cell systems. *Eur. J. Cancer* **13:** 713–720.

Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D. and Brown, F. (1989) The three-dimensional structure of foot-and-mouth disease virus at 2.9 Å resolution. *Nature* **337:** 709–716.

Agol, V. I. (1993) Poliovirus neurovirulence and its attenuation. In: *Regulation of Gene Expression in Animal Viruses*, pp. 305–321, Carrasco, L., Sonenberg, N. and Wimmer, E. (eds). Plenum Press, London, New York.

Agrawal, S., Tang, J. Y., Sun, D., Sarin, P. S. and Zamecnik, P. C. (1992) Antisense strategies: synthesis and anti-HIV activity of oligoribonucleotides and their phosphorothioate analogs. *Ann. N.Y. Acad. Sci.* **660:** 2–10.

Aktories, K. (1992) ADP-ribosylating toxins. *Curr. Top. Microbiol. Immunol.* **175:** 1–150.

Alarcón, B., Bugany, H. and Carrasco, L. (1984a) pppA2′p5A′ blocks vesicular stomatitis virus replication in intact cells. *J. Virol.* **52:** 183–187.

Alarcón, B., González, M. E. and Carrasco, L. (1984b) Antiherpesvirus action of atropine. *Antimicrob. Agents Chemother.* **26:** 702–706.

Alarcón, B., Lacal, J. C., Fernández-Sousa, J. M. and Carrasco, L. (1984c) Screening for new compounds with antiherpes activity. *Antiviral. Res.* **4:** 231–244.

Alarcón, B., Zerial, A., Dupiol, C. and Carrasco, L. (1986) Antirhinovirus compound 44,081 R.P. inhibits virus uncoating. *Antimicrob. Agents Chemother.* **30:** 31–34.

Alcalde, J., Bonay, P., Roa, A., Vilaro, S. and Sandoval, I. V. (1991) Assembly and disassembly of the Golgi complex: two processes arranged in a *cis–trans* direction. *J. Cell Biol.* **116:** 69–83.

Almela, M. J., Gonzalez, M. E. and Carrasco, L. (1991) Inhibitors of poliovirus uncoating efficiently block the early membrane permeabilization induced by virus particles. *J. Virol.* **65:** 2572–2577.

Al-Nakib, W., Higgins, P. G., Barrow, I., Tyrell, D. A. J., Lenox-Smith, I. and Ishitsuka, H. (1987) Intranasal chalcone, Ro 09-0410, as prophylaxis against rhinovirus infection in human volunteers. *J. Antimicrob. Chemother.* **20:** 887–892.

Al-Nakib, W., Higgins, P. G., Barrow, G. I., Tyrrell, D. A. J., Andries, K., Bussche, G. V., Taylor, N. and Janssen, P. A. J. (1989) Suppression of colds in human volunteers challenged with rhinovirus by a new synthetic drug (R 61837). *Antimicrob. Agents Chemother.* **33:** 522–525.

Alonso, M. A. and Carrasco, L. (1981a) Relationship between membrane permeability and the translation capacity of human HeLa cells studied by means of the ionophore nigericin. *Eur. J. Biochem.* **118:** 289–294.

Alonso, M. A. and Carrasco, L. (1981b) Permeabilization of mammalian cells to proteins by the ionophore nigericin. *FEBS Lett.* **127:** 112–114.

Alonso, M. A. and Carrasco, L. (1982a) Molecular basis of the permeabilization of mammalian cells by ionophores. *Eur. J. Biochem.* **127:** 567–569.

Alonso, M. A. and Carrasco, L. (1982b) Translation of capped viral mRNAs in poliovirus-infected HeLa cells. *EMBO J.* **1:** 913–917.

Andersen, D. O., Murray, B. K., Robins, R. K. and North, J. A. (1992) *In vitro* antiviral activity of ribavirin against picornaviruses. *Antiviral Chem. Chemother.* **3:** 361–370.

Anderson Sillman, K., Bartal, S. and Tershak, D. R. (1984) Guanidine-resistant poliovirus mutants produce modified 37-kilodalton proteins. *J. Virol.* **50:** 922–928.

Andino, R., Rieckhof, G. E. and Baltimore, D. (1990a) A functional ribonucleoprotein complex forms around the 5′ end of poliovirus RNA. *Cell* **63:** 369–380.

Andino, R., Rieckhof, G. E., Trono, D. and Baltimore, D. (1990b) Substitutions in the protease (3Cpro) gene of poliovirus can suppress a mutation in the 5′ noncoding region. *J. Virol.* **64:** 607–612.

Ando, K., Suzuki, S., Tamura, G. and Arima, K. (1968) Antiviral activity of mycophenolic acid. Studies on antiviral and antitumor antibiotics. IV. *J. Antibiot. Tokyo* **21:** 649–652.

Andries, K., Dewindt, B., De Brabander, M., Stokbroekx, R. and Janssen, P. A. (1988) *In vitro* activity of R 61837, a new antirhinovirus compound. *Arch. Virol.* **101:** 155–167.

Andries, K., Dewindt, B., Snoeks, J. and Willebrords, R. (1989) Lack of quantitative correlation between inhibition of replication of rhinoviruses by an antiviral drug and their stabilization. *Arch. Virol.* **106:** 56–61.

Anthony, D. D. and Merrick, W. C. (1991) Eukaryotic initiation factor (eIF)-4F. Implications for a role in internal initiation of translation. *J. biol. Chem.* **266:** 10218–10226.

Armstrong, J. A., Edmonds, M., Nakazato, H., Phillips, B. A. and Vaughn, M. H. (1972) Polyadenylic acid sequences in the virion RNA of poliovirus and eastern equine encephalitis virus. *Science* **176:** 526–528.

Arnold, E., Luo, M., Vriend, G., Rossmann, M. G., Palmenberg, A. C., Parks, G. D., Nicklin, M. J. and Wimmer, E. (1987) Implications of the picornavirus capsid structure for polyprotein processing. *Proc. natn. Acad. Sci. U.S.A.* **84:** 21–25.

Ash, R. J., Parker, R. A., Hagan, A. C. and Mayer, G. D. (1979) RMI 15,731 (1-[5-tetradecyloxy-2-furanyl]-ethanone), a new antirhinovirus compound. *Antimicrob. Agents Chemother.* **16:** 301–305.

Bachrach, H. L., Swaney, J. B. and Vande Woude, G. F. (1973) Isolation of the structural polypeptides of foot-and-mouth disease virus and analysis of their C-terminal sequences. *Virology* **52:** 520–528.

Badger, J., Krishnaswamy, S., Kremer, M. J., Oliveira, M. A., Rossmann, M. G., Heinz, B. A., Rueckert, R. R., Dutko, F. J. and McKinlay, M. A. (1989) Three-dimensional structures of drug-resistant mutants of human rhinovirus 14. *J. molec. Biol.* **207:** 163–174.

Badger, J., Minor, I., Kremer, M. J., Oliveira, M. A., Smith, T. J., Griffith, J. P., Guerin, D. M. A., Krishnaswamy, S., Luo, M., Rossmann, M. G., McKinlay, M. A., Diana, G. D., Dutko, F. J., Fancher, M., Rueckert, R. R. and Heinz, B. A. (1988) Structural analysis of a series of antiviral agents complexed with human rhinovirus 14. *Proc. natn. Acad. Sci. U.S.A.* **85:** 3304–3308.

Baltera, R. F. J. and Tershak, D. R. (1989) Guanidine-resistant mutants of poliovirus have distinct mutations in peptide 2C. *J. Virol.* **63:** 4441–4444.

Baltimore, D. (1966) Purification and properties of poliovirus double-stranded ribonucleic acid. *J. molec. Biol.* **18:** 421–428.

Baltimore, D., Eggers, H. J., Franklin, R. M. and Tamm, I. (1963) Poliovirus-induced RNA polymerase and the effects of virus-specific inhibitors on its production. *Biochemistry* **49:** 843–849.

Baltimore, D. and Girard, M. (1966) An intermediate in the synthesis of poliovirus RNA. *Proc. natn Acad. Sci. U.S.A.* **56:** 741–748.

Balzarini, J., Mitsuya, H., De Clercq, E. and Broder, S. (1986) Aurintricarboxylic acid and Evans Blue represent two different classes of anionic compounds which selectively inhibit the cytopathogenicity of

human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. *Biochem. biophys. Res. Commun.* **136:** 64–71.

Baron, M. H. and Baltimore, D. (1982) Purification and properties of a host cell protein required for poliovirus replication *in vitro*. *J. biol. Chem.* **257:** 12351–12358.

Barrow, G. I., Higgins, P. G., Tyrrell, D. A. J. and Andries, K. (1990) An appraisal of the efficacy of the antiviral R 61837 in rhinovirus infections in human volunteers. *Antiviral Chem. Chemother.* **1:** 279–283.

Barton, D. J. and Flanegan, J. B. (1993) Coupled translation and replication of poliovirus RNA *in vitro*: synthesis of functional 3D polymerase and infectious virus. *J. Virol.* **67:** 822–831.

Basu, A. and Modak, M. J. (1985) Observations on the suramin-mediated inhibition of cellular and viral DNA polymerases. *Biochem. biophys. Res. Commun.* **128:** 1395–1402.

Bauer, D. J., Apostolov, K. and Selway, J. W. T. (1970) Activity of methisazone against RNA viruses. *Ann. N.Y. Acad. Sci.* **173:** 314–319.

Bauer, D. J., Selway, J. W., Batchelor, J. F., Tisdale, M., Caldwell, I. C. and Young, D. A. (1981) 4′,6-Dichloroflavan (BW683C), a new antirhinovirus compound. *Nature* **292:** 369–370.

Baxt, B. (1987) Effect of lysosomotropic compounds on early events in foot-and-mouth disease virus replication. *Virus Res.* **7:** 257–271.

Beck, E., Forss, S., Strebel, K., Cattaneo, R. and Feil, G. (1983) Structure of the FMDV translation initiation site and of the structural proteins. *Nucleic Acids Res.* **11:** 7873–7885.

Belsham, G. J. (1992) Dual initiation sites of protein synthesis on foot-and-mouth disease virus RNA are selected following internal entry and scanning of ribosomes *in vivo*. *EMBO J.* **11:** 1105–1110.

Benedetto, A., Rossi, G. B., Amici, C., Belardelli, F., Cioe, L., Carruba, G. and Carrasco, L. (1980) Inhibition of animal virus production by means of translation inhibitors unable to penetrate normal cells. *Virology* **106:** 123–132.

Benziger, D. P. and Edelson, J. (1982) Disposition of arildone, an antiviral agent, after various routes of administration. *Antimicrob. Agents Chemother.* **22:** 475–481.

Bergstrom, D. E., Brattesani, A. J., Ogawa, M. K., Reddy, P. A., Schweickert, M. J., Balzarini, J. and De Clercq, E. (1984) Antiviral activity of C-5 substituted tubercidin analogues. *J. Med. Chem.* **27:** 285–292.

Bernstein, H. D., Sarnow, P. and Baltimore, D. (1986) Genetic complementation among poliovirus mutants derived from an infectious cDNA clone. *J. Virol.* **60:** 1040–1049.

Bernstein, H. D., Sonenberg, N. and Baltimore, D. (1985) Poliovirus mutant that does not selectively inhibit host cell protein synthesis. *Mol. Cell Biol.* **5:** 2913–2923.

Better, M., Bernhard, S. L., Lei, S.-P., Fishwild, D. M. and Carroll, S. F. (1992) Activity of recombinant mitogillin and mitogillin immunoconjugates. *J. biol. Chem.* **267:** 16712–16718.

Bienz, K., Egger, D., Pfister, T. and Troxler, M. (1992) Structural and functional characterization of the poliovirus replication complex. *J. Virol.* **66:** 2740–2747.

Bienz, K., Egger, D., Troxler, M. and Pasamontes, L. (1990) Structural organization of poliovirus RNA replication is mediated by viral proteins of the P2 genomic region. *J. Virol.* **64:** 1156–1163.

Bjorck, L., Grubb, A. and Kjellen, L. (1990) Cystatin C, a human proteinase inhibitor, blocks replication of herpes simplex virus. *J. Virol.* **64:** 941–943.

Bonina, L., Orzalesi, G., Merendino, R., Arena, A. and Mastroeni, P. (1982) Structure–activity relationships of new antiviral compounds. *Antimicrob. Agents Chemother.* **22:** 1067–1069.

Bonneau, A. and Sonenberg, N. (1987) Proteolysis of the p220 component of the cap-binding protein complex is not sufficient for complete inhibition of host cell protein synthesis after poliovirus infection. *J. Virol.* **61:** 986–991.

Borsa, J., Morash, B. D., Sargent, M. D., Copps, T. P., Lievaart, P. A. and Szekely, J. G. (1979) Two modes of entry of reovirus particles into L cells. *J. gen. Virol.* **45:** 161–170.

Bowman, E. J., Siebers, A. and Altendorf, K. (1988) Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms—animal cells—and plant cells. *Proc. natn. Acad. Sci. U.S.A.* **85:** 7972–7976.

Brown, D. T. and Burlingham, B. T. (1973) Penetration of host cell membranes by adenovirus 2. *J. Virol.* **12:** 386–396.

Brown, F., Planterose, D. N. and Stewart, D. L. (1961) Effect of *p*-fluorophenylalanine on the multiplication of foot-and-mouth disease virus. *Nature* **191:** 414–415.

Brown, G. C. and Ackermann, W. W. (1951) Effect of DL-ethionine on poliomyelitis virus growth in tissue culture. *Proc. Soc. exp. Biol. Med.* **77:** 367–369.

Bucknall, R. A., Swallow, D. L., Moores, H. and Harrad, J. (1973) A novel substituted guanidine with high activity *in vitro* against rhinoviruses. *Nature* **246:** 144–145.

Burkhardt, R. (1991) Fatty acids stimulate phosphatidylcholine synthesis and CTP:choline-phosphate cytidylyltransferase in type II pneumocytes isolated from adult rat lung. *Biochem. J.* **254:** 495–500.

Butterworth, B. E. and Rueckert, R. R. (1972) Kinetics of synthesis and cleavage of encephalomyocarditis virus-specific proteins. *Virology* **50:** 535–549.

Caliguiri, L. A., McSharry, J. J. and Lawrence, G. W. (1980) Effect of arildone on modifications of poliovirus *in vitro*. *Virology* **105:** 86–93.

Caliguiri, L. A. and Tamm, I. (1968a) Action of guanidine on the replication of poliovirus RNA. *Virology* **35:** 408–417.

Caliguiri, L. A. and Tamm, I. (1968b) Distribution and translation of poliovirus RNA in guanidine-treated cells. *Virology* **36:** 223–231.

Caliguiri, L. A. and Tamm, I. (1969) Membranous structures associated with translation and transcription of poliovirus RNA. *Science* **166:** 885–886.

Caliguiri, L. A. and Tamm, I. (1970a) Characterization of poliovirus-specific structures associated with cytoplasmic membranes. *Virology* **42:** 112–122.

Caliguiri, L. A. and Tamm, I. (1970b) The role of cytoplasmic membranes in poliovirus biosynthesis. *Virology* **42:** 100–111.

Caliguiri, A. C. and Tamm, I. (1973) Guanidine and 2-(a-hydroxybenzyl)-benzimidazole (HBB): selective inhibitors of picornavirus multiplication. In: *Selective Inhibitors of Viral Functions*, pp. 257–293, Carter, W. A. (ed.). CRC Press, Cleveland, Ohio.

Callahan, L. N., Phelan, M., Mallinson, M. and Norcross, M. A. (1991) Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120–CD4 interactions. *J. Virol.* **65:** 1543–1550.

Carrasco, L. (1977) The inhibition of cell functions after viral infection. A proposed general mechanism. *FEBS Lett.* **76:** 11–15.

Carrasco, L. (1978) Membrane leakiness after viral infection and a new approach to the development of antiviral agents. *Nature* **272:** 694–699.

Carrasco, L. (1981) Modification of membrane permeability induced by animal viruses early in infection. *Virology* **113:** 623–629.

Carrasco, L. (1987) Mechanisms of virus-induced cell toxicity: a general overview. In: *Mechanisms of Viral Toxicity in Animal Cells*, pp. 1–4, Carrasco, L. (ed.). CRC Press Inc., Boca Raton, FL.

Carrasco, L. and Castrillo, J. L. (1987) The regulation of translation in picornavirus infected cells. In: *Mechanisms of Viral Toxicity in Animal Cells*, pp. 115–146, Carrasco, L. (ed.). CRC Press Inc., Boca Raton, FL.

Carrasco, L. and Esteban, M. (1982) Modification of membrane permeability in vaccinia virus-infected cells. *Virology* **117:** 62–69.

Carrasco, L., Fernández-Puentes, C., Alonso, M. A., Lacal, J. C., Muñoz, A., Fernández-Sousa, J. M. and Vázquez, D. (1981) New approaches to antiviral agents. In: *Medicinal Chemistry Advances*, pp. 211–224, De las Heras, F. and Vega, D. (eds). Pergamon Press, Oxford.

Carrasco, L., Harvey, R., Blanchard, C. and Smith, A. E. (1979) Regulation of translation of eukaryotic virus mRNAs. In: *Modern Trends in Human Leukemia III*, pp. 277–281, Neth, R., Gallo, R. C., Hofschneider, P. H. and Mannweiler, K. (eds). Springer-Verlag, Berlin.

Carrasco, L., Otero, M. J. and Castrillo, J. L. (1989) Modification of membrane permeability by animal viruses. *Pharmacol. Ther.* **40:** 171–212.

Carrasco, L., Pérez, L., Irurzun, A., Lama, J., Martínez-Abarca, F., Rodrígez, P., Guinea, R., Castrillo, J. L., Sanz, M. A. and Ayala, M. J. (1993) Modification of membrane permeability by animal viruses. In: *Regulation of Gene Expression in Animal Viruses*, pp. 283–303, Carrasco, L., Sonenberg, N. and Wimmer, E. (eds). Plenum Press, London, New York.

Carrasco, L. and Smith, A. E. (1976) Sodium ions and the shut-off of host cell protein synthesis by picornaviruses. *Nature* **264:** 807–809.

Carrasco, L. and Smith, A. E. (1980) Molecular biology of animal virus infection. *Pharmac. Ther.* **9:** 311–355.

Carrasco, L. and Vázquez, D. (1973) Differences in eukaryotic ribosomes detected by the selective action of an antibiotic. *Biochim. biophys. Acta* **319:** 209–215.

Carrasco, L. and Vázquez, D. (1983) Viral translation inhibitors. In: *Antibiotics*, Vol. 6, pp. 1980–1990, Hahn, F. E. (ed.). Springer-Verlag, Berlin.

Carrasco, L. and Vázquez, D. (1984) Molecular bases for the action and selectivity of nucleoside antibiotics. *Med. Res. Rev.* **4:** 471–512.

Carrillo, E. C., Giachetti, C. and Campos, R. H. (1984) Effect of lysosomotropic agents on the foot-and-mouth disease virus replication. *Virology* **135:** 542–545.

Carrillo, E. C., Giachetti, C. and Campos, R. (1985) Early steps in FMDV replication: further analysis on the effects of chloroquine. *Virology* **147:** 118–125.

Castrillo, J. L. and Carrasco, L. (1987a) Action of 3-methylquercetin on poliovirus RNA replication. *J. Virol.* **61:** 3319–3321.

Castrillo, J. L. and Carrasco, L. (1987b) Adenovirus late protein synthesis is resistant to the inhibition of translation induced by poliovirus. *J. biol. Chem.* **262:** 7328–7334.

Castrillo, J. L., Vanden-Berghe, D. and Carrasco, L. (1986) 3-Methylquercetin is a potent and selective inhibitor of poliovirus RNA synthesis. *Virology* **152:** 219–227.

Cavener, D. and Ray, S. (1991) Eukaryotic start and stop translation sites. *Nucleic Acids Res.* **19:** 3185–3192.

Chapman, M. S., Minor, I., Rossmann, M. G., Diana, G. D. and Andries, K. (1991) Human rhinovirus 14 complexed with antiviral compound R 61837. *J. molec. Biol.* **217:** 455–463.

Charachon, G., Sobol, R. W., Bisbal, C., Salehzada, T., Silhol, M., Charubala, R., Pfleiderer, W., Lebleu, B. and Suhadolnik, R. J. (1990) Phosphorothioate analogues of (2′-5′)(A)4: agonist and antagonist activities in intact cells. *Biochemistry* **29:** 2550–2556.

Chebath, J., Benech, P., Revel, M. and Vigneron, M. (1987) Constitutive expression of (2′-5′) oligo A synthetase confers resistance to picornavirus infection. *Nature* **330:** 587–588.

Chen, S., Matsuoka, Y. and Compans, R. W. (1991) Assembly and polarized release of punta toro virus and effects of brefeldin A. *J. Virol.* **65:** 1427–1439.

Chermann, J. C., Sinoussi, F. C. and Jasmin, C. (1975) Inhibition of RNA-dependent DNA polymerase of

murine oncornaviruses by ammonium-5-tungsto-2-antimoniate. *Biochem. biophys. Res. Commun.* **65:** 1229–1236.

Cheung, P., Banfield, B. W. and Tufaro, F. (1991) Brefeldin A arrests the maduration and egress of herpes simplex virus during infection. *J. Virol.* **65:** 1893–1904.

Cho, M. W., Richards, O. C., Dmitrieva, T. M., Agol, V. and Enrenfeld, E. (1993) RNA duplex unwinding activity of poliovirus RNA-dependent RNA polymerase $3D^{pol}$. *J. Virol.* **67:** 3010–3018.

Chow, M., Newman, J. F. E., Filman, D., Hogle, J. M., Rowlands, D. J. and Brown, F. (1987) Myristilation of picornavirus capsid protein VP4 and its structural significance. *Nature* **327:** 482–486.

Clark, M., Hämemerle, T., Wimmer, E. and Dasgupta, A. (1991) Poliovirus proteinase 3C converts an active form of transcription factor IIIC to an inactive form: a mechanism for inhibition of host cell polymerase III transcription by poliovirus. *EMBO J.* **10:** 2941–2947.

Clarke, B. E., Brown, A. L., Currey, K. M., Newton, S. E., Rowlands, D. J. and Carroll, A. R. (1987) Potential secondary and tertiary structure in the genomic RNA of foot-and-mouth disease virus. *Nucleic Acids Res.* **15:** 7067–7079.

Cline, J. C., Nelson, J. D., Gerzon, K., Williams, R. H. and DeLong, D. C. (1969) *In vitro* antiviral activity of mycophenolic acid and its reversal by guanine-type compounds. *Appl. Microbiol.* **18:** 14–20.

Coccia, E. M., Romeo, G., Nissim, A., Marziali, G., Albertini, R., Affabris, E., Battistini, A., Fiorucci, G., Orsatti, R., Rossi, G. B. and Chebath, J. (1990) A full-length murine 2-5A synthetase cDNA transfected in NIH-3T3 cells impairs EMCV but not VSV replication. *Virology* **179:** 228–233.

Colonno, R. J., Callahan, P. L. and Long, W. J. (1986) Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses. *J. Virol.* **57:** 7–12.

Conti, C., Genovese, D., Santoro, R., Stein, M. L., Orsi, N. and Fiore, L. (1990) Activities and mechanisms of action of halogen-substituted flavonoids against poliovirus type 2 infection *in vitro*. *Antimicrob. Agents Chemother.* **34:** 460–466.

Contreras, A. and Carrasco, L. (1979) Selective inhibition of protein synthesis in virus-infected mammalian cells. *J. Virol.* **29:** 114–122.

Cotten, M., Wagner, E., Zatloukal, K., Phillips, S., Curiel, D. T. and Birnstiel, M. L. (1992) High-efficiency receptor-mediated delivery of small and large (48 kilobase) gene constructs using the endosome-disruption activity of defective or chemically inactivated adenovirus particles. *Proc. natn. Acad. Sci. U.S.A.* **89:** 6094–6098.

Cowen, W. F. and Heydrick, F. P. (1972) Incorporation of C18 polyunsaturated fatty acids into L cells phospholipids under normal conditions and during infection with Venezuelan equine encephalitis virus (VEE-arbovirus). *Exp. Cell Res.* **72:** 354–360.

Dales, S. (1973) Early events in cell–animal virus interactions. *Bacteriol. Rev.* **37:** 103–135.

Dales, S., Eggers, H. J., Tamm, I. and Palade, G. E. (1965) Electron microscopic study of the formation of poliovirus. *Virology* **26:** 379–389.

Dasgupta, A. (1983) Purification of host factor required for *in vitro* transcription of poliovirus RNA. *Virology* **128:** 245–251.

Dasgupta, A., Zabel, P. and Baltimore, D. (1980) Dependence of the activity of the poliovirus replicase on the host cell protein. *Cell* **19:** 423–429.

Davidoff, F. (1973) Guanidine derivatives in medicine. *N. Engl. J. Med.* **289:** 141–146.

Davies, M. V., Pelletier, J., Meerovitch, K., Sonenberg, N. and Kaufman, R. J. (1991) The effect of poliovirus proteinase 2Apro expression on cellular metabolism. Inhibition of DNA replication, RNA polymerase II transcription, and translation. *J. biol. Chem.* **266:** 14714–14720.

Dawson, K. M., Stewart, A. and Stebbing, N. (1979) Absence of significant antiviral effects of beta-gamma-methylene GTP on encephalomyocarditis virus infection of L cells and mice. *J. Gen. Virol.* **45:** 237–240.

De Clercq, E. (1979) Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor viruses. *Cancer Lett.* **8:** 9–22.

De Clercq, E. (1985) Antiviral and antimetabolic activities of neplanocins. *Antimicrob. Agents Chemother.* **28:** 84–89.

De Clercq, E., Bergstrom, D. E., Holy, A. and Montgomery, A. (1984) Broad-spectrum antiviral activity of adenosine analogues. *Antiviral Res.* **4:** 119–133.

De Clercq, E., Bernaers, R., Bergstrom, D. E., Robins, M. J., Montgomery, J. A. and Holy, A. (1986) Antirhinovirus activity of purine nucleoside analogs. *Antimicrob. Agents Chemother.* **29:** 482–487.

De Clercq, E., Bernaerts, R., Shealy, Y. F. and Montgomery, J. A. (1990) Broad-spectrum antiviral activity of carbodine, the carbocyclic analogue of cytidine. *Biochem. Pharmac.* **39:** 319–325.

De Clercq, E., Descamps, J., De Somer, P. and Holy, A. (1978) (*S*)-9-(2,3-Dihydroxypropyl)adenine: an aliphatic nucleoside analog with broad-spectrum antiviral activity. *Science* **200:** 563–565.

De Clercq, E. and Montgomery, A. (1983) Broad-spectrum antiviral activity of the carbocyclic analog of 3-deazaadenosine. *Antiviral Res.* **3:** 17–24.

De Clercq, E., Murase, J. and Marquez, V. E. (1991) Broad-spectrum antiviral and cytocidal activity of cyclopentenylcytosine, a carbocyclic nucleoside targeted at CTP synthetase. *Biochem. Pharmac.* **41:** 1821–1829.

Degols, G., Leonetti, J. P., Gagnor, C., Lemaitre, M. and Lebleu, B. (1989) Antiviral activity and possible mechanisms of action of oligonucleotides-poly(l-lysine) conjugates targeted to vesicular stomatitis virus mRNA and genomic RNA. *Nucleic Acids Res.* **17:** 9341–9350.

de la Torre, J. C., Alarcón, B., Martínez-Salas, E., Carrasco, L. and Domingo, E. (1987) Ribavirin cures cells of a persistent infection with foot-and-mouth disease virus *in vitro*. *J. Virol.* **61:** 233–235.

de la Torre, J. C., Wimmer, E. and Holland, J. J. (1990) Very high frequency of reversion to guanidine resistance in clonal pools of guanidine-dependent type 1 poliovirus. *J. Virol.* **64:** 664–671.

Delong, D. and Reed, S. E. (1980) Inhibition of rhinovirus replication in organ culture by a potential antiviral drug. *J. Infect. Dis.* **141:** 87–91.

De Maeyer, E., Galasso, G. and Schellekens, H. (1981) *The Biology of the Interferon System.* Elsevier/North-Holland Biomedical Press, Amsterdam.

Desideri, N., Sestili, I., Stein, M. L., Conti, C., Tomao, P. and Orsi, N. (1990) Synthesis and anti-rhinovirus 1B activity of oxazolinylflavans. *Antiviral Chem. Chemother.* **1,** 307–312.

Diana, G. and Mallamo, J. P. (1993) Correlation of pocket size and shape with antiviral activity. *J. molec. Biol.* **230:** 226–227.

Diana, G. D., McKinlay, M. A., Brisson, C. J., Zalay, E. S., Miralles, J. V. and Salvador, U. J. (1985a) Isoxazoles with antipicornavirus activity. *J. med. Chem.* **28:** 748–752.

Diana, G. D., McKinlay, M. A., Otto, M. J., Akullian, V. and Oglesby, C. (1985b) [[4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles. Inhibitors of picornavirus uncoating. *J. med. Chem.* **28:** 1906–1910.

Diana, G. D., Nitz, T. J., Mallamo, J. P. and Treasurywala, A. (1993) Antipicornavirus compounds: use of rational drug design and molecular modelling. *Antiviral Chem. Chemother.* **4:** 1–10.

Diana, G. D., Otto, M. J. and McKinlay, M. A. (1985c) Inhibitors of picornavirus uncoating as antiviral agents. *Pharmac. Ther.* **29:** 287–297.

Diana, G. D., Salvador, U. J., Zalay, E. S., Carabateas, P. M., Williams, G. L., Collins, J. C. and Pancic, F. (1977a) Antiviral activity of some b-diketones. 2. Aryloxy alkyl diketones. *In vitro* activity against both RNA and DNA viruses. *J. med. Chem.* **20:** 757–761.

Diana, G. D., Salvador, U. J., Zalay, E. S., Johnson, R. E., Collins, J. C., Johnson, D., Hinshaw, W. B., Lorenz, R. R., Thielking, W. H. and Pancic, F. (1977b) Antiviral activity of some b-diketones. 1. Aryl alkyl diketones. *In vitro* activity against both RNA and DNA viruses. *J. med. Chem.* **20:** 750–756.

Diana, G. D., Treasurywala, A. M., Bailey, T. R., Oglesby, R. C., Pevear, D. C. and Dutko, F. J. (1990) A model for compounds active against human rhinovirus-14 based on X-ray crystallography data. *J. med. Chem.* **33:** 1306–1311.

Dimmock, N. J. (1982) Review article initial stages in infection with animal viruses. *J. Gen. Virol.* **59:** 1–22.

Dinter, Z. and Bengston, Z. (1964) Suppression of the inhibitory action of guanidine on virus multiplication by some amino acids. *Virology* **24:** 254–261.

Domingo, E. (1989) RNA virus evolution and the control of viral disease. *Prog. Drug Res.* **33:** 93–133.

Domingo, E. (1992) Genetic variation and quasi-species. *Curr. Opin. Genetic Dev.* **2:** 61–63.

Doms, R. W., Russ, G. and Yewdell, J. W. (1989) Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum. *J. Cell Biol.* **109:** 61–72.

Donaldson, J. G., Finazzi, D. and Klausner, R. D. (1992) Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. *Nature* **360:** 350–352.

Dorsett, P. H., Kerstine, E. E. and Powers, L. J. (1975) Letter: Antiviral activity of gossypol and apogossypol. *J. Pharm. Sci.* **64:** 1073–1075.

Drake, J. W. (1993) Rates of spontaneous mutation among RNA viruses. *Proc. natn. Acad. Sci. U.S.A.* **90:** 4171–4175.

Dröse, S., Bindseil, K. U., Bowman, E. J., Siebers, A., Zeeck, A. and Altendorf, K. (1993) Inhibitory effect of modified bafilomycins and concanamycins on P- and V-type adenosinetriphosphatases. *Biochemistry* **32:** 3902–3906.

Dunnebacke, T. H., Levinthal, J. D. and Williams, R. C. (1969) Entry and release of poliovirus as observed by electron microscopy of cultured cells. *J. Virol.* **4:** 505–513.

Durkin, J. P. and Shier, W. T. (1981) Staphylococcal delta toxin stimulates endogenous phospholipase A2 activity and prostaglandin synthesis in fibroblasts. *Biochim. biophys. Acta* **814:** 319–326.

Egberts, E., Hackett, P. B. and Traub, P. (1977) Alteration of the intracellular energetic and ionic conditions by mengovirus infection of Ehrlich ascites tumor cells and its influence on protein synthesis in the midphase of infection. *J. Virol.* **22:** 591–597.

Eggers, H. J. (1976) Successful treatment of entrovirus-infected mice by 2-(a-hydroxybenzil)-benzimidazole and guanidine. *J. exp. Med.* **143:** 1367–1382.

Eggers, H. J. (1977) Selective inhibition of uncoating of echovirus 12 by rhodanine. A study on early virus–cell interactions. *Virology* **78:** 241–252.

Eggers, H. J. (1982) Benzimidazoles. In: *Chemotherapy of Viral Infections*, pp. 377–417, Came, P. E. and Caliguiri, L. A. (eds). Springer Verlag, Berlin.

Eggers, H. J. (1985) Antiviral agents against picornaviruses. *Antiviral. Res (Suppl. 1)* 57–65.

Eggers, H. J., Bode, B. and Brown, D. T. (1979) Cytoplasmic localization of the uncoating of picornaviruses. *Virology* **92:** 211–218.

Eggers, H. J. and Rosenwirth, B. (1988) Isolation and characterization of an arildone-resistant poliovirus 2 mutant with an altered capsid protein VP1. *Antiviral. Res.* **9:** 23–35.

Eggers, H. J. and Tamm, I. (1962) On the mechanism of selective inhibition of enterovirus multiplication by 2-(alpha-hydroxybenzyl)-benzimidazole. *Virology* **18:** 426–438.

Eggers, H. J. and Tamm, I. (1963a) Inhibition of *Enterovirus* ribonucleic acid synthesis by 2-(alpha-hydroxybenzyl)-benzimidazole. *Nature* **197:** 1327–1328.

Eggers, H. J. and Tamm, I. (1963b) Drug dependence of entroviruses: variants of coxsackie A9 and ECHO 13 viruses that require 2-(a-hydroxybenzyl)-benzimidazole for growth. *Virology* **20:** 62–74.

Eggers, H. J. and Tamm, I. (1965) Coxsackie A9 virus: mutation from drug dependence to drug independence. *Science* **148:** 97–98.

Endo, Y., Mitsui, K., Motizuki, M. and Tsurugi, K. (1987) The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. The site and the characteristics of the modification in 28 S ribosomal RNA caused by the toxins. *J. biol. Chem.* **262:** 5908–5912.

Endo, Y. and Tsurugi, K. (1987) RNA N-glycosidase activity of ricin A-chain. Mechanism of action of the toxic lectin ricin on eukaryotic ribosomes. *J. biol. Chem.* **262:** 8128–8130.

Eppstein, D. A., Marsh, V., Schryver, B. B., Larsen, M. A., Barnett, J. W., Verheyden, J. P. H. and Prisbe, J. (1982) Analogs of $(A2'p)_nA$. Correlation of structure of analogs of $ppp(A2'p)_2A$ and $(A2'p)_2A$ with stability and biological activity. *J. biol. Chem.* **257:** 13390–13397.

Eppstein, D. A., Schryvr, B. B. and Marsh, Y. V. (1986) Stereoconfiguration markedly affects the biochemical and biological properties of phosphorothioate analogs of 2-5A core, $(A2'p5')_2A$. *J. biol. Chem.* **261:** 5999–6003.

Etchison, D. and Ehrenfeld, E. (1981) Comparison of replication complexes synthesizing poliovirus RNA. *Virology* **111:** 33–46.

Etchison, D., Milburn, S. C., Edery, I., Sonenberg, N. and Hershey, J. W. (1982) Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000-dalton polypeptide associated with eucaryotic initiation factor 3 and a cap binding protein complex. *J. biol. Chem.* **257:** 14806–14810.

Everaert, L., Vrijsen, R. and Boeye, A. (1989) Eclipse products of poliovirus after cold-synchronized infection of HeLa cells. *Virology* **171:** 76–82.

Falk, M. M., Grigera, P. R., Bergmann, I. E., Zibert, A., Multhaup, G. and Beck, E. (1990) Foot-and-mouth disease virus protease 3C induces specific proteolytic cleavage of host cell histone H3. *J. Virol.* **64:** 748–756.

Feduchi, E., Esteban, M. and Carrasco, L. (1988) Reovirus type 3 synthesizes proteins in interferon-treated HeLa cells without reversing the antiviral state. *Virology* **164:** 420–426.

Felix, H. (1982) Permeabilized cells. *Anal. Biochem.* **120:** 211–234.

Fenwick, M. L. and Cooper, P. D. (1962) Early interactions between poliovirus and ERK cells. Some observations on the nature and significance of the rejected particles. *Virology* **18:** 212–223.

Fernandez, H., Banks, G. and Smith, R. (1986) Ribavirin: a clinical overview. *Eur. J. Epidemiol.* **2:** 1–14.

Fernandez-Larsson, R. and Patterson, J. L. (1990) Ribavirin is an inhibitor of human immunodeficiency virus reverse transcriptase. *Am. Soc. Pharm. Exp. Therapy* **38:** 766–770.

Fernández-Puentes, C. and Carrasco, L. (1980) Viral infection permeabilizes mammalian cells to protein toxins. *Cell* **20:** 769–775.

Filman, D. J., Syed, R., Chow, M., Macadam, A. J., Minor, P. D. and Hogle, J. M. (1989) Structural factors that control conformational transitions and serotype specificity in type 3 poliovirus. *EMBO J.* **8:** 1567–1579.

FitzGerald, D. J., Padmanabhan, R., Pastan, I. and Willingham, M. C. (1983) Adenovirus-induced release of epidermal growth factor and pseudomonas toxin into the cytosol of KB cells during receptor-mediated endocytosis. *Cell* **32:** 607–617.

Flanegan, J. B. and Baltimore, D. (1977) Poliovirus-specific primer-dependent RNA polymerase able to copy poly(A). *Proc. natn. Acad. Sci. U.S.A.* **74:** 3677–3680.

Flanegan, J. B., Petterson, R. F., Ambros, V., Hewlett, N. J. and Baltimore, D. (1977) Covalent linkage of a protein to a defined nucleotide sequence at the 5′-terminus of virion and replicative intermediate RNAs of poliovirus. *Proc. natn. Acad. Sci. U.S.A.* **74:** 961–965.

Flanegan, J. B. and Van Dyke, T. A. (1979) Isolation of a soluble and template-dependent poliovirus RNA polymerase that copies virion RNA *in vitro*. *J. Virol.* **32:** 155–161.

Fletcher, R. D., Hirschfield, J. E. and Forbes, M. (1965) A common mode of antiviral action for ammonium ions and various amines. *Nature* **207:** 664–665.

Fox, M. P., Bopp, L. H. and Pfau, C. J. (1977) Contact inactivation of RNA and DNA viruses by *N*-methyl isatin beta-thiosemicarbazone and $CuSO_4$. *Ann. N.Y. Acad. Sci.* **284:** 533–543.

Fox, M. P., Otto, M. J. and McKinlay, M. A. (1986) Prevention of rhinovirus and poliovirus uncoating by WIN 51711, a new antiviral drug. *Antimicrob. Agents Chemother.* **30:** 110–116.

Fricks, C. E. and Hogle, J. M. (1990) Cell-induced conformational change in poliovirus: externalization of the amino terminus of VP1 is responsible for liposome binding. *J. Virol.* **64:** 1934–1945.

Friedman, R. M. (1970) Basis for variable response of arboviruses to guanidine treatment. *J. Virol.* **6:** 628–636.

Fujiwara, T., Oda, K., Yokota, S., Takatsuki, A. and Ikehara, Y. (1988) Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. *J. biol. Chem.* **263:** 18545–18552.

Fukuchi, K., Sakagami, H., Ikeda, M., Kawazoe, Y., Ohara, T., Konno, K., Ichikawa, S., Hata, N., Kondo, H. and Nonoyama, M. (1989a) Inhibition of herpes virus infection by pine cone antitumor substances. *Antiviral Res.* **9:** 313–317.

Fukuchi, K., Sakagami, H., Okuda, T., Hatano, T., Tanuma, S., Kitajima, K., Inoue, Y., Inoue, S.,

Ichikawa, S., Nonoyama, M. and Konno, K. (1989b) Inhibition of herpes simplex virus infection by tannins and related compounds. *Antiviral. Res.* **11:** 285–297.

Galabov, A. S. (1979) Thiourea derivatives as specific inhibitors of picorna viruses. *Arzneimittelforschung* **29:** 1863–1868.

Galabov, A. S., Velichkova, E. H. and Vassilev, G. N. (1977) Antiviral activity of *N*-phenyl-*N'*-arylthiourea derivatives against some rhinoviruses. *Chemotherapy* **23:** 81–89.

Garry, R. F. (1989) Alteration of intracellular monovalent cation concentrations by a poliovirus mutant which encodes a defective 2A protease. *Virus Res.* **13:** 129–141.

Garry, R. F., Bishop, J. M., Parker, S., Westbrook, K., Lewis, G. and Waite, M. R. (1979) Na^+ and K^+ concentrations and the regulation of protein synthesis in Sindbis virus-infected chick cells. *Virology* **96:** 108–120.

Gerzon, K., DeLong, D. C. and Cline, J. C. (1971) C-nucleosides: aspects of chemistry and mode of action. *Pure Appl. Chem.* **28:** 489–497.

Gessa, G. L., Loddo, B., Brotz, G., Schivo, M. L., Tagliamonte, A., Spanedda, A., Bo, G. and Ferrari, W. (1966) Selective inhibition of poliovirus growth by D-penicillamine *in vitro*. *Virology* **30:** 618–622.

Giranda, V. L., Heinz, B. A., Oliveira, M. A., Minor, I., Kim, K. H., Kolatkar, P. R., Rossmann, M. G. and Rueckert, R. R. (1992) Acid-induced structural changes in human rhinovirus 14: possible role in uncoating. *Proc. natn. Acad. Sci. U.S.A.* **89:** 10213–10217.

Girard, M. (1969) *In vitro* synthesis of poliovirus ribonucleic acid: role of the replicative intermediate. *J. Virol.* **3:** 376–384.

Girard, M. and Baltimore, D. (1967) The poliovirus replication complex: site for synthesis of poliovirus RNA. *J. molec. Biol.* **24:** 59–74.

González, M. E., Alarcón, B. and Carrasco, L. (1987) Polysaccharides as antiviral agents: antiviral activity of carrageenan. *Antimicrob. Agents Chemother.* **31:** 1388–1393.

González, M. E., Almela, M. J., Yacout, M. and Carrasco, L. (1990a) 6-(3,4-Dichlorophenoxy)-3-(ethylthio)-2-pyridincarbonitrile inhibits poliovirus uncoating. *Antimicrob. Agents Chemother.* **34:** 1259–1261.

Gonzalez, M. E. and Carrasco, L. (1987) Animal viruses promote the entry of polysaccharides with antiviral activity into cells. *Biochem. biophys. Res. Commun.* **146:** 1303–1310.

González, M. E., Martínez-Abarca, F. and Carrasco, L. (1990b) Flavonoids: potent inhibitors of poliovirus RNA synthesis. *Antivir. Chem. Chemother.* **1:** 203–209.

Goodchild, J., Agrawal, S., Civeira, M. P., Sarin, P. S., Sun, D. and Zamecnik, P. C. (1988) Inhibition of human immunodeficiency virus replication by antisense oligodeoxynucleotides. *Proc. natn. Acad. Sci. U.S.A.* **85:** 5507–5511.

Gorbalenya, A. E., Koonin, E. V. and Wolf, Y. I. (1990) A new superfamily of putative NTP-binding domains encoded by genomes of small DNA and RNA viruses. *FEBS Lett.* **262:** 145–148.

Goswami, B. B., Borek, E., Sharma, O. K., Fujitaki, J. and Smith, R. A. (1979) The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. *Biochem. biophys. Res. Commun.* **89:** 830–836.

Goswami, B. B., Gosselin, G., Imbach, J. L. and Sharma, O. K. (1984) Mechanism of inhibition of herpesvirus growth by 2'-5'-linked trimer of 9-b-d-xylofuranosyladenine. *Virology* **137:** 400–407.

Goulet, N. R., Cochran, K. W. and Brown, G. C. (1960) Differential and specific inhibition of ECHO viruses by plant extracts. *Proc. Soc. Exp. Biol. Med.* **103:** 96–100.

Grado, C. and Ohlbaum, A. (1973) The effect of rifampicin, actinomycin D and mitomycin C on poliovirus and foot-and-mouth disease virus replication. *J. gen. Virol.* **21:** 297–303.

Grant, R. A., Filman, D. J., Fujinami, R. S., Icenogle, J. P. and Hogle, J. M. (1992) Three-dimensional structure of Theiler virus. *Proc. natn. Acad. Sci. U.S.A.* **89:** 2061–2065.

Green, G. D. J. and Shaw, E. (1981) Peptidyl diazomethyl ketones are specific inactivators of thiol. *J. biol. Chem.* **256:** 1923–1928.

Green, R. H. (1948) Inhibition of influenza virus by tannic acid. *Proc. Soc. Exp. Biol. Med.* **67:** 483–484.

Green, R. H. (1949) Inhibition of influenza virus by extracts of tea. *Proc. Soc. Exp. Biol. Med.* **71:** 84–85.

Greve, J. M., Davis, G., Meyer, A. M., Forte, C. P., Yost, S. C., Marlor, C. W., Kamarck, M. E. and McClelland, A. (1989) The major human rhinovirus receptor is ICAM-1. *Cell* **56:** 839–847.

Grigera, P. R. and Tisminetzky, S. G. (1984) Histone H3 modification in BHK cells infected with foot-and-mouth disease virus. *Virology* **136:** 10–19.

Gromeier, M. and Wetz, K. (1990) Kinetics of poliovirus uncoating in HeLa cells in a nonacidic environment. *J. Virol.* **64:** 3590–3597.

Gruenberger, M., Pevear, D., Diana, G. D., Kuechler, E. and Blaas, D. (1991) Stabilization of human rhinovirus serotype 2 against pH-induced conformational change by antiviral compounds. *J. gen. Virol.* **72:** 431–433.

Grunberg, E. and Prince, H. N. (1968) The antiviral activity of 3,4-dihydro-1-isoquinolineacetamide hydrochloride *in vitro*, *in ovo*, and in small laboratory animals (33335). *Proc. Soc. Exp. Biol. Med.* **129:** 422–430.

Grunberg, E. and Prince, H. N. (1970) Experimental methodology and the search for effective antiviral agents. *Ann. N.Y. Acad. Sci.* **173:** 122–130.

Guinea, R. and Carrasco, L. (1990) Phospholipid biosynthesis and poliovirus genome replication, two coupled phenomena. *EMBO J.* **9:** 2011–2016.

Guinea, R. and Carrasco, L. (1991) Effects of fatty acids on lipid synthesis and viral RNA replication in poliovirus-infected cells. *Virology* **185:** 473–476.

Guinea, R., López-Rivas, A. and Carrasco, L. (1989) Modification of phospholipase C and phospholipase A2 activities during poliovirus infection. *J. biol. Chem.* **264:** 21923–21927.

Gurgo, C., Bridges, S. and Green, M. (1982) Rifamycins. In: *Chemotherapy of Viral Infections*, pp. 519–555, Came, P. E. and Caliguire, L. A. (eds). Springer-Verlag, Berlin.

Gutowski, G. E., Sweeney, M. J., DeLong, D. C., Hamill, R. L., Gerzon, K. and Dyke, R. W. (1975) Biochemistry and biological effects of the pyrazofurins (pyrazomycins): initial clinical trial. *Ann. N.Y. Acad. Sci.* **255:** 544–551.

Guttman, N. and Baltimore, D. (1977) A plasma membrane component able to bind and alter virions of poliovirus type 1: studies on cell-free alteration using a simplified assay. *Virology* **82:** 25–36.

Hahn, F. E. (1979) Virazole (Ribavirin). In: *Antibiotics*, Vol. V/Part 2, *Mechanism of Action of Antieukaryotic and Antiviral Compounds*, pp. 439–458, Hahn, F. E. (ed.). Springer-Verlag, Berlin.

Hambidge, S. J. and Sarnow, P. (1992) Translational enhancement of the poliovirus 5′ noncoding region mediated by virus-encoded polypeptide 2A. *Proc. natn. Acad. Sci. U.S.A.* **89:** 10272–10276.

Hanecak, R., Semler, B. L., Anderson, C. W. and Wimmer, E. (1982) Proteolytic processing of poliovirus polypeptides: antibodies to polypeptide P3-7c inhibit cleavage at glutamine-glycine pairs. *Proc. natn. Acad. Sci. U.S.A.* **79:** 3973–3977.

Harber, J. and Wimmer, E. (1993) Aspects of the molecular biology of poliovirus replication. In: *Regulation of Gene Expression in Animal Viruses*, pp. 189–224, Carrasco, L., Sonenberg, N. and Wimmer, E. (eds). Plenum Press, London, New York.

Harborne, J. B. (1988) *The Flavonoids: Advances in Research Since 1980.* Chapman and Hall, New York.

Harnden, M. R., Bailey, S., Boyd, M. R., Wilkinson, J. B. and Wright, N. D. (1979) Easily hydrolyzable, water-soluble derivatives of (+ / −)-alpha-5-[1-(indol-3-yl)ethyl]-2-methylamino-delta-2-thiazoline-4-one, a novel antiviral compound. *J. med. Chem.* **22:** 191–195.

Hartley, M. R., Legname, G., Osborn, R., Chen, Z. and Lord, J. M. (1991) Single-chain ribosome inactivating proteins from plants depurinate *Escherichia coli* 23S ribosomal RNA. *FEBS Lett.* **290:** 65–68.

Haugh, M. C., Cayley, P. J., Serafinowska, H. T., Norman, D. G., Reese, C. B. and Kerr, I. M. (1983) Analogues and analogue inhibitors of ppp(A2′p)$_n$A. Their stability and biological activity. *Eur. J. Biochem.* **131:** 77–84.

Hayden, F. G. (1985) Clinical applications of antiviral agents for chemoprophylaxis and therapy of respiratory viral infections. *Antiviral. Res. (Suppl. 1)* 229–239.

Hayden, F. G. and Gwaltney, J. M., Jr (1982) Prophylactic activity of intranasal enviroxime against experimentally induced rhinovirus type 39 infection. *Antimicrob. Agents Chemother.* **21:** 892–897.

Hayden, F. G., Andries, K. and Janssen, P. A. J. (1992) Safety and efficacy of intranasal pirodavir (R 77975) in experimental rhinovirus infection. *Antimicrob. Agents Chemother.* **36:** 727–732.

Hayden, F. G., Gwaltney, J. M. and Colonno, R. J. (1988) Modification of experimental rhinovirus infection by receptor blockade. *Antiviral. Res.* **9:** 233–247.

Hellen, C. U., Krausslich, H. G. and Wimmer, E. (1989) Proteolytic processing of polyproteins in the replication of RNA viruses. *Biochemistry* **28:** 9881–9890.

Hellen, C. U. T. and Wimmer, E. (1992a) Viral proteases as targets for chemotherapeutic intervention. *Curr. Opin. Biotechnol.* **3:** 643–649.

Hellen, C. U. T. and Wimmer, E. (1992b) Maturation of poliovirus capsid proteins. *Virology* **187:** 391–397.

Helms, J. B. and Rothman, J. E. (1992) Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. *Nature* **360:** 352–354.

Hewlett, M. J., Rozenblatt, S., Ambros, V. and Baltimore, D. (1977) Separation and quantitation of intracellular forms of poliovirus RNA by agarose gel electrophoresis. *Biochemistry* **16:** 2763–2767.

Hiebsch, R. R., Raub, T. J. and Wattenberg, B. W. (1991) Primaquine blocks transport by inhibiting the formation of functional transport vesicles. Studies in a cell-free assay of protein transport through the Golgi apparatus. *J. biol. Chem.* **266:** 20323–20328.

Hoekstra, D. and Kok, J. W. (1989) Entry mechanisms of enveloped viruses. Implications for fusion of intracellular membranes. *Biosci. Rep.* **9:** 273–305.

Hogle, J. M., Chow, M. and Filman, D. J. (1985) Three-dimensional structure of poliovirus at 2.9 Å resolution. *Science* **229:** 1358–1365.

Holland, J. J., de la Torre, J. C. and Steinhauer, D. A. (1992) RNA virus populations as quasispecies. *Curr. Top. Microbiol. Immunol.* **176:** 1–20.

Holsey, C., Cragoe, E. J. J. and Nair, C. N. (1990) Evidence for poliovirus-induced cytoplasmic alkalinization in HeLa cells. *J. Cell Physiol.* **142:** 586–591.

Hoover-Litty, H. and Greve, J. M. (1993) Formation of rhinovirus-soluble ICAM-1 complexes and conformational changes in the virion. *J. Virol.* **67:** 390–397.

Horwitz, S. B., Chang, C. K. and Grollman, A. P. (1972) Antiviral action of camptothecin. *Antimicrob. Agents Chemother.* **2:** 395–401.

Huggins, J. W., Robins, R. K. and Canonico, P. G. (1984) Synergistic antiviral effects of ribavirin and the

C-nucleoside analogs tiazofurin and selenazofurin against togaviruses, bunyaviruses, and arenaviruses. *Antimicrob. Agents Chemother.* **26:** 476–480.

Hunziker, W., Whitney, J. A. and Mellman, I. (1992) Brefeldin A and the endocytic pathway: possible implications for membrane traffic and sorting. *FEBS Lett.* **307:** 93–96.

Iizuka, N., Kuge, S. and Nomoto, A. (1987) Complete nucleotide sequence of the genome of coxsackievirus B1. *Virology* **156:** 64–73.

Ikuta, K. and Luftig, R. B. (1986) Inhibition of cleavage of Moloney leukemia virus gag and env coded precursor polyproteins by cerulenin. *Virology* **154:** 195–206.

Imai, J., Johnston, I. and Torrence, P. (1982) Chemical modification potentiates the biological activities of 2-5A and its congeners. *J. biol. Chem.* **257:** 12739–12745.

Irurzun, A., Perez, L. and Carrasco, L. (1992) Involvement of membrane traffic in the replication of poliovirus genomes: effects of Brefeldin A. *Virology* **191:** 166–175.

Irurzun, A., Perez, L. and Carrasco, L. (1993) Enhancement of phospholipase C activity during poliovirus infection. *J. gen. Virol.* **74:** 1063–1071.

Irvin, J. D. and Uckun, F. M. (1992) Pokeweed antiviral protein: ribosome inactivation and therapeutic applications. *Pharmac. Ther.* **55:** 279–302.

Ishitsuka, H., Ninomiya, Y. T., Ohsawa, C., Fujiu, M. and Suhara, Y. (1982a) Direct and specific inactivation of rhinovirus by chalcone Ro 09-0410. *Antimicrob. Agents Chemother.* **22:** 617–621.

Ishitsuka, H., Ninomiya, Y. and Suhara, Y. (1986) Molecular basis of drug resistance to new antirhinovirus agents. *J. Antimicrob. Chemother. (Suppl. B)* **18:** 11–18.

Ishitsuka, H., Ohsawa, C., Ohiwa, T., Umeda, I. and Suhara, Y. (1982b) Antipicornavirus flavone Ro 09-0179. *Antimicrob. Agents Chemother.* **22:** 611–616.

Jackson, R. J., Howell, M. T. and Kaminski, A. (1990) The novel mechanism of initiation of picornavirus RNA translation. *Trends Biochem. Sci.* **15:** 477–483.

Jacobsen, H. J., Epstein, D. A., Friedmann, R. M., Safer, B. and Torrence, P. F. (1983) Double-stranded RNA-dependent phosphorylation of protein P1 and eukaryotic initiation factor 2a does not correlate with protein synthesis inhibition in a cell-free system from interferon-treated mouse L cells. *Proc. natn. Acad. Sci. U.S.A.* **80:** 41–45.

Jacobson, M. F., Asso, J. and Baltimore, D. (1970) Further evidence on the formation of poliovirus proteins. *J. molec. Biol.* **49:** 657–669.

Jacobson, M. F. and Baltimore, D. (1968) Polypeptide cleavages in the formation of poliovirus proteins. *Proc. natn. Acad. Sci. U.S.A.* **61:** 77–84.

Jacobson, S. J., Konings, D. A. M. and Sarnow, P. (1993) Biochemical and genetic evidence for a pseudoknot structure at the 3t terminus of the poliovirus RNA genome and its role in viral RNA amplification. *J. Virol.* **67:** 2961–2971.

James, W. (1991) Towards gene-inhibition therapy: a review of progress and prospects in the field of antiviral antisense nucleic acids and ribozymes. *Antivir. Chem. Chemother.* **2:** 191–214.

Jang, S. K., Krausslich, H. G., Nicklin, M. J., Duke, G. M., Palmenberg, A. C. and Wimmer, E. (1988) A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during *in vitro* translation. *J. Virol.* **62:** 2636–2643.

Jentsch, K. D., Hunsmann, G., Hartmann, H. and Nickel, P. (1987) Inhibition of human immunodeficiency virus type I reverse transcriptase by suramin-related compounds. *J. gen. Virol.* **68:** 2183–2192.

Jimenez, A. and Vazquez, D. (1985) Plant and fungal protein and glycoprotein toxins inhibiting eukaryote protein synthesis. *Annu. Rev. Microbiol.* **39:** 649–672.

Joachims, M. and Etchison, D. (1992) Poliovirus infection results in structural alteration of a microtubule-associated protein. *J. Virol.* **66:** 5797–5804.

Johnson, K. and Sarnow, P. (1991) Three poliovirus 2B mutants exhibit noncomplementable defects in viral RNA amplification and display dosage-dependent dominance over wild-type poliovirus. *J. Virol.* **65:** 4341–4349.

Jubelt, B., Wilson, A. K., Ropka, S. L., Guidinger, P. L. and McKinlay, M. A. (1989) Clearance of a persistent human enterovirus infection of the mouse central nervous system by the antiviral agent disoxaril. *J. Infect. Dis.* **159:** 866–871.

Kaljot, K. T., Shaw, R. D., Rubin, D. H. and Greenberg, H. B. (1988) Infectious rotavirus enters cells by direct cell membrane penetration, not by endocytosis. *J. Virol.* **62:** 1136–1144.

Kalko, S. G., Cachau, R. E. and Silva, A. M. (1992) Ion channels in icosahedral virus: a comparative analysis of the structures and binding sites at their fivefold axes. *Biophys. J.* **63:** 879–1182.

Kaminski, A., Howell, M. and Jackson, R. (1990) Initiation of encephalomyocarditis virus RNA translation: the authentic initiation site is not selected by a scanning mechanism. *EMBO J.* **9:** 3753–3759.

Kaplan, G., Freistadt, M. S. and Racaniello, V. R. (1990a) Neutralization of poliovirus by cell receptors expressed in insect cells. *J. Virol.* **64:** 4697–4702.

Kaplan, G., Peters, D. and Racaniello, V. R. (1990b) Poliovirus mutants resistant to neutralization with soluble cell receptors. *Science* **250:** 1596–1599.

Katz, E. and Feliz, H. (1977) Synthesis of vaccinia viral antigens in HeLa cells in the presence of isatin beta-thiosemicarbazone. *Antimicrob. Agents Chemother.* **11:** 202–208.

Kelley, J. L., Linn, J. A. and Selway, J. W. (1990) Antirhinovirus activity of 6-anilino-9-benzyl-2-chloro-9H-purines. *J. med. Chem.* **33:** 1360–1363.

Kenny, M. T., Dulworth, J. K., Bargar, T. M., Torney, H. L., Graham, M. C. and Manelli, A. M. (1986) *In vitro* antiviral activity of the 6-substituted 2-(3′,4′-dichlorophenoxy)-2*H*-pyrano[2,3-b] pyridines MDL 20,610, MDL 20,646, and MDL 20,957. *Antimicrob. Agents Chemother.* **30:** 516–518.

Kenny, M. T., Dulworth, J. K. and Torney, H. L. (1985) *In vitro* and *in vivo* antipicornavirus activity of some phenoxypyridinecarbonitriles. *Antimicrob. Agents Chemother.* **28:** 745–750.

Kenny, M. T., Torney, H. L. and Dulworth, J. K. (1988) Mechanism of action of the antiviral compound MDL 20,610. *Antiv. Res.* **9:** 249–261.

Kerr, I. M., Cayley, P. J., Silverman, R. H. and Knight, M. (1982) The antiviral action of interferon. *Philos. Trans. R. Soc. London Biol.* **299:** 59–67.

Kerr, I. M. and Martin, E. M. (1971) Virus protein synthesis in animal cell-free systems: nature of the products synthesized in response to ribonucleic acid of encephalomyocarditis virus. *J. Virol.* **7:** 438–447.

Kerr, I. M. and Stark, G. R. (1992) The antiviral effects of the interferons and their inhibition. *J. Interferon Res.* **12:** 237–240.

Kettner, C. A. and Korant, B. D. (1987) Method for preparing specific inhibitors of virus-specific proteases. US Patent 4,644,055.

Kim, K. H., Willingmann, P., Gong, Z. X., Kremer, M. J., Chapman, M. S., Minor, I., Oliveira, M. A., Rossmann, M. G., Andries, K., Diana, G. D., Dutko, F. J., McKinlay, M. A. and Pevear, D. C. (1993) A comparison of the anti-rhinoviral drug binding pocket in HRV14 and HRV1A. *J. molec. Biol.* **230:** 206–227.

Kim, K. S., Sapienza, V. J. and Carp, R.I. (1980) Antiviral activity of arildone on deoxyribonucleic acid and ribonucleic acid viruses. *Antimicrob. Agents Chemother.* **18:** 276–280.

Kim, S., Smith, T. J., Chapman, M. S., Rossmann, M. G., Pevear, D. C., Dutko, F. J., Felock, P. J., Diana, G. D. and McKinlay, M. A. (1989) Crystal structure of human rhinovirus serotype 1A (HRV1A). *J. molec. Biol.* **210:** 91–111.

Kirkegaard, K. (1992) Genetic analysis of picornavirus. *Current Opinion Genetics Develop.* **2:** 64–70.

Kirsi, J. J., McKernan, P. A., Burns, N. J., North, J. A., Murray, B. K. and Robins, R. K. (1984) Broad-spectrum synergistic antiviral activity of selenazofurin and ribavirin. *Antimicrob. Agents Chemother.* **26:** 466–475.

Kitamura, N., Semler, B. L., Rothberg, P. G., Larsen, G. R., Adler, C. J., Dorner, A. J., Emini, E. A., Hanecak, R., Lee, J. J., van-der-Werf, S., Anderson, C. W. and Wimmer, E. (1981) Primary structure, gene organization and polypeptide expression of poliovirus RNA. *Nature* **291:** 547–553.

Klausner, R. D., Donaldson, J. G. and Lippincott-Schwartz, J. (1992) Brefeldin A: insights into the control of membrane traffic and organelle structure. *J. Cell Biol.* **116:** 1071–1080.

Kleina, L. G. and Grubman, M. J. (1992) Antiviral effects of a thiol protease inhibitor on foot-and-mouth disease virus. *J. Virol.* **66:** 7168–7175.

Knowalchuk, J. and Speirs, J. I. (1978) Antiviral effect of apple beverages. *Appl. Environ Microbiol.* **36:** 798–801.

Koch, F. and Koch, G. (1985) *The Molecular Biology of Poliovirus.* Springer Verlag, Vienna.

Kohn, A., Gitelman, J. and Inbar, M. (1980) Unsaturated free fatty acids inactivate animal enveloped viruses. *Arch. Virol.* **66:** 301–307.

Koike, S., Horie, H., Ise, I., Okitsu, A., Yoshida, M., Iizuka, N., Takeuchi, K., Takegami, T. and Nomoto, A. (1990) The poliovirus receptor protein is produced both as membrane-bound and secreted forms. *EMBO J.* **9:** 3217–3224.

Koike, S., Ise, I. and Nomoto, A. (1991a) Functional domains of the poliovirus receptor. *Proc. natn. Acad. Sci. U.S.A.* **88:** 4104–4108.

Koike, S., Ise, I., Sato, Y., Yonekawa, H., Gotoh, O. and Nomoto, A. (1992) A second gene for the African green monkey poliovirus receptor that has no putative *N*-glycosylation site in the functional N-terminal immunoglobulin-like domain. *J. Virol.* **66:** 7059–7066.

Koike, S., Taya, C., Kurata, T., Abe, S., Ise, I., Yonekawa, H. and Nomoto, A. (1991b) Transgenic mice susceptible to poliovirus. *Proc. natn. Acad. Sci. U.S.A.* **88:** 951–955.

Komatsu, K., Inazuki, J., Hosoya, J. and Satoh, S. (1986) Beneficial effect of new thiol protease inhibitors, epoxide derivatives, on dystrophic mice. *Exp. Neurol.* **91:** 23–29.

Konowalchuk, J. and Speirs, J. I. (1976) Virus inactivation by grapes and wines. *Appl. Environ. Microbiol.* **32:** 757–763.

Korant, B., Chow, N., Lively, M. and Powers, J. (1979) Virus-specified protease in poliovirus-infected HeLa cells. *Proc. natn. Acad. Sci. U.S.A.* **76:** 2992–2995.

Korant, B. D. (1972) Cleavage of viral precursor proteins *in vivo* and *in vitro*. *J. Virol.* **10:** 751–759.

Korant, B. D. (1987) Viral protein cleavages: target for viral therapy. In: *Umezawa Memorial Symposium*, pp. 138–149, Aoyagi, T. (ed.). Japan Science Press, Tokyo.

Korant, B. D. (1990) Strategies to inhibit viral polyprotein cleavages. *Ann. N.Y. Acad. Sci.* **616:** 252–257.

Korant, B. D. (1991) Viral protein cleavages as a drug design target. *Acta Biol. Hung.* **43:** 203–211.

Korant, B. D., Brzin, J. and Turk, V. (1985) Cystatin, a protein inhibitor of cysteine proteases alters viral protein cleavages in infected human cells. *Biochem. biophys. Res. Commun.* **127:** 1072–1076.

Korant, B. D., Kauer, J. C. and Butterworth, B. E. (1974) Zinc ions inhibit replication of rhinoviruses. *Nature* **248:** 588–590.

Korant, B. D., Towatari, T., Ivanoff, L., Petteway, S., Jr, Brzin, J., Lenarcic, B. and Turk, V. (1986) Viral therapy: prospects for protease inhibitors. *J. Cell Biochem.* **32:** 91–95.

Kozak, M. (1989) The scanning model for translation: an update. *J. Cell Biol.* **108:** 229–241.
Kozak, M. (1992) Regulation of translation in eukaryotic systems. *Annu. Rev. Cell Biol.* **8:** 197–225.
Krausslich, H. G. and Wimmer, E. (1988) Viral proteinases. *Annu. Rev. Biochem.* **57:** 701–754.
Krishnaswamy, S. and Rossmann, M. G. (1990) Structural refinement and analysis of Mengo virus. *J. molec. Biol.* **211:** 803–844.
Kronenberger, P., Vrijsen, R. and Boeyé, A. (1991) Chloroquine induces empty capsid formation during poliovirus eclipse. *J. Virol.* **65:** 7008–7011.
Kronenberger, P., Vrijsen, R. and Boeyé, A. (1992) Compartmentalization of subviral particles during poliovirus eclipse in HeLa cells. *J. gen. Virol.* **73:** 1739–1744.
Kucera, L. S. and Herrmann, E. C. (1967) Antiviral substances in plants of the mint family (*Labiatae*). I. Tannin of *Melissa officinalis*. II. Non-tannin polyphenol of *Melissa officinalis*. *Proc. Soc. Exp. Biol. Med.* **124:** 865–878.
Kuhrt, M. F., Fancher, M. J., Jasty, V., Pancic, F. and Came, P. E. (1979) Preliminary studies of the mode of action of arildone, a novel antiviral agent. *Antimicrob. Agents Chemother.* **15:** 813–819.
Kumar, R., Choubey, K., Lengyel, P. and Sen, G. C. (1988) Studies on the role of the 2′-5′-oligoadenylate synthetase-RNase L pathway in beta interferon-mediated inhibition of encephalomyocarditis virus replication. *J. Virol.* **62:** 3175–3181.
Kuwata, T., Fuse, A. and Morinaga, N. (1977) Effects of ouabain on the anticellular and antiviral activities of human and mouse interferon. *J. gen. Virol.* **34:** 537–540.
Lacal, J. C. and Carrasco, L. (1982) Relationship between membrane integrity and the inhibition of host translation in virus-infected mammalian cells. Comparative studies between encephalomyocarditis virus and poliovirus. *Eur. J. Biochem.* **127:** 359–366.
Lacal, J. C. and Carrasco, L. (1983a) Modification of membrane permeability in poliovirus-infected HeLa cells: effect of guanidine. *J. gen. Virol.* **64:** 787–793.
Lacal, J. C. and Carrasco, L. (1983b) Antiviral effects of hygromycin B, a translation inhibitor nonpermeant to uninfected cells. *Antimicrob. Agents Chemother.* **24:** 273–275.
Lacal, J. C., Vázquez, D., Fernández-Sousa, J. M. and Carrasco, L. (1980) Antibiotics that specifically block translation in virus-infected cells. *J. Antibiot. Tokyo* **33:** 441–446.
La Colla, P., Marcialis, M. A., Mereu, G. P. and Loddo, B. (1972) Specific inhibition of poliovirus induced blockade of cell protein synthesis by a thiopyrimidine derivative. *J. gen. Virol.* **17:** 13–18.
Lain, S., Riechmann, J. L., Martin, M. T. and Garcia, J. A. (1989) Homologous potyvirus and flavivirus proteins belonging to a superfamily of helicase-like proteins. *Gene* **82:** 357–362.
Lama, J. and Carrasco, L. (1992a) Inducible expression of a toxic poliovirus membrane protein in *Escherichia coli*. Comparative studies using different expression systems based on T7 promoters. *Biochem. biophys. Res. Commun.* **188:** 972–981.
Lama, J. and Carrasco, L. (1992b) Expression of poliovirus nonstructural proteins in *Escherichia coli* cells. Modification of membrane permeability induced by 2B and 3A. *J. biol. Chem.* **267:** 15932–15937.
Lama, J., Guinea, R., Martinez-Abarca, F. and Carrasco, L. (1992) Cloning and inducible synthesis of poliovirus nonstructural proteins. *Gene* **117:** 185–192.
Lanker, S., Müller, P. P., Altmann, M., Goyer, C., Sonenberg, N. and Trachsel, H. (1992) Interactions of the eIF-4F subunits in the yeast *Saccharomyces cerevisiae*. *J. biol. Chem.* **267:** 21167–21171.
Larin, N. M., Copping, M. P., Herbst-Laier, R. H., Roberts, B. and Wenham, R. B. M. (1965) Antiviral activity of gliotoxin. *Chemoterapia* **10:** 12–23.
Laurence, L., Marti, J., Roux, D. and Cailla, H. (1984) Immunological evidence for the *in vivo* occurrence of (2′-5′)adenylyladenosine oligonucleotides in eukaryotes and prokaryotes. *Proc. natn. Acad. Sci. U.S.A.* **81:** 2322–2326.
Lawson, M. A. and Semler, B. L. (1990) Picornavirus protein processing—enzymes, substrates, and genetic regulation. *Curr. Top. Microbiol. Immunol.* **161:** 49–89.
Lee, S. B. and Esteban, M. (1993) The interferon-induced double-stranded RNA-activated human p68 protein kinase inhibits the replication of vaccinia virus. *Virology* **193:** 1037–1041.
Lee, T., Crowell, M., Shearer, M. H., Aron, G. M. and Irvin, J. D. (1990) Poliovirus-mediated entry of pokeweed antiviral protein. *Antimicrob. Agents Chemother.* **34:** 2034–2037.
Lee, Y. F., Nomoto, A., Detjen, B. M. and Wimmer, E. (1977) A protein covalently linked to poliovirus genome RNA. *Proc. natn. Acad. Sci. U.S.A.* **74:** 59–63.
Leiter, J. M., Agrawal, S., Palese, P. and Zamecnik, P. C. (1990) Inhibition of influenza virus replication by phosphorothioate oligodeoxynucleotides. *Proc. natn. Acad. Sci. U.S.A.* **87:** 3430–3434.
Lejbkowicz, F., Goyer, C., Darveau, A., Neron, S., Lemieux, R. and Sonenberg, N. (1992) A fraction of the mRNA 5′ cap-binding protein, eukaryotic initiation factor 4E, localizes to the nucleus. *Proc. natn. Acad. Sci. U.S.A.* **89:** 9612–9616.
Lentz, T. L. (1990) The recognition event between virus and host cell receptor: a target for antiviral agents. *J. gen. Virol.* **71:** 751–766.
Leonetti, J. P., Machy, P., Degols, G., Lebleu, B. and Leserman, L. (1990) Antibody-targeted liposomes containing oligodeoxyribonucleotides complementary to viral RNA selectively inhibit viral replication. *Proc. natn. Acad. Sci. U.S.A.* **87:** 2448–2451.
Levandowski, R. A., Pachucki, C. T., Rubenis, M. and Jackson, G. G. (1982) Topical enviroxime against rhinovirus infection. *Antimicrob. Agents Chemother.* **22:** 1004–1007.

Levinson, W. (1975) Inhibition of viruses, tumors, and pathogenic microorganisms by isatin beta-thiosemicarbazone and other thiosemicarbazones. In: *Selective Inhibitors of Viral Functions*, pp. 213–226, Carter, W. A. (ed.). CRC Press Inc., Boca Raton, FL.

Levintow, L., Thorén, M. M., Darnell, J. E. and Hooper, J. L. (1962) Effect of *p*-fluorophenylalanine and puromycin of the replication of poliovirus. *Virology* **16:** 220–229.

Lewis, J. A. (1988) Induction of an antiviral state by interferon in the absence of elevated levels of 2,5-oligo(A) synthetase and eIF-2 kinase. *Virology* **162:** 118–127.

Li, C. P. (1960) Antimicrobial effect of abalone juice. *Proc. Soc. Exp. Biol. Med.* **103:** 522–524.

Li, J. P. and Baltimore, D. (1988) Isolation of poliovirus 2C mutants defective in viral RNA synthesis. *J. Virol.* **62:** 4016–4021.

Li, J. P. and Baltimore, D. (1990) An intragenic revertant of a poliovirus 2C mutant has an uncoating defect. *J. Virol.* **64:** 1102–1107.

Lippincott-Schwartz, J. (1993) Bidirectional membrane traffic between the endoplasmic reticulum and Golgi apparatus. *Trends Cell Biol.* **3:** 81–88.

Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S. and Klausner, R. D. (1989) Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* **56:** 801–813.

Lippincott-Schwartz, J., Yuan, L., Tipper, C., Amherdt, M., Orci, L. and Klausner, R. D. (1991) Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. *Cell* **67:** 601–616.

Lloyd, R. E. and Bovee, M. (1993) Persistent infection of human erythroblastoid cells by poliovirus. *Virology* **194:** 200–209.

Lloyd, R. E., Grubman, M. J. and Ehrenfeld, E. (1988) Relationship of p220 cleavage during picornavirus infection to 2A proteinase sequencing. *J. Virol.* **62:** 4216–4223.

Loddo, B., Gessa, G. L., Schivo, M. L., Spanedda, A., Brotzu, G. and Ferrari, W. (1966) Antagonism of the guanidine interference with poliovirus replication by simple methylated and ethylated compounds. *Virology* **28:** 707–712.

Loddo, B., Mutoni, S., Spanedda, A., Brotzu, G. and Ferrari, W. (1963) Guanidine conditional infectivity of ribonucleic acid extracted from a strain of guanidine-dependent polio-1 virus. *Nature* **197:** 315.

Lonberg Holm, K., Gosser, L. B. and Kauer, J. C. (1975) Early alteration of poliovirus in infected cells and its specific inhibition. *J. gen. Virol.* **27:** 329–342.

Lopez Guerrero, J. A., Martinez Abarca, F., Fresno, M., Carrasco, L. and Alonso, M. A. (1991) Cell type determines the relative proportions of (−) and (+) strand RNA during poliovirus replication. *Virus. Res.* **20:** 23–29.

Lopez Pila, J. M., Kopecka, H. and Vanden Berghe, D. (1989) Lack of evidence for strand-specific inhibition of poliovirus RNA synthesis by 3-methylquercetin. *Antiviral Res.* **11:** 47–53.

López-Rivas, A., Castrillo, J. L. and Carrasco, L. (1987) Cation content in poliovirus-infected HeLa cells. *J. gen. Virol.* **68:** 335–342.

Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold, E., Rossmann, M. G., Boege, U., Scraba, D. G., Duke, G. M. and Palmenberg, A. C. (1987) The atomic structure of Mengo virus at 3.0 Å resolution. *Science* **235:** 182–191.

Lwoff, A. (1965) The specific effectors of viral development. *Biochem. J.* **96:** 289–302.

Machamer, C. E., Mentone, S. A., Rose, J. K. and Farquhar, M. G. (1992) The E1 glycoprotein of an avian coronavirus is targeted to the *cis* Golgi complex. *Proc. natn. Acad. Sci. U.S.A.* **87:** 6944–6948.

Madshus, I. H., Olsnes, S. and Sandvig, K. (1984a) Different pH requirements for entry of the two picornaviruses, human rhinovirus 2 and murine encephalomyocarditis virus. *Virology* **139:** 346–357.

Madshus, I. H., Olsnes, S. and Sandvig, K. (1984b) Requirements for entry of poliovirus RNA into cells at low pH. *EMBO J.* **3:** 1945–1950.

Madshus, I. H., Olsnes, S. and Sandvig, K. (1984c) Mechanism of entry into the cytosol of poliovirus type 1: requirement for low pH. *J. Cell Biol.* **98:** 1194–1200.

Marlin, S. D., Staunton, D. E., Springer, T. A., Stratowa, C., Sommergruber, W. and Merluzzi, V. J. (1990) A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature* **344:** 70–72.

Marsh, M. and Helenius, A. (1989) Virus entry into animal cells. *Adv. Virus. Res.* **36:** 107–151.

Marshall, W. S. and Caruthers, M. H. (1993) Phosphorodithioate DNA as a potential therapeutic drug. *Science* **259:** 1564–1570.

Martin, S., Casasnovas, J. M., Staunton, D. E. and Springer, T. A. (1993) Efficient neutralization and disruption of rhinovirus by chimeric ICAM-1/immunoglobulin molecules. *J. Virol.* **67:** 3561–3568.

Mason, P. W., Baxt, B., Brown, F., Harber, J., Murdin, A. and Wimmer, E. (1993) Antibody-complexed foot-and-mouth disease virus, but not poliovirus, can infect normally insusceptible cells via the Fc receptor. *Virology* **192:** 568–577.

Matsumoto, S., Stanfield, F. J., Goore, M. Y. and Haff, R. F. (1972) The antiviral activity of a triazinoindole (SK&F 30097) (36163). *Proc. Soc. Exp. Biol. Med.* **139:** 455–460.

Matthews, T. J., Lawrence, M. K., Nair, C. D. G. and Tyrell, D. A. J. (1976) Antiviral activity in milk of possible clinical importance. *Lancet* **ii:** 1387–1389.

Maynell, L. A., Kirkegaard, K. and Klymkowsky, M. W. (1992) Inhibition of poliovirus RNA synthesis by brefeldin A. *J. Virol.* **66:** 1985–1994.

McClure, M. O., Whitby, D., Patience, C., Gooderham, N. J., Bradshaw, A., Cheingsong-Popov, R., Weber, J. N., Davies, D. S., Cook, G. M. W., Keynes, R. J. and Weiss, R. A. (1991) Dextrin sulphate and fucoidan are potent inhibitors of HIV infection *in vitro*. *Antivir. Chem. Chemother.* **2:** 149–156.

McDougall, J. K. (1969) Antiviral action of gliotoxin. *Arch. Gesamte. Virusforsch.* **27:** 255–267.

McKinlay, M. A. (1985) WIN 51711, a new systematically active broad-spectrum antipicornavirus agent. *J. Antimicrob. Chemother.* **16:** 284–286.

McKinlay, M. A., Pevear, D. C. and Rossmann, M. G. (1992) Treatment of the picornavirus common cold by inhibitors of viral uncoating and attachment. *Annu. Rev. Microbiol.* **46:** 635–654.

McKinlay, M. A. and Rossmann, M. G. (1989) Rational design of antiviral agents. *Annu. Rev. Pharmac. Toxicol.* **29:** 111–122.

McKinlay, M. A. and Steinberg, B. A. (1986) Oral efficacy of WIN 51711 in mice infected with human poliovirus. *Antimicrob. Agents Chemother.* **29:** 30–32.

McNair, A. N. B. and Kerr, I. M. (1992) Viral inhibition of the interferon system. *Pharmac. Ther.* **56:** 79–95.

McSharry, J. J., Caliguiri, L. A. and Eggers, H. J. (1979) Inhibition of uncoating of poliovirus by arildone, a new antiviral drug. *Virology* **97:** 307–315.

McSharry, J. J. and Pancic, F. (1982) Arildone: a b-diketone. In: *Chemotherapy of Viral Infections*, pp. 419–444, Came, P. E. and Caliguiri, L. A. (eds). Springer Verlag, Berlin.

Meerovitch, K., Nicholson, R. and Sonenberg, N. (1991) *In vitro* mutational analysis of *cis*-acting RNA translational elements within the poliovirus type 2 5′ untranslated region. *J. Virol.* **65:** 5895–5901.

Meerovitch, K. and Sonenberg, N. (1993) Studies on the mechanism of internal initiation of translation on poliovirus RNA. In: *Regulation of Gene Expression in Animal Viruses*, pp. 245–254, Carrasco, L., Sonenberg, N. and Wimmer, E. (eds). Plenum Press, London, New York.

Meerovitch, K., Svitkin, Y. V., Lee, H. S., Lejbkowicz, F., Kenan, D. J., Chan, E. K. L., Agol, V. I., Keene, J. D. and Sonenberg, N. (1993) La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J. Virol.* **67:** 3798–3807.

Mehdi, S. (1991) Cell-penetrating inhibitors of calpain. *TIBS* **16:** 150–153.

Mendelsohn, C. L., Wimmer, E. and Racaniello, V. R. (1989) Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* **56:** 855–865.

Merryman, P., Jaffe, I. A. and Ehrenfeld, E. (1974) Effect of D-penicillamine on poliovirus replication in HeLa cells. *J. Virol.* **13:** 881–887.

Meurs, E. F., Watanabe, Y., Kadereit, S., Barber, G. N., Katze, M. G., Chong, K., Williams, B. R. G. and Hovanessian, A. G. (1992) Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukaryotic initiation factor 2 and partial resistance to encephalomyocarditis virus growth. *J. Virol.* **66:** 5805–5814.

Meyer, W. J., Gidwitz, S., Ayers, V. K., Schoepp, R. J. and Johnston, R. E. (1992) Conformational alteration of Sandbis virion glycoproteins induced by heat, reducing agents, or low pH. *J. Virol.* **66:** 3504–3513.

Michel, K. H., Chaney, M. O., Jones, N. D., Hoehn, M. M. and Nagarajan, R. (1974) Epipolythiopiperazine-dione antibiotics from *Penicillium turbatum*. *J. Antibiot. Tokyo* **27:** 57–64.

Miller, F. A., Rightsel, W. A., Sloan, B. J., Ehrlich, J., French, J. C. and Bartz, Q. R. (1963) Antiviral activity of tenuazonic acid. *Nature* **200:** 1338–1339.

Miller, K. D., Miller, G. G., Sanders, M. and Fellowes, O. N. (1977) Inhibition of virus-induced plaque formation by a toxic derivative of purified cobra neurotoxin. *Biochim. biophys. Acta* **496:** 192–196.

Miller, P. A., Milstrey, K. P. and Trown, P. W. (1968) Specific inhibition of viral ribonucleic acid replication by gliotoxin. *Science* **159:** 431–432.

Minor, P. D. (1990) Antigenic structure of picornaviruses. In: *Current Topics in Microbiology 161 and Immunology, Picornaviruses*, pp. 121–155, Racaneillo, V. R. (ed.). Springer Verlag, Berlin, Heidelberg.

Minor, P. D. (1992) The molecular biology of poliovaccines. *J. gen. Virol.* **73:** 3065–3077.

Minor, P. D., Macadam, A., Cammack, N., Dunn, G. and Almond, J. W. (1990) Molecular biology and the control of viral vaccines. *FEMS. Microbiol. Immunol.* **2:** 207–213.

Minor, P. D., Pipkin, P. A., Hockley, D., Schild, G. C. and Almond, J. W. (1984) Monoclonal antibodies which block cellular receptors of poliovirus. *Virus Res.* **1:** 203–212.

Misumi, Y., Miki, K., Takatsuki, A., Tamura, G. and Ikehara, Y. (1986) Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J. biol. Chem.* **261:** 11398–11403.

Mitsuya, H., Popovic, M., Yarchoan, R., Matsushita, S., Gallo, R. C. and Broder, S. (1984) Suramin protection of T cells *in vitro* against infectivity and cytopathic effect of HTLV-III. *Science* **226:** 172–174.

Patterson, J. L. and Fernandez Larsson, R. (1990) Molecular mechanisms of action of ribavirin. *Rev. Infect. Dis.* **12:** 1139–1146.

Paul, A. V., Schultz, A., Pincus, S. E., Oroszlan, S. and Wimmer, E. (1987) Capsid protein VP4 of poliovirus is *N*-myristoylated. *Proc. natn. Acad. Sci. U.S.A.* **84:** 7827–7831.

Pearson, G. D. and Zimmerman, E. F. (1969) Inhibition of poliovirus replication by *N*-methylisatin-beta-4′:4′-dibutylthiosemicarbazone. *Virology* **38:** 641–650.

Pelletier, J. and Sonenberg, N. (1988) Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **334:** 320–325.

Pellkofer, R., Marsh, D., Hoffmann-Bleihauer, P. and Sandhoff, K. (1982) Melittin stimulates incorpor-

ation and degradation of sphyngomyelin in synaptosomal plasma membranes. *J. Neurochem.* **38:** 1230–1235.

Pennington, T. H. (1977) Isatin-beta-thiosemicarbazone causes premature cessation of vaccinia virus-induced late post-replicative polypeptide synthesis. *J. gen. Virol.* **35:** 567–571.

Pérez, L. and Carrasco, L. (1991) Cerulenin—an inhibitor of lipid synthesis-blocks vesicular stomatitis virus RNA replication. *FEBS Lett.* **280:** 129–133.

Pérez, L. and Carrasco, L. (1992) Lack of direct correlation between p220 cleavage and the shut-off of host translation after poliovirus infection. *Virology* **189:** 178–186.

Pérez, L. and Carrasco, L. (1993) Entry of poliovirus into cells does not require a low-pH step. *J. Virol.* **67:** 4543–4548.

Pérez, L., Guinea, R. and Carrasco, L. (1991) Synthesis of Semliki Forest virus RNA requires continuous lipid synthesis. *Virology* **183:** 74–82.

Pevear, D. C., Fancher, M. J., Felock, P. J., Rossmann, M. G., Miller, M. S., Diana, G., Treasurywala, A. M., McKinlay, M. A. and Dutko, F. J. (1989) Conformational change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors. *J. Virol.* **63:** 2002–2007.

Pfau, C. J. (1982) The thiosemicarbazones. In: *Chemotherapy of Viral Infections*, pp. 147–204, Came, P. E. and Caliguiri, L. A. (eds). Springer Verlag, Berlin.

Philipson, L., Bengtsson, S. and Dinter, Z. (1966) The reversion of guanidine inhibition of poliovirus synthesis. *Virology* **29:** 317.

Phillpotts, R. J., Wallace, J., Tyrrell, D. A., Freestone, D. S. and Shepherd, W. M. (1983a) Failure of oral 4′,6-dichloroflavan to protect against rhinovirus infection in man. *Arch. Virol.* **75:** 115–121.

Phillpotts, R. J., Wallace, J., Tyrrell, D. A. J. and Tagart, V. B. (1983b) Therapeutic activity of enviroxime against rhinovirus infection in volunteers. *Antimicrob. Agents Chemother.* **23:** 671–675.

Pincus, S. E., Diamond, D. C., Emini, E. A. and Wimmer, E. (1986) Guanidine-selected mutants of poliovirus: mapping of point mutations to polypeptide 2C. *J. Virol.* **57:** 638–646.

Pincus, S. E., Rohl, H. and Wimmer, E. (1987) Guanidine-dependent mutants of poliovirus: identification of three classes with different growth requirements. *Virology* **157:** 83–88.

Pincus, S. E. and Wimmer, E. (1986) Production of guanidine-resistant and -dependent poliovirus mutants from cloned cDNA: mutations in polypeptide 2C are directly responsible for altered guanidine sensitivity. *J. Virol.* **60:** 793–796.

Pinto, C. A., Bahnsen, H. P., Ravin, L. J., Haff, R. F. and Pagano, J. F. (1972) The antiviral effect of a triazinoindole (SK&F 40491) in rhinovirus infected gibbons. *Proc. Soc. Exp. Biol. Med.* **141:** 467–474.

Pinto, L. H., Holsinger, L. J. and Lamb, R. A. (1992) Influenza virus M_2 protein has ion channel activity. *Cell* **69:** 517–528.

Planterose, D. N. (1969) Antiviral and cytotoxic effects of mycophenolic acid. *J. gen. Virol.* **4:** 629–630.

Pleij, C. W. (1990) Pseudoknots: a new motif in the RNA game. *Trends Biochem. Sci.* **15:** 143–147.

Pohjanpelto, P. (1958) Stabilization of poliovirus by cystine. *Virology* **6:** 472–487.

Pompei, R., Flore, O., Marccialis, M. A., Pani, A. and Loddo, B. (1979) Glycyrrhizic acid inhibits virus growth and inactivates virus particles. *Nature* **281:** 689–690.

Powers, R. D., Gwaltney, J. M. J. and Hayden, F. G. (1982) Activity of 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (MDL-860) against picornaviruses *in vitro*. *Antimicrob. Agents Chemother.* **22:** 639–642.

Putnak, J. R. and Phillips, B. A. (1981) Picornaviral structure and assembly. *Microbiol. Rev.* **45:** 287–315.

Racaniello, V. R. (1990) Cell receptors for picornaviruses. *Curr. Top. Microbiol. Immunol.* **161:** 1–22.

Racaniello, V. R. and Baltimore, D. (1981a) Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. *Proc. natn. Acad. Sci. U.S.A.* **78:** 4887–4891.

Racaniello, V. R. and Baltimore, D. (1981b) Cloned poliovirus complementary DNA is infectious in mammalian cells. *Science* **214:** 916–919.

Rawlings, N. D. and Barrett, A. J. (1993) Evolutionary families of peptidases. *Biochem. J.* **290:** 205–218.

Reed, S. E. (1980) The assessment of antirhinovirus compounds with clinical potential. In: *Developments in Antiviral Therapy*, pp. 157–170, Collier, L. H. and Oxford, J. (eds). Academic Press, London.

Ren, R., Costantini, F., Gorgacz, E. J., Lee, J. J. and Racaniello, V. R. (1990) Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell* **63:** 353–362.

Ren, R. and Racaniello, V. R. (1992) Human poliovirus receptor gene expression and poliovirus tissue tropism in transgenic mice. *J. Virol.* **66:** 296–304.

Rhoads, R. E. (1988) Cap recognition and the entry of mRNA into the protein synthesis initiation cycle. *Trends Biochem. Sci.* **13:** 52–56.

Rhoads, R. E. (1993) Regulation of eukaryotic protein synthesis by initiation factors. *J. biol. Chem.* **268:** 3017–3020.

Rice, A. P., Duncan, R., Hershey, W. B. and Kerr, I. M. (1985) Double-stranded RNA-dependent protein kinase and 2-5A system are both activated in interferon-treated, encephalomyocarditis virus-infected HeLa cells. *J. Virol.* **54:** 894–898.

Richards, O. C. and Ehrenfeld, E. (1990) Poliovirus RNA replication. *Curr. Top. Microbiol. Immunol.* **161:** 89–121.

Rightsel, W. A., Dice, J. R., McAlpine, R. J., Timm, E. A., McLean, I. W., Dixon, G. J. and Schabel, F. M. (1961) Antiviral effect of guanidine. *Science* **134:** 558–559.

Rinehart, K. L., Jr and Gloer, J. B. (1981) Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. *Science* **212:** 933–935.

Robins, R. K., Revankar, G. R., McKernan, P. A., Murray, B. K., Kirsi, J. J. and North, J. A. (1985) The importance of IMP dehydrogenase inhibition in the broad spectrum antiviral activity of ribavirin and selenazofurin. *Adv. Enzyme Regul.* **24:** 29–43.

Rodriguez, P. L. and Carrasco, L. (1992) Gliotoxin: Inhibitor of poliovirus RNA synthesis that blocks the viral RNA polymerase $3D^{pol}$. *J. Virol.* **66:** 1971–1976.

Rodríguez, P. L. and Carrasco, L. (1993) Poliovirus protein 2C has ATPase and GTPase activities. *J. biol. Chem.* **268:** 8105–8110.

Rombaut, B., Andries, K. and Boeye, A. (1991) A comparison of WIN 51711 and R 78206 as stabilizers of poliovirus virions and procapsids. *J. gen. Virol.* **72:** 2153–2157.

Rosenwirth, B. and Eggers, H. J. (1977) Echovirus 12-induced host cell shutoff is prevented by rhodanine. *Nature* **267:** 370–371.

Rosenwirth, B. and Eggers, H. J. (1979) Early processes of echovirus 12-infection: elution, penetration, and uncoating under the influence of rhodanine. *Virology* **97:** 241–255.

Rossmann, M. G. (1988) Antiviral agents targeted to interact with viral capsid proteins and a possible application to human immunodeficiency virus. *Proc. natn. Acad. Sci. U.S.A.* **85:** 4625–4627.

Rossmann, M. G. (1989a) The structure of antiviral agents that inhibit uncoating when complexed with viral capsids. *Antiviral. Res.* **11:** 3–13.

Rossmann, M. G. (1989b) The canyon hypothesis. Hiding the host cell receptor attachment site on a viral surface from immune surveillance. *J. biol. Chem.* **264:** 14587–14590.

Rossman, M. G. and Johnson, J. E. (1989) Icosahedral RNA virus structure. *Annu. Rev. Biochem.* **58:** 533–573.

Rossmann, M. G., Arnold, E., Erickson, J. W., Frankenberger, E. A., Griffith, J. P., Hecht, H.-J., Johnson, J. E., Kamer, G., Luo, M., Mosser, A. G., Rueckert, R. R., Sherry, B. and Vriend, G. (1985) Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* **317:** 145–153.

Rothman, J. E. and Orci, L. (1992) Molecular dissection of the secretory pathway. *Nature* **355:** 409–415.

Rueckert, R. R. and Wimmer, E. (1984) Systematic nomenclature of picornavirus proteins. *J. Virol.* **50:** 957–959.

Sabin, A. B. and Fieldsteel, A. H. (1962) Antipoliomyelitic activity of human and bovine colostrum and milk. *Pedriat.* **29:** 105–115.

Saborio, J. L., Pong, S. S. and Koch, G. (1974) Selective and reversible inhibition of initiation of protein synthesis in mammalian cells. *J. molec. Biol.* **85:** 195–211.

Sakai, A., Ebina, T. and Ishida, N. (1983) Interferon induction in mice by oral administration of K-582, a new peptide antibiotic. *J. Antibiotics* **36:** 335–338.

Sanders, M., Soret, M. G. and Akin, B. A. (1953) Neurotoxoid interference with two human strains of poliomyelitis in Rhesus monkeys. *Ann. N.Y. Acad. Sci.* **58:** 1–12.

Sanders, M., Soret, M. G., Akin, B. A. and Roizin, L. (1958) Neurotoxoid interference in Macacus rhesus infected intramuscularly with poliovirus. *Science* **127:** 594–596.

Sarnow, P. (1989) Role of 3'-end sequences in infectivity of poliovirus transcripts made *in vitro*. *J. Virol.* **63:** 467–470.

Sarnow, P., Bernstein, H. D. and Baltimore, D. (1986) A poliovirus temperature-sensitive RNA synthesis mutant located in a noncoding region of the genome. *Proc. natn. Acad. Sci. U.S.A.* **83:** 571–575.

Sarnow, P., Jacobson, S. J. and Najita, L. (1990) Poliovirus genetics. *Curr. Top. Microbiol. Immunol.* **161:** 155–188.

Saunders, K. and King, A. M. (1982) Guanidine-resistant mutants of aphthovirus induce the synthesis of an altered nonstructural polypeptide, P34. *J. Virol.* **42:** 389–394.

Sawai, H., Iami, J., Lesiak, K., Johnston, M. I. and Torrence, F. (1983) Cordycepin analogues of 2-5A and its derivatives. Chemical synthesis and biological activity. *J. biol. Chem.* **258:** 1671–1677.

Schaeffer, H. J., Beauchamp, L., De Miranda, P., Elion, G. B., Bauer, D. J. and Collins, P. (1978) 9-(2-Hydroxyethoxymethyl) guanine activity against viruses of the herpes group. *Nature* **272:** 583–585.

Scharff, M. D. and Levintow, L. (1963) Quantitative study of the formation of poliovirus antigens in infected HeLa cells. *Virology* **19:** 491–500.

Schiff, G. M., Sherwood, J. R., Young, E. C. and Mason, L. J. (1992) Prophylactic efficacy of WIN 54954 in prevention of experimental human coxsackievirus A21 infection and illness. *Antiviral Res.* **17:** 92.

Schlesinger, M. J. and Malfer, C. (1982) Cerulenin blocks fatty acid acylation of glycoproteins and inhibits vesicular stomatitis and Sindbis virus particle formation. *J. biol. Chem.* **257:** 9887–9890.

Schneider, D. L. (1987) The proton pump ATPase of lysosomes and related organelles of the vacuolar apparatus. *Biochim. biophys. Acta* **895:** 1–10.

Schols, D., Pauwels, R., Desmyter, J. and De Clercq, E. (1990) Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T-cells persistently infected with HIV-1. *Virology* **175:** 556–561.

Schrader, A. P. and Westaway, E. G. (1990) Successful competition in translation by the flavivirus Kunjin with poliovirus during co-infections in Vero cells. *Arch. Virol.* **114:** 75–89.

Seglen, P. O. (1983) Inhibitors of lysosomal function. *Methods Enzymol.* **96:** 737–764.

Sehgal, P. B., Pfeffer, L. M. and Tamm, I. (1982) Interferon and its inducers. In: *Chemotherapy of Viral Infections*, pp. 205–312, Came, P. E. and Caliguiri, L. A. (eds). Springer Verlag, Berlin.

Semler, B. L., Johnson, V. H. and Tracy, S. (1986) A chimeric plasmid from cDNA clones of poliovirus and coxsackievirus produces a recombinant virus that is temperature-sensitive. *Proc. natn. Acad. Sci. U.S.A.* **83:** 1777–1781.

Semler, B. L., Kuhn, R. J. and Wimmer, E. (1988) Replication of the poliovirus genome. In: *RNA Genetics*, Vol. I, *RNA-Directed Virus Replication*, pp. 23–49, Domingo, E., Holland, J. J. and Ahlquist, P. (eds). CRC Press Inc., Boca Raton, FL.

Shannon, W. M. (1984) Mechanisms of action and pharmacology: chemical agents. In: *Antiviral Agents and Viral Diseases of Man*, pp. 55–121, Galasso, G. J., Merigan, T. C. and Buchanan, R. A. (eds). Raven Press, New York.

Shepard, D. A., Heinz, B. A. and Rueckert, R. R. (1993) WIN 52035-2 inhibits both attachment and eclipse of human rhinovirus 14. *J. Virol.* **67:** 2245–2254.

Shope, R. E. (1953a) An antiviral substance from *Penicillium funiculosum*: III. General properties and characteristics of helenine. *J. exp. Med.* **97:** 639–650.

Shope, R. E. (1953b) An antiviral substance from *Penicillium funiculosum*: I. Effect upon infection in mice with Swine influenza virus and Columbia SK encephalomyelitis virus. *J. exp. Med.* **97:** 601–625.

Shope, R. E. (1953c) An antiviral substance from *Penicillium funiculosum*: II. Effect of helenine upon infection in mice with Semliki Forest virus. *J. exp. Med.* **97:** 627–638.

Shugar, D. (1974) Progress with antiviral agents. *FEBS Lett.* **40:** S48–S62.

Sidwell, R. W., Robins, R. K. and Hillyard, I. W. (1979) Ribavirin: an antiviral agent. *Pharmac. Ther.* **6:** 123–146.

Siegl, G. and Eggers, H. J. (1982) Failure of guanidine and 2-(alpha-hydroxybenzyl)benzimidazole to inhibit replication of hepatitis A virus in vitro. *J. gen. Virol.* **61:** 111–114.

Silverman, R. H., Cayley, P. J., Knight, M., Gilbert, C. S. and Kerr, I. M. (1982) Control of the ppp(A2′p)$_n$A system in HeLa cells. Effects of interferon and virus infection. *Eur. J. Biochem.* **124:** 131–138.

Singh, K. R. P., Pavri, K. and Anderson, C. R. (1963) Experimental transovarial transmission of Kyasanur Forest disease virus in *Haemaphysalis spinigera*. *Nature* **199:** 513.

Singh, S. B., Cordingley, M. G., Ball, R. G., Smith, J. L., Dombrowski, A. W. and Goetz, M. A. (1991) Structure and stereochemistry of thysanone: a novel human rhinovirus 3C-protease inhibitor from *Thysanophora penicilloides*. *Tetrahedron Lett.* **32:** 5279–5282.

Skiles, J. W. and McNeil, D. (1990) Spiro indolinone beta-lactams, inhibitors of poliovirus and rhinovirus 3c-proteinases. *Tetrahedron Lett.* **31:** 7277–7280.

Smith, R. A. and Kirkpatrick, W. (1980) *Ribavirin: A Broad Spectrum Antiviral Agent*. Academic Press, New York.

Smith, T. J., Kremer, M. J., Luo, M., Vriend, G., Arnold, E., Kamer, G., Rossmann, M. G., McKinlay, M. A., Diana, G. D. and Otto, M. J. (1986) The site of attachment in human rhinovirus 14 for antiviral agents that inhibit uncoating. *Science* **233:** 1286–1293.

Smyth, M., Hall, J., Fry, E., Stuart, D., Stanway, G. and Hyypiä, T. (1993) Preliminary crystallographic analysis of coxsackievirus A9. *J. molec. Biol.* **230:** 667–669.

Sola, A., Rodríguez, S., García Gancedo, A., Vilas, P. and Gil-Fernández, C. (1986a) Inactivation and inhibition of African swine fever virus by monoolein-monolinolein and gamma-linolenyl alcohol. *Arch. Virol.* **88:** 285–292.

Sola, A., Rodríguez, S., Gil-Fernández, C., Alarcón, B., González, M. E. and Carrasco, L. (1986b) New agents active against African swine fever virus. *Antimicrob. Agents Chemother.* **29:** 284–288.

Sonenberg, N. (1990) Poliovirus translation. *Curr. Top. Microbiol. Immunol.* **161:** 23–47.

Sonenberg, N. (1991) Picornavirus RNA translation continues to surprise. *TIG* **7:** 105–106.

Spector, D. H. and Baltimore, D. (1975a) Polyadenylic acid on poliovirus RNA. II. Poly(A) on intracellular RNAs. *J. Virol.* **15:** 1418–1431.

Spector, D. H. and Baltimore, D. (1975b) Polyadenylic acid on poliovirus RNA IV. Poly(U) in replicative intermediate and double-stranded RNA. *Virology* **67:** 498–505.

Spector, D. H., Villa-Komaroff, L. and Baltimore, D. (1975) Studies on the function of polyadenylic acid on poliovirus RNA. *Cell* **6:** 41–44.

Srivastava, P. C., Pickering, M. V., Allen, L. B., Streeter, D. G., Campbell, M. T., Witkowski, J. T., Sidwell, R. W. and Robins, R. K. (1977) Synthesis and antiviral activity of certain thiazole C-nucleosides. *J. med. Chem.* **20:** 256–262.

Staeheli, P. (1990) Interferon-induced proteins and the antiviral state. *Adv. Virus Res.* **38:** 147–200.

Stanway, G. (1990) Structure, function and evolution of picornaviruses. *J. gen. Virol.* **71:** 2483–2501.

Staunton, D. E., Dustin, M. L., Erickson, H. P. and Springer, T. A. (1990) The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. *Cell* **61:** 243–254.

Staunton, D. E., Merluzzi, V. J., Rothlein, R., Barton, R., Marlin, S. D. and Springer, T. A. (1989) A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* **56:** 849–853.

Stebbing, N. (1984) Antiviral effects of single-stranded polynucleotides and their mode of action. In: *Viral Chemotherapy*, Vol. 1, pp. 437–479, Shugar, D. (ed.). Pergamon Press, Oxford.

Stebbing, N., Grantham, C. A. and Kaminski, F. (1976) Investigation of the anti-viral mechanism of poly I

and poly C against encephalomyocarditis virus infection in the absence of interferon induction in mice. *J. gen. Virol.* **32:** 25–35.
Stebbing, N., Grantham, C. A., Lindley, I. J., Eaton, M. A. and Carey, N. H. (1977) *In vivo* antiviral activity of polynucleotide mimics of strategic regions in viral RNA. *Ann. N.Y. Acad. Sci.* **284:** 682–696.
Steele, F. M. and Black, F. L. (1967) Inactivation and heat stabilization of poliovirus by 2-thiouracil. *J. Virol.* **1:** 653–658.
Stein, I. M. and Micklus, M. J. (1973) Concentrations in serum and urinary excretion of guanidine, 1-methylguanidine and 1,1-dimethylguanidine in renal failure. *Clin. Chem.* **19:** 583–585.
Stephenson, M. L. and Zamecnik, P. C. (1978) Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc. natn. Acad. Sci. U.S.A.* **75:** 285–288.
Strebel, K. and Beck, E. (1986) A second protease of foot-and-mouth disease virus. *J. Virol.* **58:** 893–899.
Streeter, D. G., Witkowski, J. T., Khare, G. P., Sidwell, R. W., Bauer, R. J., Robins, R. K. and Simon, L. N. (1973) Mechanism of action of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proc. natn. Acad. Sci. U.S.A.* **70:** 1174–1178.
Streissle, G., Paessens, A. and Oediger, H. (1985) New antiviral compounds. *Adv. Virus Res.* **30:** 83–138.
Stringellow, D. A. (1981) Induction of interferon with low molecular weight compounds: fluorenone esters, ethers (Tilorone), and pyramidinones. *Meth. Enzymol.* **78:** 262–284.
Sturzenbecker, L. J., Nibert, M., Furlong, D. and Fields, B. N. (1987) Intracellular digestion of reovirus particles requires a low pH and is an essential step in the viral infectious cycle. *J. Virol.* **61:** 2351–2361.
Suhadolnik, R. J. (1967a) Gliotoxin. In: *Antibiotics*, Vol. II, *Biosynthesis*, pp. 29–31, Gottlieb, D. and Shaw, P. D. (eds). Springer Verlag, Berlin.
Suhadolnik, R. J. (1967b) Cordycepin, Psicofuranine, Decoyinine, Tubercidin and Toyocamycin. In: *Antibiotics*, Vol. II, *Biosynthesis*, pp. 400–409, Gottlieb, D. and Shaw, P. D. (eds). Springer Verlag, Berlin.
Summers, D. F. and Maizel, J. V. J. (1968) Evidence for large precursor proteins in poliovirus synthesis. *Proc. natn. Acad. Sci. U.S.A.* **59:** 966–971.
Summers, D. F. and Maizel, J. V. J. (1971) Determination of the gene sequence of poliovirus with pactamycin. *Proc. natn. Acad. Sci. U.S.A.* **68:** 2852–2856.
Summers, D. F., Shaw, E. N., Stewart, M. L. and Maizel, J. V. J. (1972) Inhibition of cleavage of large poliovirus-specific precursor proteins in infected HeLa cells by inhibitors of proteolytic enzymes. *J. Virol.* **10:** 880–884.
Svensson, U. (1985) Role of vesicles during adenovirus 2 internalization into HeLa cells. *J. Virol.* **55:** 442–449.
Taber, R., Rekosh, D. and Baltimore, D. (1971) Effect of pactamycin on synthesis of poliovirus proteins: a method for genetic mapping. *J. Virol.* **8:** 395–401.
Takeda, N., Kuhn, R. J., Yang, C. F., Takegami, T. and Wimmer, E. (1986) Initiation of poliovirus plus-strand RNA synthesis in a membrane complex of infected HeLa cells. *J. Virol.* **60:** 43–53.
Takegami, T., Kuhn, R. J., Anderson, C. W. and Wimmer, E. (1983) Membrane-dependent uridylylation of the genome-linked protein VPg of poliovirus. *Proc. natn. Acad. Sci. U.S.A.* **80:** 7447–7451.
Tamm, I. and Sehgal, P. B. (1978) Halobenzimidazole ribosides and RNA synthesis of cells and viruses. *Adv. Virus Res.* **22:** 187–258.
Tamura, G., Ando, K., Suzuki, S., Takatsuki, A. and Arima, K. (1968) Antiviral activity of brefeldin A and verrucarin A. *J. Antibiot. Tokyo* **21:** 160–161.
Tang, J., Colacino, J. M., Larsen, S. H. and Spitzer, W. (1990) Virucidal activity of hypericin against enveloped and non-enveloped DNA and RNA viruses. *Antiviral Res.* **13:** 313–325.
Taylor, J. L. and Grossberg, S. E. (1990) Recent progress in interferon research: molecular mechanisms of regulation, action, and virus circumvention. *Virus Res.* **15:** 1–25.
Tershak, D. R. (1974) Guanidine inhibition of poliovirus growth. Partial elimination by protease antagonists and low temperature. *Can. J. Microbiol.* **20:** 817–824.
Tershak, D. R. (1982). Inhibition of poliovirus polymerase by guanidine *in vitro*. *J. Virol.* **41:** 313–318.
Tershak, D. R., Mitchell, W. and Garfinkle, B. (1972) Effect of guanidine on the growth of LSc poliovirus. *Can. J. Microbiol.* **18:** 747–755.
Tershak, D. R., Yin, F. H. and Korant, B. D. (1982) Guanidine. In: *Chemotherapy of Viral Infections*, pp. 343–377, Came, P. E. and Caliguiri, L. A. (eds). Springer Verlag, Berlin.
Thomas, A. A., ter Haar, E., Wellink, J. and Voorma, H. O. (1991) Cowpea mosaic virus middle component RNA contains a sequence that allows internal binding of ribosomes and that requires eukaryotic initiation factor 4F for optimal translation. *J. Virol.* **65:** 2953–2959.
Tisdale, M. and Selway, J. W. T. (1983) Inhibition of an early stage of rhinovirus replication by dichloroflavan (BW683C). *J. gen. Virol.* **64:** 795–803.
Tobin, G. J., Young, D. C. and Flanegan, J. B. (1989) Self-catalyzed linkage of poliovirus terminal protein VPg to poliovirus RNA. *Cell* **59:** 511–519.
Togo, Y., Schwartz, A. R. and Hornick, R. B. (1973a) Antiviral effect of 3,4-dihydro-1-isoquinolineacetamide hydrochloride in experimental human rhinovirus infection. *Antimicrob. Agents Chemother.* **4:** 612–616.
Togo, Y., Schwartz, A. R. and Hornick, R. B. (1973b) Failure of a 3-substituted triazinoindole in the prevention of experimental human rhinovirus infection. *Chemotherapy* **18:** 17–26.
Tomassini, J. E., Graham, D., DeWitt, C. M., Lineberger, D. W., Rodkey, J. A. and Colonno, R. J. (1989)

cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. *Proc. natn. Acad. Sci. U.S.A.* **86:** 4907–4911.

Tonew, E., Indulen, M. K. and Dzeguze, D. R. (1982) Antiviral action of dipyridamole and its derivatives against influenza virus A. *Acta Virol. Praha* **26:** 125–129.

Torney, H. L., Dulworth, J. K. and Steward, D. L. (1982) Antiviral activity and mechanism of action of 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (MDL-860). *Antimicrob. Agents Chemother.* **22:** 635–638.

Toyoda, H., Nicklin, M. J., Murray, M. G., Anderson, C. W., Dunn, J. J., Studier, F. W. and Wimmer, E. (1986) A second virus-encoded proteinase involved in proteolytic processing of poliovirus polyprotein. *Cell* **45:** 761–770.

Trown, P. W. (1968) Antiviral activity of *N,N'*-dimethyl-epidithiapiprazinedione, a synthetic compound related to the gliotoxins, LL-S88alpha and beta, chetomin and the sporidesmins. *Biochem. biophys. Res. Commun.* **33:** 402–407.

Trown, P. W. and Bilello, J. A. (1972) Mechanism of action of gliotoxin: elimination of activity by sulfhydryl compounds. *Antimicrob. Agents Chemother.* **2:** 261–266.

Troxler, M., Egger, D., Pfister, T. and Bienz, K. (1992) Intracellular localization of poliovirus RNA by *in situ* hybridization at the ultrastructural level using single-stranded riboprobes. *Virology* **191:** 687–697.

Turner, R. B., Dutko, F. J., Goldstein, N. H., Lockwood, G. and Hayden, F. G. (1993) Efficacy of oral WIN 54954 for prophylaxis of experimental rhinovirus infection. *Antimicrob. Agents Chemother.* **37:** 297–300.

Tyrrell, D. A. J. (1984) Antirhinovirus drugs. In: *Targets for the Design of Antiviral Agents*, pp. 189–202, De Clercq, E. and Walder, R. T. (eds). Plenum Press, New York.

Ulmer, J. B. and Palade, G. E. (1990) Effects of brefeldin A on the processing of viral envelope glycoproteins in murine erythroleukemia cells. *J. biol. Chem.* **266:** 9173–9179.

Ulug, E. T., Garry, R. F. and Bose, H. R., Jr (1987) Cell killing by enveloped RNA viruses. In: *Mechanisms of Viral Toxicity in Animal Cells*, pp. 91–144, Carrasco, L. (ed.). CRC Press Inc., Boca Raton, FL.

Urzainqui, A. and Carrasco, L. (1988) Post-translational modifications of poliovirus proteins. *Biochem. biophys. Res. Commun.* **158:** 263–271.

Urzainqui, A. and Carrasco, L. (1989) Degradation of cellular proteins during poliovirus infection: studies by two-dimensional gel electrophoresis. *J. Virol.* **63:** 4729–4735.

Ussery, M. A., Irvin, J. D. and Hardesty, B. (1977) Inhibition of poliovirus replication by a plant antiviral peptide. *Ann. N.Y. Acad. Sci.* **284:** 431–440.

Van Aerschot, A., Herdewijn, P., Janssen, G., Cools, M. and De Clercq, E. (1989) Synthesis and antiviral activity evaluation of 3'-fluoro-3'-deoxyribonucleosides: broad-spectrum antiviral activity of 3'-fluoro-3'-deoxyandenosine. *Antiviral Res.* **12:** 133–150.

Van Hoof, L., Vanden Berghe, D. A., Hatfield, G. M. and Vlietinck, A. J. (1984) Plant antiviral agents; V. 3-Methoxyflavones as potent inhibitors of viral-induced block of cell synthesis. *Planta Med.* **50:** 513–517.

Varga, M. J., Weibull, C. and Everitt, E. (1991) Infectious entry pathway of adenovirus type 2. *J. Virol.* **65:** 6061–6070.

Vázquez, D. (1979) *Inhibitors of Protein Biosynthesis*. Springer Verlag, Berlin, Heidelberg, New York.

Veckenstedt, A., Béládi, I. and Mucsi, I. (1978) Effect of treatment with certain flavonoids on mengo virus-induced encephalitis in mice. *Arch. Virol.* **57:** 255–260.

Verhaegen-Lewalle, M., Kuwata, T., Zhang, Z.-X., DeClercq, E., Cantell, K. and Content, J. (1982) 2-5A Synthetase activity induced by interferon a, b and g in human cell lines differing in their sensitivity to the anticellular and antiviral activities of these interferons. *Virology* **117:** 425–434.

Vlietinck, A. J., Vanden Berghe, D. A., Van Hoof, L. M., Vrijsen, R. and Boeye, A. (1986) Antiviral activity of 3-methoxyflavones. *Prog. Clin. Biol. Res.* **213:** 537–540.

Wagner, E., Cotten, M., Foisner, R. and Birnstiel, M. L. (1991) Transferrin–polycation–DNA complexes: the effect of polycations on the structure of the complex and DNA delivery to cells. *Proc. natn. Acad. Sci. U.S.A.* **88:** 4255–4259.

Wagner, E., Plank, C., Zatloukal, K., Cotten, M. and Birnstiel, M. L. (1992a) Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin–polylysine–DNA complexes: toward a synthetic virus-like gene-transfer vehicle. *Proc. natn. Acad. Sci. U.S.A.* **89:** 7934–7938.

Wagner, E., Zatloukal, K., Cotten, M., Kirlappos, H., Mechtler, K., Curiel, D. T. and Birnstiel, M. L. (1992b) Coupling of adenovirus to transferrin–polylysine–DNA complexes greatly enhances receptor-mediated gene delivery and expression of transfected genes. *Proc. natn. Acad. Sci. U.S.A.* **89:** 6099–6103.

Werner, G. H., Jasmin, C. and Chermann, J. C. (1976) Effect of ammonium 5-tungsto-2-antimoniate on encephalomyocarditis and vesicular stomatitis virus infections in mice. *J. Gen. Virol.* **31:** 59–64.

Whealy, M. E., Card, J. P., Meade, R. P., Robbins, A. K. and Enquist, L. W. (1991) Effect of brefeldin A on alphaherpesvirus membrane protein glycosylation and virus egress. *J. Virol.* **65:** 1066–1081.

White, J. M. (1990) Viral and cellular membrane fusion proteins. *Annu. Rev. Physiol.* **52:** 675–697.

Wigle, D. T. and Smith, A. E. (1973) Specificity in initiation of protein synthesis in a fractionated mammalian cell-free system. *Nature New. Biol.* **242:** 136–140.

Wiley, D. C. and Skehel, J. J. (1987) The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu. Rev. Biochem.* **56:** 365–394.

Williams, B. R. G., Golgher, R. R., Brown, R. E., Gilbert, C. S. and Kerr, I. M. (1979) Natural occurrence of 2-5A in interferon-treated EMC virus-infected L cells. *Nature* **282:** 582–586.

Williams, B. R. G., Kerr, I. M., Gilbert, C. S., White, C. N. and Ball, L. A. (1978) Synthesis and breakdown

of pppA2′p5′p5′A and transient inhibition of protein synthesis in extracts from interferon-treated and control cells. *Eur. J. Biochem.* **92:** 455–562.

Williams, R. H., Lively, D. H., DeLong, D. C., Cline, J. C., Sweeney, M. J., Poore, G. A. and Larsen, S. H. (1968) Micophenolic acid: antiviral and antitumor properties. *J. Antibiotics* **21:** 463–464.

Wimmer, E., Kuhn, R. J., Pincus, S., Yang, C. F., Toyoda, H., Nicklin, M. J. and Takeda, N. (1987) Molecular events leading to picornavirus genome replication. *J. Cell Sci.* **7:** 251–276.

Witkowski, J. T., Robins, R. K., Sidwell, R. W. and Simon, L. N. (1972) Design, synthesis, and broad spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides. *J. Med. Chem.* **15:** 1150–1154.

Wu, M., Davidson, N. and Wimmer, E. (1978) An electron microscope study of the proteins attached to polio virus RNA and its replicative form (RF). *Nucleic Acids Res.* **5:** 4711–4723.

Wu, Y., Zhang, D. Y. and Kramer, F. R. (1992) Amplifiable messenger RNA. *Proc. natn. Acad. Sci. U.S.A.* **89:** 11769–11773.

Wyckoff, E. E., Hershey, J. W. B. and Ehrenfeld, E. (1990) Eukaryotic initiation factor 3 is required for poliovirus 2A protease-induced cleavage of the p220 component of eukaryotic initiation factor 4F. *Proc. natn. Acad. Sci. U.S.A.* **87:** 9529–9533.

Wyckoff, E. E., Lloyd, R. E. and Ehrenfeld, E. (1992) Relationship of eukaryotic initiation factor 3 to poliovirus-induced p220 cleavage activity. *J. Virol.* **66:** 2943–2951.

Wyde, P. R., Six, H. R., Wilson, S. Z., Gilbert, B. E. and Knight, V. (1988) Activity against rhinoviruses, toxicity, and delivery in aerosol of enviroxime in liposomes. *Antimicrob. Agents Chemother.* **32:** 890–895.

Yamada, Y., Natsumeda, Y. and Weber, G. (1988) Action of the active metabolites of tiazofurin and ribavirin on purified IMP dehydrogenase. *Biochemistry* **27:** 2193–2196.

Yamaizumi, M., Uchida, T. and Okada, Y. (1979) Macromolecules can penetrate the host cell membrane during the early period of incubation with HVJ (Sendai virus). *Virology* **95:** 218–221.

Yamazi, Y., Takahashi, M. and Todome, Y. (1970) Inhibition of poliovirus by effect of a methylthiopyrimidine derivative. *Proc. Soc. Exp. Biol. Med.* **133:** 674–677.

Yeates, T. O., Jacobson, D. H., Martin, A., Wychowski, C., Girard, M., Filman, D. J. and Hogle, J. M. (1991) Three-dimensional structure of a mouse-adapted type 2/type 1 poliovirus chimera. *EMBO J.* **10:** 2331–2341.

Yogo, Y. and Wimmer, E. (1972) Polyadenylic acid at the 3′-terminus of poliovirus RNA. *Proc. natn. Acad. Sci. U.S.A.* **69:** 1877–1882.

Zatloukal, K., Wagner, E., Cotten, M., Phillips, S., Plank, C., Steinlein, P., Curiel, D. T. and Birnstiel, M. L. (1992) Transferrinfection: a highly efficient way to express gene constructs in eukaryotic cells. *Ann. N.Y. Acad. Sci.* **660:** 136–153.

Zeichhardt, H., Otto, M. J., McKinlay, M. A., Willingmann, P. and Habermehl, K. O. (1987) Inhibition of poliovirus uncoating by disoxaril (WIN 51711). *Virology* **160:** 281–285.

Zeichhardt, H., Wetz, K., Willingmann, P. and Habermehl, K. O. (1985) Entry of poliovirus type 1 and Mouse Elberfeld (ME) virus into HEp-2 cells: receptor-mediated endocytosis and endosomal or lysosomal uncoating. *J. gen. Virol.* **66:** 483–492.

Zenke, M., Steinlein, P., Wagner, E., Cotten, M., Beug, H. and Birnstiel, M. L. (1990) Receptor-mediated endocytosis of transferrin-polycation conjugates: an efficient way to introduce DNA into hematopoietic cells. *Proc. natn. Acad. Sci. U.S.A.* **87:** 3655–3659.

Zerial, A., Werner, G. H., Phillpotts, R. J., Willmann, J. S., Higgins, P. G. and Tyrrell, D. A. (1985) Studies on 44 081 R.P., a new antirhinovirus compound, in cell cultures and in volunteers. *Antimicrob. Agents Chemother.* **27:** 846–850.

Zeuzem, S., Feick, P., Zimmermann, P., Haase, W., Kahn, R. A. and Schulz, I. (1992) Intravesicular acidification correlates with binding of ADP-ribosylation factor to microsomal membranes. *Proc. natn. Acad. Sci. U.S.A.* **89:** 6619–6623.

Zhang, A., Nanni, R. G., Li, T., Arnold, G. F., Oren, D. A., Jacobo-Molina, A., Williams, R. L., Kamer, G., Rubenstein, D. A., Li, Y., Rozhon, E., Cox, S., Buontempo, P., O'Connell, J., Schwartz, J., Miller, G., Bauer, B., Versace, R., Pinto, P., Ganguly, A., Girijavallabhan, V. and Arnold, E. (1993) Structure determination of antiviral compound SCH 38057 complexed with human rhinovirus 14. *J. molec. Biol.* **230:** 857–867.

Zhou, A., Hassel, B. A. and Silverman, R. H. (1993) Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. *Cell* **72:** 753–765.

Zuckerman, A. J. (1987) Screening of antiviral drugs for hepadna virus infection in Pekin ducks: a review. *J. Virol. Methods* **17:** 119–126.