Biochem. J. (1982) **205**, 1–13
Printed in Great Britain

REVIEW ARTICLE

Specific targets for antiviral drugs

Erik DE CLERCQ
Rega Institute for Medical Research, Katholieke Universiteit Leuven, 10 Minderbroedersstraat, B-3000 Leuven, Belgium

Introduction

For a long time the general assumption has prevailed that in their replicative cycle viruses strictly follow the normal metabolic pathways of the cell and that it would be impossible to block virus replication without affecting normal cell metabolism. This concept is no longer tenable. In recent years it has become increasingly evident that viruses and virus-infected cells exhibit some characteristics which are quantitatively or qualitatively different from normal uninfected cells (De Clercq, 1979). These differences are most clearly shown by the herpesviruses, which are capable of inducing a wide variety of specific enzymes, i.e. thymidine (deoxycytidine) kinase, DNA polymerase, 3′→5′-exodeoxyribonuclease, ribonucleotide reductase and dUTP pyrophosphatase. These enzymes are either absent from the uninfected cell or fundamentally different from their normal cellular counterparts. By virtue of a broadened substrate specificity, virus-induced enzymes like the herpesvirus-encoded thymidine (deoxycytidine) kinase may recognize as substrate thymidine and deoxycytidine analogues which are not recognized as substrate by the cellular kinases and thereby restrict their phosphorylation and antiviral action to the virus-infected cell.

Other specific events that occur only in virus-infected cells and could therefore serve as appropriate targets for the action of antiviral drugs are: (i) fusion of the virions with the cell membrane, as required for virus penetration and release; (ii) virus uncoating, which may also require the interaction of virus and cellular membranes; (iii) proteolytic cleavage of viral precursor proteins, which is an essential step in the assembly of virions (i.e. ortho- and paramyxoviruses, picorna- and togaviruses); (iv) synthesis of viral DNA or RNA, which is often achieved by virus-specific DNA polymerases (e.g. herpesviruses) or RNA polymerases (e.g. myxoviruses); (v) maturation of viral mRNA (5′-capping, methylation, 3′-adenylation) which may impose greater demands on the cell than the maturation of cellular mRNA; (vi) glycosylation of viral proteins, which is required for the assembly of enveloped viruses (i.e. herpes-, toga-, rhabdo- and myxoviruses). Most of the antiviral agents that have been reported to date act at one or more of these targets. Accordingly, these compounds display considerable specificity in their antiviral action. Some of the compounds have been introduced in clinical medicine for the treatment of virus diseases and other compounds are being explored for their clinical potentials. Irrespective of their clinical usefulness, the antiviral agents have been grouped in different classes, according to their chemical structure, target of action and antiviral spectrum (Table 1). For the most relevant compounds the structural formulae are presented in Fig. 1. Their targets in the replicative cycle of the virus are depicted in Fig. 2. A considerable number of the recently developed antiviral agents are effective against herpesviruses. The antiviral specificity of these compounds depends strongly on two virus-induced enzymes, thymidine (deoxycytidine) kinase and DNA polymerase (Fig. 3)

Abbreviations used: for compounds, see Fig. 1; for viruses: HSV_1, herpes simplex virus type 1; HSV_2, herpes simplex virus type 2; VZV, varicella zoster virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PRV, pseudorabies virus; IBRV, infectious bovine rhinotracheitis virus; HVS, herpesvirus saimiri; B, herpesvirus simiae; VSV, vesicular stomatitis virus. The abbreviated names of the compounds followed by MP, DP or TP correspond to the 5′-monophosphate, 5′-diphosphate or 5′-triphosphate of the compound.

Antiviral drugs

5-Iodo-2′-deoxyuridine

5-Iodo-2′-deoxyuridine (idoxuridine, 5-iodo-dUrd) (Fig. 1) may be considered as the prototype of the antiviral drugs. For almost 20 years it has been applied (as 0.1% eye drops) in the topical treatment of HSV_1 keratitis. Yet, 5-iodo-dUrd is not highly specific in its antiviral activity. It is phosphorylated in both virus-infected and uninfected cells, and in its phosphorylated form it could interfere with different steps that lead to the incorporation of thymidine into DNA (i.e. thymidine kinase, dTMP kinase, DNA polymerase). However, the main target for the

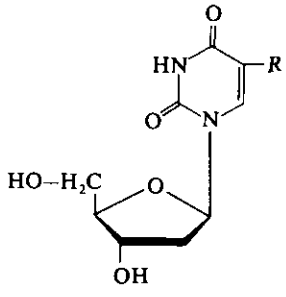
0306-3275/82/070001-13$01.50/1

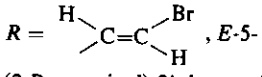
Table 1. *Antiviral compounds, their targets and antiviral spectrum*

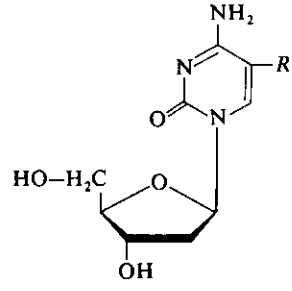
Compound	Target	Antiviral spectrum
5-Iodo-dUrd via io^{5}dUTP	DNA (incorporation)	DNA viruses (e.g. herpesviruses: HSV_1, HSV_2, . . .)
5-CF_3-dUrd via tfm^{5}dUMP	Thymidylate synthetase	DNA viruses (e.g. herpesviruses: HSV_1, HSV_2, . . .)
BV-dUrd via BV-dUTP	DNA polymerase DNA (incorporation)	Herpesviruses: HSV_1, VZV, PRV, IBRV, HVS, . . .
5-Iodo-dCyd via io^{5}dUTP	DNA (incorporation)	DNA viruses (e.g. herpesviruses: HSV_1, HSV_2, . . .)
Ara-A via Ara-AMP, Ara-ADP and Ara-ATP	DNA polymerase Ribonucleotide reductase *S*-Adenosylhomocysteine hydrolase 3′-Adenylation of mRNA DNA (incorporation)	DNA viruses (e.g. herpesviruses: HSV_1, HSV_2, . . .) Rhabdoviruses (i.e. VSV)
AI-ddUrd via AI-ddUTP	DNA (incorporation)	HSV_1
Ara-T via Ara-TTP	DNA polymerase DNA (incorporation)	Herpesviruses: HSV_1, HSV_2, VZV, . . .
Acyclovir via acyclovir triphosphate	DNA polymerase DNA (incorporation)	Herpesviruses: HSV_1, HSV_2, VZV, EBV, B, . . .
FIAra-C via FIAra-CTP	DNA polymerase DNA (incorporation)	Herpesviruses: HSV_1, HSV_2, VZV, . . .
Phosphonoformic acid	DNA polymerase	Herpesviruses (HSV_1, HSV_2, VZV, . . .) Retroviruses Hepatitis B virus
Ribavirin via ribavirin 5′monophosphate via ribavirin 5′-triphosphate	IMP dehydrogenase mRNA guanylyltransferase	All major DNA and RNA viruses, in particular myxoviruses (e.g. influenza) and arenaviruses (e.g. Lassa)
DHP-Ade	*S*-Adenosylhomocysteine hydrolase Adenosine deaminase	Several DNA and RNA viruses, in particular rhabdoviruses (e.g. VSV) and reoviruses (e.g. rota)
3-Deaza-adenosine	Viral DNA synthesis *S*-Adenosylhomocysteine hydrolase	Retroviruses (e.g. Rous sarcoma virus) and, probably, several other viruses
Specific oligodeoxynucleotides e.g. d(A-A-T-G-G-T-A-A-A-A-T-G-G)	Viral genome (hybridization)	Retroviruses (e.g. Rous sarcoma virus, and, possibly, several other viruses)
Benzimidazole derivatives, e.g. Enviroxime	Viral RNA synthesis	Picornaviruses (entero- and rhinoviruses)
Diarylamidine derivatives	Trypsin-like proteinases DNA polymerase	Respiratory syncytial virus, and, probably, several other viruses
Specific oligopeptides, e.g. Z-D-Phe-L-Phe-Gly	Virus-cell fusion, virus penetration	Orthomyxoviruses (e.g. influenza) and paramyxoviruses (e.g. measles)
Reverse transcriptase inhibitors	Reverse transcriptase	Retroviruses
Adamantane derivatives, e.g. Amantadine	Virus uncoating	Influenza A virus
Arildone	Virus uncoating	DNA viruses (e.g. herpesviruses: HSV_1, HSV_2, . . .) and RNA viruses (e.g. picornaviruses: rhino, polio, . . .)
Interferon (or interferon inducers) via (2′-5′)pppA(pA)$_n$	Viral protein synthesis	DNA and RNA viruses
Protein synthesis inhibitors via leaky membranes	Viral protein synthesis	DNA and RNA viruses
Glycosylation inhibitors, e.g. 2-deoxy-D-glucose	Virus assembly	Enveloped viruses (e.g. herpes-, toga-, myxo- and rhabdoviruses)

antiviral action of 5-iodo-dUrd is DNA itself (Prusoff & Goz, 1973). 5-Iodo-dUrd is readily incorporated into DNA and this incorporation may disturb the normal flow of genetic information during the replication of DNA or its transcription to RNA. Since 5-iodo-dUrd is incorporated into DNA of both virus-infected and uninfected cells, it is difficult to imagine how this incorporation could lead to any antiviral specificity. In addition to 5-iodo-dUrd, several other 5-substituted 2′-deoxyuridines

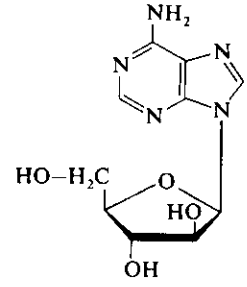
are incorporated into DNA: e.g. 5-vinyl-, 5-ethyl-, 5-propyl- and 5-hydroxymethyl-2′-deoxyuridine. Some of these dUrd derivatives, e.g. 5-ethyl-dUrd and 5-propyl-dUrd, display a remarkable selectivity as antiherpes agents (De Clercq *et al.*, 1978*c*). This selectivity may be related to a specific phosphorylation by the herpesvirus-induced thymidine kinase and, consequently, a preferential incorporation of the dUrd analogue into DNA of the virus-infected cell.



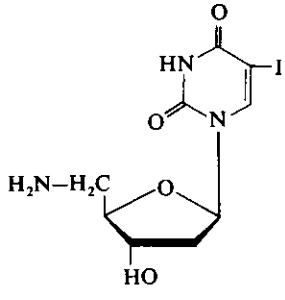
R = I, 5-Iodo-2′-deoxyuridine (5-iodo-dUrd)
R = CF_3, 5-Trifluoromethyl-2′-deoxyuridine(5-CF_3-dUrd)
R =  , *E*-5-(2-Bromovinyl)-2′-deoxyuridine (BV-dUrd)



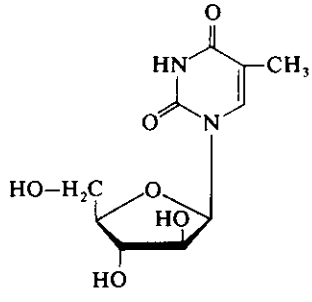
R = I, 5-Iodo-2′-deoxycytidine (5-iodo-dCyd)



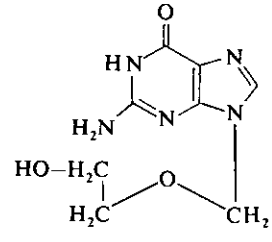
9-β-D-Arabinofuranosyladenine-(Ara-A)



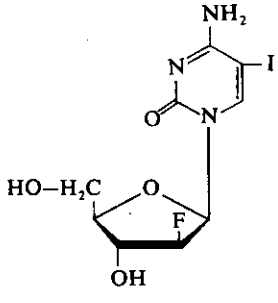
5-Amino-5-iodo-2′,5′-dideoxy-uridine (AI-ddUrd)



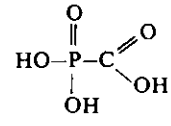
1-β-D-Arabinofuranosylthymine (Ara-T)



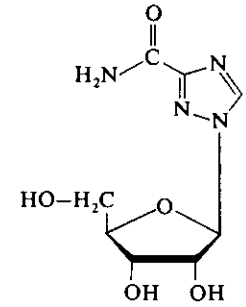
9-(2-Hydroxyethoxymethyl)-guanine acyclovir



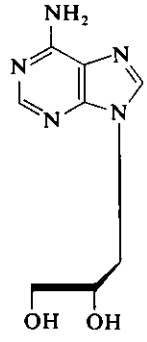
2′-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAra-C)



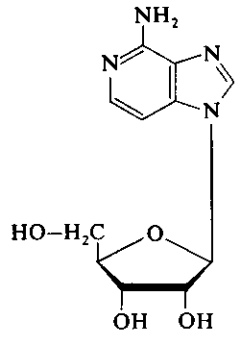
Phosphonoformic acid



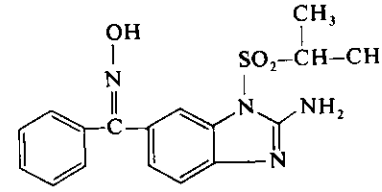
Ribavirin



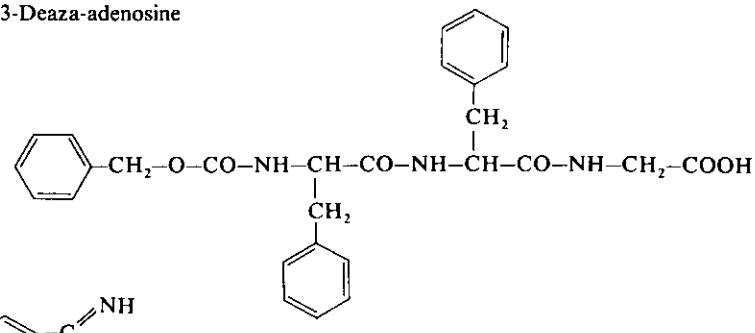
(*S*)-9-(2,3-Dihydroxypropyl) adenine (DHP-Ade)



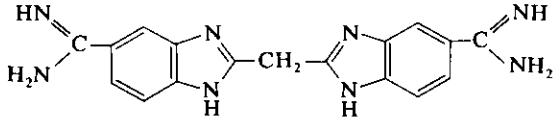
3-Deaza-adenosine



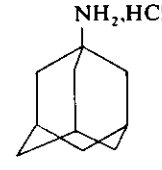
2-Amino-1-(isopropylsulphonyl)-6-benzimidazole phenyl ketone oxime (Enviroxime)



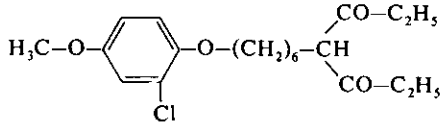
Z-D-Phe-L-Phe-Gly



bis-(5-Amidino-2-benzimidazolyl)methane



1-Adamantanamine hydrochloride (Amantadine)



4-[6-(2-Chloro-4-methoxy)phenoxy]hexylheptan-3,5-dione (Arildone)

Fig. 1. *Structures of and abbreviations for antiviral agents*

5-Trifluoromethyl-2′-deoxyuridine

5-Trifluoromethyl-2′-deoxyuridine (trifluridine, 5-CF_3-dUrd) (Fig. 1) and other 5-substituted 2′-deoxyuridines with a strong electron-withdrawing 5-substituent, such as 5-fluoro-dUrd, 5-nitro-dUrd, 5-formyl-dUrd and 5-ethynyl-dUrd, mainly act at the thymidylate synthetase level (De Clercq *et al.*, 1978*b*). As a result, the biosynthesis *de novo* of dTTP is shut off and DNA synthesis is severely impaired. To act as dTMP synthetase inhibitors, 5-fluoro-dUrd and its congeners must first be phosphorylated to their 5′-monophosphate form. Little, if any, viral specificity could be expected from these compounds, unless they would be specifically phosphorylated by the virus-induced cell. This does not appear to be the case. Hence, all 5-substituted dUrd analogues belonging to the class of thymidylate synthetase inhibitors are fairly cytotoxic and their antiviral activity is primarily a sequel of their suppressive effect on host DNA synthesis. One member of this group, 5-CF_3-dUrd, is licensed for clinical use (as 1% eye drops) for the topical

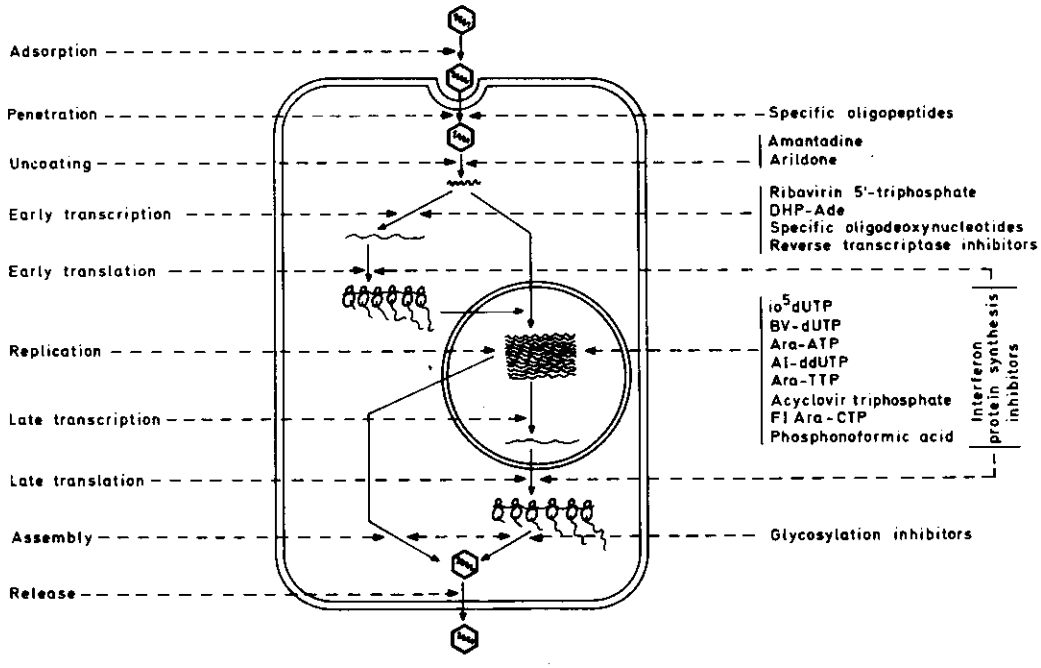


Fig. 2. *Highly schematic diagram of the virus replicative cycle showing the points of attack for antiviral drugs*

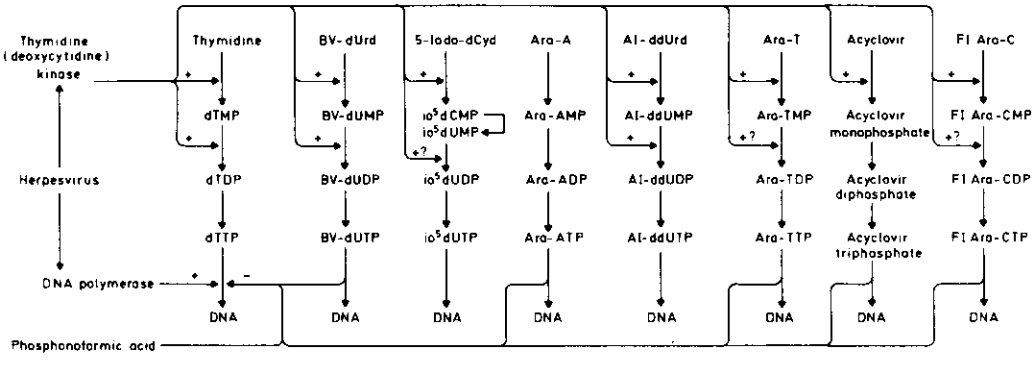


Fig. 3. *Role of virus-induced thymidine (deoxycytidine) kinase and DNA polymerase in the selective antiherpes activity of BV-dUrd, 5-iodo-dCyd, Ara-A, AI-ddUrd, Ara-T, acyclovir, FIAra-C and phosphonoformic acid*

For BV-dUrd, ARa-T, acyclovir and FIAra-C the selective antiherpes activity depends on both thymidine (deoxycytidine) kinase and DNA polymerase. For AI-ddUrd and 5-iodo-dCyd it depends on virus-induced thymidine (deoxycytidine) kinase only and for Ara-A and phosphonoformic acid it depends on viral DNA polymerase only. In addition to the indicated pathway, FIAra-C may also be metabolized to the 5'-mono-, di- and triphosphates of FIAra-U, FAra-U, FAra-C and FAra-T and these 5'-triphosphates may interact with DNA polymerase and eventually be incorporated into DNA.

treatment of HSV_1 keratitis. Although 5-CF_3-dUrd appears to be more efficacious for this purpose than 5-iodo-dUrd and less toxic for the eye than one would expect from its toxicity in cell culture, it is not a safe drug (Cassiman *et al.*, 1981) and should therefore be superseded by equally (or more) effective and less toxic drugs such as BV-dUrd and acyclovir.

E-5-(2-Bromovinyl)-2'-deoxyuridine

E-5-(2-bromovinyl)-2'-dexoyuridine (BV-dUrd) (Fig. 1), and *E*-5-(2-halogenovinly)-dUrd derivatives in general (De Clercq *et al.*, 1979, 1980*b*), differ from 5-iodo-dUrd and 5-CF_3-dUrd in that they are specifically phosphorylated by the herpesvirus-induced thymidine kinase (Cheng *et al.*, 1981), which restricts their action to the herpesvirus-infected cell. BV-dUrd is a very potent inhibitor of HSV_1, VZV, PRV, IBRV, HVS and, possibly, other herpesviruses. It is less active against HSV_2. The discriminating behaviour of BV-dUrd toward HSV_1 and HSV_2 may be related to the fact that the HSV_1-induced thymidine kinase converts BV-dUrd successively to its 5'-monophosphate and 5'-diphosphate, whereas the HSV_2-induced thymidine kinase stops at the 5'-monophosphate stage (Descamps & De Clercq, 1981). Upon further conversion (by cellular enzymes) to the 5'-triphosphate, BV-dUrd would interact with DNA polymerase, thereby inhibiting HSV_1 DNA polymerase to a greater degree than cellular DNA polymerases (Allaudeen *et al.*, 1981). Thus, at least two viral enzymes would contribute to the antiviral specificity of BV-dUrd: thymidine kinase and DNA polymerase. BV-dUrd can also be incorporated into DNA, but since it is specifically phosphorylated by the virus-infected cell, its incorporation into DNA is mainly confined to the virus-infected cell (Allaudeen *et al.*, 1982). To what extent this incorporation contributes to the antiviral effects of BV-dUrd is at present unclear. BV-dUrd has proven effective in a variety of experimental HSV_1 and VZV infections in animal models and initial clinical results indicate that it is also efficacious in humans, i.e. as 0.1% eye drops in the topical treatment of HSV_1 keratitis (including stromal keratitis) (Maudgal *et al.*, 1981), and administered orally (at 5–15 mg/kg per day) in the systemic treatment of mucocutaneous HSV_1 and VZV infections in cancer patients (e.g. leukaemic children) (De Clercq *et al.*, 1980*a*).

5-Iodo-2'-deoxycytidine

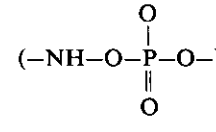
5-Iodo-2'-deoxycytidine (5-iodo-dCyd) (Fig. 1) and 5-bromo-, 5-ethynyl- and 5-nitro-2'-deoxycytidine are more specific in their antiviral activity than their 2'-deoxyuridine counterparts (Schildkraut *et al.*, 1975; Dobersen *et al.*, 1976; De Clercq *et al.*, 1982). This increased specificity may be attributed to the fact that the 2'-deoxycytidines, in addition to a specific phosphorylation by the virus-induced thymidine (deoxycytidine) kinase, require a second step for their activation, that is, deamination by dCMP deaminase. Whether this dCMP deaminase is also virus-encoded is still a matter of conjecture. Following phosphorylation and deamination, 5-substituted 2'-deoxycytidines would behave as their dUrd counterparts, and thus interfere with dTMP synthetase (i.e. 5-nitro-dUMP) or DNA polymerase (i.e. BV-dUTP) or be incorporated into DNA (i.e. 5-iodo-dUTP). There is only scanty information on the antiviral activity of dCyd derivatives in animal models or humans. Available evidence suggests that 5-iodo-dCyd is as effective as 5-iodo-dUrd in the topical treatment of HSV_1 keratitis.

9-β-D-Arabinofuranosyladenine

9-β-D-Arabinofuranosyladenine (vidarabine, Ara-A) (Fig. 1) interacts with a number of enzymes, i.e. DNA polymerase, *S*-adenosylhomocysteine hydrolase and ribonucleotide reductase. It also interferes with the polyadenylation of mRNA and is incorporated into DNA via internucleotide linkage. It is difficult to sort out from this variety of interactions which is responsible for the antiviral effects of the compound. Incorporation into DNA does not appear to account for the antiviral action of Ara-A, since substantially more compound is incorporated into cellular DNA than into viral DNA (Pelling *et al.*, 1981). From a clinical viewpoint Ara-A can be considered as a successful antiviral drug. Placebo-controlled clinical trials have established the efficacy of Ara-A (when administered as an intravenous infusion at 15 mg/kg per day) in the treatment of HSV_1 encephalitis, neonatal HSV_2 infections and VZV infections in immunocompromised patients. Ara-A has also been licensed for use (as a 3% eye ointment) in the topical treatment of HSV_1 keratitis.

5'-Amino-5-iodo-2',5'-dideoxyuridine

5'-Amino-5-iodo-2',5'-dideoxyuridine (AI-ddUrd) (Fig. 1) is a more selective inhibitor of HSV_1 replication than is its parent compound 5-iodo-dUrd. It owes this selectivity to a specific phosphorylation by the virus-induced thymidine kinase which converts AI-ddUrd to its 5'-diphosphate, AI-ddUDP (Chen & Prusoff, 1979). Upon subsequent phosphorylation (by cellular kinases) to AI-ddUTP, AI-ddUrd is internally incorporated into DNA. Since the initial phosphorylation of AI-ddUrd is restricted to the virus-infected cell, it is primarily incorporated into viral DNA (Chen *et al.*, 1976). This incorporation leads to extensive DNA breakage because of the formation of the relatively labile phosphoramidate



bonds (Fischer *et al.*, 1980). Despite its selective anti-HSV_1 activity it is doubtful whether AI-ddUrd will ever be used as an antiviral drug since it is much

less potent than other selective antiherpes agents such as BV-dUrd, acyclovir and FIAra-C.

1-β-D-Arabinofuranosylthymine

1-β-D-Arabinofuranosylthymine (Ara-T) (Fig. 1) and several other 5-substituted arabinofuranosyluracil derivatives, e.g. 5-ethyl-1-β-D-arabinofuranosyluracil and *E*-5-(2-bromovinyl)1-β-D-arabinofuranosyluracil (BVAra-U), follow a strategy in their antiviral action similar to that of BV-dUrd: they are specifically phosphorylated by the herpesvirus-induced thymidine kinase (Gentry & Aswell, 1975; Aswell *et al.*, 1977) and in their 5'-triphosphate form they inhibit the DNA polymerase reaction. It is not clearly established whether Ara-TTP inhibits viral DNA polymerase to a great degree than it does cellular DNA polymerases (Matsukage *et al.*, 1978; Müller *et al.*, 1979). To what extent Ara-T is incorporated into viral or cellular DNA is also a matter for further study. Ara-T has proven effective against HSV_1 encephalitis in mice (Machida *et al.*, 1980), but further experiments in animal models are needed before Ara-T can be submitted to clinical trials.

9-(2-Hydroxyethoxymethyl)guanine

9-(2-Hydroxyethoxymethyl)guanine (acyclovir) (Fig. 1), an acyclic analogue of guanosine, resembles most other selective antiherpes drugs in that it cannot act at its target until it has first been activated by a virus-specified kinase (Elion *et al.*, 1977; Fyfe *et al.*, 1978). Although it is generally assumed that the selectivity of acyclovir as an antiherpes agent depends at least partially on a specific phosphorylation by the virus-induced thymidine kinase (Fyfe *et al.*, 1978), an analysis of the herpesviruses that are susceptible to acyclovir and their thymidine (deoxycytidine) kinase-inducing capabilities suggests that the virus-induced deoxycytidine kinase rather than thymidine kinase accounts for the specific phosphorylation of acyclovir. For HSV_1, HSV_2 and VZV the deoxycytidine kinase and thymidine kinase activities reside within the same molecular entity, and these viruses are highly susceptible to the inhibitory effects of acyclovir. Yet, other herpesviruses, i.e. PRV, IBRV and HVS, which encode a thymidine kinase that has no deoxycytidine kinase activity, are not inhibited by acyclovir. These viruses are fully susceptible to BV-dUrd, suggesting that in its antiviral activity BV-dUrd truly depends on phosphorylation by the virus-induced thymidine kinase. The viral deoxycytidine kinase converts acyclovir to its monophosphate, which is then further phosphorylated by a cellular GMP kinase (Miller & Miller, 1980). In its triphosphate form acyclovir interacts with DNA polymerase, thereby inhibiting HSV_1 DNA polymerase to a greater degree than it inhibits cellular DNA polymerases (Furman *et al.*, 1979). Acyclovir may also be incorporated into DNA, but since it does not provide the necessary 3'-hydroxyl group for further chain elongation, its incorporation leads to premature termination of DNA synthesis (Furman *et al.*, 1980). Because of its specific phosphorylation by the herpesvirus-infected cell, the inhibitory effects of acyclovir on DNA synthesis are primarily confined to viral DNA. Acyclovir has already been the subject of extensive clinical studies. It has proven efficacious (when administered intravenously at 750 mg/m^2 per day) in the prophylaxis and treatment of mucocutaneous HSV_1 infections in immunocompromised patients (i.e. bone-marrow-transplant recipients) (Mitchell *et al.*, 1981; Saral *et al.*, 1981). A similar dosage regimen was also found effective in shortening the duration of acute pain and accelerating the healing of herpes zoster lesions in both immunocompetent and immunosuppressed patients (Selby *et al.*, 1979; Peterslund *et al.*, 1981). Furthermore, acyclovir would seem valuable in the topical treatment of HSV_1 keratitis (as a 3% eye ointment) and genital herpes (as a 5% cream).

2'-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine

2'-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAra-C) (Fig. 1) is another selective antiherpes drug which depends on a specific phosphorylation by the virus-induced thymidine (deoxycytidine) kinase (Watanabe *et al.*, 1979; Lopez *et al.*, 1980). Once it has been converted to its 5'-triphosphate it may interfere with viral DNA polymerase or be incorporated into DNA. In addition, FIAra-C may give rise to a number of metabolites, such as FIAra-U (2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil), FAra-C (2'-fluoro-1-β-D-arabinofuranosylcytosine), FIAra-T (2'-fluoro-5-methyl-1-β-D-arabinofuranosyluracil) and FAra-U (2'-fluoro-1-β-D-arabinofuranosyluracil), and the triphosphates of these metabolites may also interfere with DNA polymerization and eventually be incorporated into DNA. Little information is available on the clinical value of FIAra-C. Beneficial effects have been noted when it was administered intravenously (at $\geqslant$ 120 mg/m^2 per day) to immunocompromised patients with VZV infections.

Phosphonoformic acid

Phosphonoformic acid (Fig. 1) and its predecessor, phosphonoacetic acid, do not require a previous activation step to act at their target. They interact directly with DNA polymerase at the pyrophosphate-binding site (Leinbach *et al.*, 1976). Some viral DNA polymerases, e.g. HSV_1, retrovirus and hepatitis B virus DNA polymerase, are more sensitive to the inhibitory effects of phosphonoformic acid than are the cellular DNA polymerases (Helgstrand *et al.*, 1978; Sundquist &

Öberg, 1979). This explains why phosphonoformic acid specifically inhibits viral DNA synthesis in HSV_1-infected cells (Larsson & Öberg, 1981). Phosphonoformic acid is being pursued as a topical drug for the treatment of herpesvirus infections. Promising results have been obtained with a 3% phosphonoformic acid cream in the topical treatment of herpes labialis, and these studies will be extended to patients with genital herpes.

Ribavirin

Ribavirin (Fig. 1) is active against a broad range of DNA and RNA viruses (Smith & Kirkpatrick, 1980). To exert its antiviral action it must first be phosphorylated and this phosphorylation can be carried out by cellular kinases. In its 5′-monophosphate form, ribavirin inhibits IMP dehydrogenase, a crucial enzyme in the biosynthesis of GTP (Streeter *et al.*, 1973). As a result, the supply of GTP is shut off. Concomitantly, dTTP formation is also suppressed (Drach *et al.*, 1981). The mechanism of the latter effect has not been elucidated. In its 5′-triphosphate form, ribavirin inhibits mRNA guanylyltransferase, the first step in the capping of mRNA (Goswami *et al.*, 1979). This enzyme transfers GMP from GTP to the 5′-terminus of acceptor mRNA, and since both cellular and viral mRNAs (except picornaviral mRNAs) require capping, it is difficult to imagine how an inhibitory action at this level could confer any viral specificity unless one assumes that, for an effective translation, viral mRNAs depend more heavily on the 5′-cap than do their cellular counterparts. The synthesis of influenza viral mRNA appears to be a peculiar case. During the transcription process, influenza RNA polymerase uses as primer dinucleotides such as ApG or GpG, and this primer function can also be ensured by cellular mRNAs from which the 5′-cap and the adjacent 10–15 nucleotides may be transferred to the viral mRNA (Bouloy *et al.*, 1978; Plotch *et al.*, 1979). The apparent cannibalization of cellular mRNA to provide primers for viral RNA transcription is unique to influenza virus, and if this 5′-cap transfer reaction were the size of attack of ribavirin 5′-triphosphate, it could well explain the relatively specific activity of ribavirin against influenza virus. Although the efficacy of ribavirin as an anti-influenza agent has been demonstrated in mice, no beneficial effects were noted with oral ribavirin treatment in a natural outbreak of influenza A in humans (Smith *et al.*, 1980). Perhaps, ribavirin may yield better results if given as an aerosol by the intranasal route. This route of administration would maximize drug levels in the respiratory tract mucosa while minimizing systemic toxicity. Another potential lead for the clinical use of ribavirin may be arenavirus infections, e.g. Lassa fever. In rhesus monkeys ribavirin provided a protective effect against an experimental Lassa fever virus infection (Jahrling *et al.*, 1980), which indicates that ribavirin may be valuable in the treatment of humans infected by this life-threatening virus.

(S)-9-(2,3-dihydroxypropyl)adenine

(*S*)-9-(2,3-dihydroxypropyl)adenine (DHP-Ade) (Fig. 1), an acyclic analogue of adenosine, is active against several DNA and RNA viruses, i.e. vaccinia, VSV, rabies, rota and Rous sarcoma virus (De Clercq *et al.*, 1978*a*). Its activity against Rous sarcoma virus may be mediated by an inhibitory effect on $p60^{src}$, a virus-specified protein kinase of 60 000 daltons that is responsible for the transformation of chick embryo fibroblasts (Kara *et al.*, 1979). While DHP-Ade itself is not a substrate for adenosine aminohydrolase (deaminase), it inhibits the deamination of adenosine and Ara-A by adenosine deaminase. However, the principal mode of action of DHP-Ade is an inhibition of *S*-adenosylhomocysteine hydrolase (Votruba & Holy, 1980) and, consequently, an accumulation of *S*-adenosylhomocysteine, which is an inhibitor of the methyl transfer from *S*-adenosylmethionine to a number of acceptor molecules, e.g. viral mRNA. Viral mRNA requires this methylation for its maturation. Thus, an inhibitory effect of DHP-Ade on *S*-adenosylhomocysteine hydrolase would finally be reflected by an inhibition of virus replication. DHP-Ade has not yet been the subject of clinical studies. In animal experiments it afforded some protection against rabies and rotavirus infections, two viral diseases for which there is no alternative therapy. It is noteworthy that DHP-Ade, when administered orally to mice at subtoxic doses, caused a complete but reversible inhibition of spermatogenesis (De Clercq *et al.*, 1981). These findings suggest that DHP-Ade could also be pursued as an antifertility agent.

3-Deaza-adenosine

3-Deaza-adenosine (Fig. 1) and its carbocyclic analogue, 3-deaza-aristeromycin, represent a new class of antiviral agents whose potentials have not been fully explored. Like other adenosine analogues, e.g. Ara-A and DHP-Ade, 3-deaza-adenosine is an inhibitor of *S*-adenosylhomocysteine hydrolase (Bader *et al.*, 1978). As a result of this inhibitory activity, 3-deaza-adenosine may interfere with the methylation of mRNA at large and viral mRNA in particular. As has been demonstrated with avian sarcoma virus as a model, 3-deaza-adenosine may also exert a selective inhibitory effect on viral RNA transcription (Stoltzfus & Montgomery, 1981). However, the precise mechanism of this selective inhibition is not known, and nor are the possible therapeutic applications of 3-deaza-adenosine.

Specific oligo(deoxy)ribonucleotides

Specific oligo(deoxy)ribonucleotides that are of sufficient length (≥12 nucleotide units) and complementary to a well-defined nucleotide sequence of the viral genome may be expected to hybridize with the viral genome (provided that the latter is not shielded by proteins). This hybridization may lead to a block in the replication or transcription of the viral genome (if ±DNA, −RNA or +RNA) or translation of the viral genome (if +RNA). For example, an oligonucleotide complementary to the 3′-terminus of the influenza (−) RNA genome may prevent its transcription to mRNA and thereby interrupt the virus replicative cycle. However, this premise has so far not been borne out: an oligodeoxyribonucleotide complementary to the 12 terminal nucleotides of the 3′-end of the influenza RNA genome did not inhibit transcription of the viral RNA to a greater extent than did non-complementary oligodeoxyribonucleotides (Stridh *et al.*, 1981). More encouraging are the results reported with a tridecanucleotide, d(A-A-T-G-G-T-A-A-A-A-T-G-G), complementary to 13 nucleotides of the 3′- and 5′-reiterated terminal sequences of the Rous sarcoma virus genome. This tridecanucleotide was found to inhibit both virus production and cell transformation (Zamecnik & Stephenson, 1978). Obviously, additional data, including animal experiments, are needed before the therapeutic usefulness of the oligonucleotide-hybridization approach can be fully evaluated.

Benzimidazole derivatives

Benzimidazole derivatives, e.g. 2-(α-hydroxybenzyl)benzimidazole, have been known for some time as rather selective inhibitors of enteroviruses (Tamm *et al.*, 1969). Their mechanism of action was attributed to an inhibition of viral RNA synthesis. Recently, a new benzimidazole derivative called enviroxime [2-amino-1-(isopropylsulphonyl)-6-benzimidazole phenyl ketone oxime] (Fig. 1), was reported to be a potent inhibitor of rhinovirus replication in human embryonic nasal organ cultures (De Long & Reed, 1980). Although the exact mode of action and therapeutic usefulness of enviroxime remain to be established, its potent antirhinovirus activity suggests that this class of compounds should be further pursued for their antiviral potentials.

Diarylamidine derivatives

Diarylamidine derivatives, originally developed as antitrypanosomal agents, appear to possess an activity spectrum that is much wider than protozoa and spans bacteria, fungi and viruses as well. At the molecular level, diarylamidine derivatives are targeted at the DNA polymerase (i.e. reverse transcriptase) (De Clercq & Dann, 1980) and arginine- and lysine-specific esteroproteinases (i.e. trypsin-like proteinases) (Tidwell *et al.*, 1978). The inhibitory effects on DNA polymerase seem to be related to a specific interaction of the diamidines with A·T base pairs and depend on structural requirements that are clearly different from those that are responsible for antiproteolytic activity. Several diamidines, e.g. bis-(5-amidino-2-benzimidazolyl)methane (Fig. 1), exert a strong inhibitory effect on cell fusion induced by respiratory syncytial virus and this inhibitory effect may be related to their antiproteolytic activity (Dubovi *et al.*, 1981). As has been postulated for other myxoviruses, e.g. influenza and measles virus, the infectivity of respiratory syncytial virus may require the aid of a trypsin-like proteinase, and, to the extent that this proteinase is specific for respiratory syncytial virus-infected cells, one may expect diamidines to exhibit some specificity in their antiviral action. Proteolytic cleavage is crucial to the assembly of most, if not all, viruses and to the activity of some viral enzymes, e.g. RNA polymerases of picornaviruses and togaviruses (Korant, 1981). This proteolytic cleavage may be achieved by either cellular or viral proteinases. Considering the specificity of the proteinases involved, and the profound effects that minor structural variations have on the antiproteolytic activity of diarylamidine derivatives, it should be possible, at least in principle, to design specific inhibitors for each of these proteinases and for the viral functions they are entangled with.

Specific oligopeptides

Specific oligopeptides have been synthesized with amino acid sequences that mimic those of the *N*-terminal regions of the paramyxovirus F_1 polypeptide and orthomyxovirus HA_2 polypeptide (Richardson *et al.*, 1980). These polypeptides are involved in virus penetration. F is a glycoprotein responsible for the cell fusion caused by paramyxovirus (e.g. measles) and is activated by proteolytic cleavage by a host proteinase to yield two disulphide-linked subunits, F_1 and F_2. Similarly, the haemagglutinin (HA) protein of orthomyxoviruses (influenza) is cleaved by a host proteinase to yield the disulphide-linked subunits HA_1 and HA_2. As a result of this cleavage, new *N*-termini are generated on both the F_1 and HA_2 subunits, and synthetic oligopeptides which resemble these *N*-termini have been found to inhibit specifically the infectivity of ortho- and paramyxoviruses. Among the oligopeptides synthesized, Z-D-Phe-L-Phe-Gly-D-Ala-D-Val-D-Ile-Gly, Z-D-Phe-L-Phe-Gly (Fig. 1) and Z-D-Phe-L-Phe-L-Arg(NO_2) (where Z is carbobenzoxy) (Norrby, 1971) emerged as the most potent inhibitors of measles virus, whereas Z-Gly-L-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly were the most effective inhibitors of influenza A virus (Richardson *et al.*, 1980). These oligopeptides may interfere with the binding of the *N*-termini of the viral

glycoproteins at specific cell membrane sites and thereby prevent the initiation of virus infection. The premise that the initiation of virus infection can be blocked by oligopeptides homologous to a specific region of the viral envelope glycoproteins can probably be extended to viruses other than the myxovirus group. However, it remains to be established by studies with animal models whether such approach has any chemotherapeutic value.

Reverse transcriptase inhibitors

Reverse transcriptase inhibitors encompass a great variety of compounds which achieve their inhibitory effects on reverse transcriptase (RNA-directed DNA polymerase) by any of the following mechanisms: binding to the enzyme (e.g. the rifamycins), binding to the template (e.g. ethidium bromide, diarylamidine derivatives), acting as a substrate analogue [e.g. Ara-CTP (1-β-D-arabinofuranosylcytosine-5'-triphosphate)], acting as a template analogue [e.g. poly(2-fluoroadenylic acid)] or acting as a pyrophosphate analogue (e.g. phosphonoformic acid). For other reverse transcriptase inhibitors, i.e. 5-tungsto-2-antimoniate, the mode of action is still unknown. The rationale for the development of reverse transcriptase inhibitors is obviously based upon the premise that retrovirses (RNA tumour viruses), and the reverse transcriptase associated therewith, would be implicated in some forms of human cancer. Reverse transcriptase may be required for the initiation of cell transformation by retroviruses. Hence, inhibitors of reverse transcriptase may be useful in the prevention of the malignant disease and maintenance of remission. In as far as the neoplasia proceeds through virus spread and infection of new cells, reverse transcriptase inhibitors might be applicable at any stage of tumour development. While some of the reverse transcriptase inhibitors, e.g. 5-tungsto-2-antimoniate (Larnicol *et al.*, 1981) and diarylamidine derivatives, have demonstrated a marked anti-leukaemic activity in mice, their potential clinical value remains to be established.

Adamantane derivatives

1-Adamantanamine (Amantadine) (Fig. 1) and α-methyl-1-adamantanemethylamine (Rimantadine), are specifically active against influenza A virus infections. Both compounds have been licensed for clinical use. Ideally, these drugs should be administered prophylactically, but, even if they are given within 48 h after onset of the clinical symptoms, some benefit may be expected. Amantadine can be given orally (at 200 mg/day for an adult person) or intranasally (as an aerosol). The target for the antiviral action of Amantadine and Rimantadine has been identified as an event that occurs immediately after virus infection but precedes the primary transcriptions of the viral genome. Most probably this event corresponds to virus uncoating (Kato & Eggers, 1969; Skehel *et al.*, 1978; Koff & Knight, 1979).

Arildone

Arildone {4-[6-(2-chloro-4-methoxy)phenoxy]-hexylheptan-3,5-dione} (Fig. 1) represents a relatively new class of antiviral agents (aryloxy alkyl diketones) which selectively inhibit the replication of some RNA and DNA viruses, i.e. rhino, polio, HSV_1 and HSV_2 (Diana *et al.*, 1977). Arildone interacts with the protein capsid of the virus and protects it from uncoating (McSharry *et al.*, 1979). Arildone, as well as its pyrazole derivative {4-[6-(2-chloro-4-methoxy)phenoxy]hexyl-3,5-diethyl-1*H*-pyrazole methanesulphonate} (Pancic *et al.*, 1981), have been found effective in the topical treatment of HSV_1 and HSV_2 infections in mice and guinea pigs, and are now being evaluated as topical antiherpes drugs against HSV_1 and HSV_2 infections in humans.

Interferon and interferon inducers

These are capable of generating an abundance of biological effects (which are clearly beyond the scope of the present review). Predominant among these effects is an inhibition of protein synthesis, and this inhibition may be achieved by either of the following pathways: (i) activation of a protein kinase which leads to the phosphorylation of initiation factor eIF-2, inhibition of Met-$tRNA_f$ binding to 40S ribosomal subunits, and, consequently, inhibition of the formation of protein synthesis-initiating complexes, or (ii) induction of a (2'-5') $(A)_n$ synthetase which converts ATP into oligonucleotides generically called (2'-5')pppA$(pA)_n$ (since they contain 2',5'- instead of the usual 3',5'-phosphodiester bonds); these (2'-5')adenylates then activate a latent endoribonuclease which degrades mRNA and thereby shuts off protein synthesis. In as far as the synthesis of (2'-5')pppA$(pA)_n$ is confined to the cells or cell compartments involved in virus multiplication, (2'-5')pppA$(pA)_n$ may primarily affect viral protein synthesis and thus will exhibit some viral specificity in its mode of action. (2'-5')pppA$(pA)_n$, or analogues thereof, may also serve as a possible alternative to interferon (or inducers) in the treatment of virus infections. However, before (2'-5')pppA$(pA)_n$, or its analogues, could ever qualify for this purpose, they should satisfy several conditions, i.e. they should resist premature degradation by phosphodiestereases, enter the virus-infected cell, and inhibit viral protein synthesis to a greater degree than normal protein synthesis. The 3'-deoxy analogue of (2'-5')pppA$(pA)_n$, (2'-5')ppp3'dA$(p3'dA)_n$, appears to meet some of these requirements; it is not hydrolysed by 2',5'-phosphodiesterase under conditions where (2'-5')pppA-

$(pA)_n$ is entirely degraded (Doetsch *et al.*, 1981*b*), and when used in its core form, (2′-5′)-3′dA(p3′dA)$_n$, it inhibits transformation of Epstein–Barr virus-infected lymphocytes (Doetsch *et al.*, 1981*a*). The therapeutic potentials of this and other (2′-5′)$(A)_n$ analogues need further study.

Protein synthesis inhibitors

Protein synthesis inhibitors, such as hygromycin B and guanosine 5′-[β,γ-methylene]triphosphate, which are normally impermeable to uninfected cells may be able to enter virus-infected cells and thereby specifically inhibit viral protein synthesis. This assumption is based upon the fact that virus-infected cells gradually become leaky as the virus life cycle goes on (Carrasco, 1978; Benedetto *et al.*, 1980). Although announced as a novel approach to antiviral chemotherapy, the premises of membrane leakiness have so far not been borne out. No significant antiviral effects were noted with guanosine 5′-[β,γ-methylene]triphosphate in cell cultures or animals infected with encephalomyocarditis virus (Dawson *et al.*, 1979). Apparently, virus-infected cells become permeable to protein synthesis inhibitors too late for sufficient inhibition of viral protein synthesis to be obtained.

Glycosylation inhibitors

Glycosylation inhibitors, e.g. 2-deoxy-D-glucose, D-glucosamine and tunicamycin, have been shown to inhibit the multiplication of a number of enveloped RNA and DNA viruses. These inhibitory effects have been generally attributed to a deficient glycosylation of the viral glycoproteins, and a loss of the proper function of these glycoproteins. The mode of action of the glycosylation inhibitors is not fully understood, except, perhaps, for tunicamycin which prevents the formation of *N*-acetylglucosamine–dolichylphosphate, an intermediate in the synthesis of the core oligosaccharides of glycoproteins. As a result of an inhibition of their glycosylation, viral glycoproteins may be hampered in their transfer from the rough endoplasmic reticulum to the plasma membrane (Morrison *et al.*, 1978), where they are supposed to participate in the assembly ('budding') of the enveloped virions. Thus, an inhibition of the glycosylation process may finally be reflected by an impairment of virus assembly (Leavitt *et al.*, 1977). Under conditions where assembly takes place and virus particles are produced, the infectivity of these virus particles may be impaired (Chatterjee *et al.*, 1981). There are, however, several other possible mechanisms by which glycosylation inhibitors could interfere with virus multiplication; for example, 2-deoxy-D-glucose and tunicamycin may inhibit the glycosylation of host cell glycoproteins essential for viral DNA synthesis (Radsak & Weder, 1981). In addition, 2-deoxy-D-glucose may also affect cellular processes other than glycosylation. Therefore, future attempts should be directed at developing glycosylation inhibitors which specifically interfere with virus replication. Although 2-deoxy-D-glucose cannot be considered as a truly specific antiviral agent, it has been advocated for the topical treatment of genital HSV infections in humans (Blough & Giuntoli, 1979).

Conclusion

This review was primarily addressed to a description of the molecular targets for the antiviral action of a selected group of compounds. Each class of compounds was represented by its most relevant congeners and no attempts were made to examine the structure–function relationships within each particular class. Consequently, the compounds that have been discussed should not necessarily be regarded as the end products of our search for selective antiviral agents. On the contrary, they should be models for the development of more potent and more selective compounds. Possible exceptions to this rule are the highly selective antiherpes agents, i.e. BV-dUrd and acyclovir. Most other compounds, e.g. benzimidazole and diarylamidine derivatives, specific oligopeptides and oligonucleotides, are susceptible to further adjustments and refinements. More active and/or specific derivatives could be made from these compounds by applying the appropriate chemical modifications. The targets for an attack on virus replication should thereby provide essential background information. Keeping pace with the progress in molecular virology that may be anticipated for the next few years, these viral targets would become better defined, and this should, in turn, facilitate the development of more effective antiviral agents.

References

Allaudeen, H. S., Kozarich, J. W., Bertino, J. R. & De Clercq, E. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2698–2702

Allaudeen, H. S., Chen, M. S., Lee, J. J., De Clercq, E. & Prusoff, W. H. (1982) *J. Biol. Chem.* **257**, 603–606

Aswell, J. F., Allen, G. P., Jamieson, A. T., Campbell, D. E. & Gentry, G. A. (1977) *Antimicrob. Agents Chemother.* **12**, 243–254

Bader, J. P., Brown, N. R., Chiang, P. K. & Cantoni, G. L. (1978) *Virology* **89**, 494–505

Benedetto, A., Rossi, G. B., Amici, C., Belardelli, F., Cioé, L., Carruba, G. & Carrasco, L. (1980) *Virology* **106**, 123–132

Blough, H. A. & Giuntoli, R. L. (1979) *J. Am. Med. Assoc.* **241**, 2798–2801

Bouloy, M., Plotch, S. J. & Krug, R. M. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4886–4890

Carrasco, L. (1978) *Nature (London)* **272**, 694–699

Cassiman, J. J., De Clercq, E., Jones, A. S., Walker, R. T. & Van Den Berghe, H. (1981) *Br. Med. J.* **283**, 817–818

Chatterjee, S., Bradac, J. & Hunter, E. (1981) *J. Virol.* **38**, 770–776

Chen, M. S. & Prusoff, W. H. (1979) *J. Biol. Chem.* **254**, 10449–10452

Chen, M. S., Ward, D. C. & Prusoff, W. H. (1976) *J. Biol. Chem.* **251**, 4833–4838

Cheng, Y.-C., Dutschman, G., De Clercq, E., Jones, A. S., Rahim, S. G., Verhelst, G. & Walker, R. T. (1981) *Mol. Pharmacol.* **20**, 230–233

Dawson, K. M., Stewart, A. & Stebbing, N. (1979) *J. Gen. Virol.* **45**, 237–240

De Clercq, E. (1979) *Arch. Int. Physiol. Biochim.* **87**, 353–395

De Clercq, E. & Dann, O. (1980) *J. Med. Chem.* **23**, 787–795

De Clercq, E., Descamps, J., De Somer, P. & Holy, A. (1978*a*) *Science* **200**, 563–565

De Clercq, E., Descamps, J., Huang, G.-F. & Torrence, P. F. (1978*b*) *Mol. Pharmacol.* **14**, 422–430

De Clercq, E., Descamps, J. & Shugar, D. (1978*c*) *Antimicrob. Agents Chemother.* **13**, 545–547

De Clercq, E., Descamps, J., De Somer, P., Barr, P. J., Jones, A. S. & Walker, R. T. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2947–2951

De Clercq, E., Degreef, H., Wildiers, J., De Jonge, G., Drochmans, A., Descamps, J. & De Somer, P. (1980*a*) *Br. Med. J.* **281**, 1178

De Clercq, E., Descamps, J., Verhelst, G., Walker, R. T., Jones, A. S., Torrence, P. F. & Shugar, D. (1980*b*) *J. Infect. Dis.* **141**, 563–574

De Clercq, E., Leyten, R., Sobis, H., Matousek, J., Holy, A. & De Somer, P. (1981) *Toxicol. Appl. Pharmacol.* **59**, 441–451

De Clercq, E., Balzarini, J., Descamps, J., Huang, G.-F., Torrence, P. F., Bergstrom, D. E., Jones, A. S., Serafinowski, P., Verhelst, G. & Walker, R. T. (1982) *Mol. Pharmacol.* **21**, 217–223

De Long, D. C. & Reed, S. E. (1980) *J. Infect. Dis.* **141**, 87–91

Descamps, J. & De Clercq, E. (1981) *J. Biol. Chem.* **256**, 5973–5976

Diana, G. D., Salvador, U. J., Zalay, E. S., Carabateas, P. M., Williams, G. L., Collins, J. C. & Pancic, F. (1977) *J. Med. Chem.* **20**, 757–761

Dobersen, M. J., Jerkofsky, M. & Greer, S. (1976) *J. Virol.* **20**, 478–486

Doetsch, P. W., Suhadolnik, R. J., Sawada, Y., Mosca, J. D., Flick, M. B., Reichenbach, N. L., Dang, A. Q., Wu, J. M., Charubala, R., Pfleiderer, W. & Henderson, E. E. (1981*a*) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6699–6703

Doetsch, P., Wu, J. M., Sawada, Y. & Suhadolnik, R. J. (1981*b*) *Nature (London)* **291**, 355–358

Drach, J. C., Thomas, M. A., Barnett, J. W., Smith, S. H. & Shipman, C., Jr. (1981) *Science* **212**, 549–551

Dubovi, E. J., Geratz, J. D., Shaver, S. R. & Tidwell, R. R. (1981) *Antimicrob. Agents Chemother.* **19**, 649–656

Elion, G. B., Furman, P. A., Fyfe, J. A., De Miranda, P., Beauchamp, L. & Schaeffer, H. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5716–5720

Fischer, P. H., Chen, M. S. & Prusoff, W. H. (1980) *Biochim. Biophys. Acta* **606**, 236–245

Furman, P. A., St. Clair, M. H., Fyfe, J. A., Rideout, J. L., Keller, P. M. & Elion, G. B. (1979) *J. Virol.* **32**, 72–77

Furman, P. A., McGuirt, P. V., Keller, P. M., Fyfe, J. A. & Elion, G. B. (1980) *Virology* **102**, 420–430

Fyfe, J. A., Keller, P. M., Furman, P. A., Miller, R. L. & Elion, G. B. (1978) *J. Biol. Chem.* **253**, 8721–8727

Gentry, G. A. & Aswell, J. F. (1975) *Virology* **65**, 294–296

Goswami, B. B., Borek, E., Sharma, O. K., Fujitaki, J. & Smith, R. A. (1979) *Biochem. Biophys. Res. Commun.* **89**, 830–836

Helgstrand, E., Eriksson, B., Johansson, N. G., Lannerö, B., Larsson, A., Misiorny, A., Noren, J. O., Sjöberg, B., Stenberg, K., Stening, G., Stridh, S., Öberg, B., Alenius, S. & Philipson, L. (1978) *Science* **201**, 819–821

Jahrling, P. B., Hesse, R. A., Eddy, G. A., Johnson, K. M., Callis, R. T. & Stephen, E. L. (1980) *J. Infect. Dis.* **141**, 580–589

Kara, J., Vacha, P. & Holy, A. (1979) *FEBS Lett.* **107**, 187–192

Kato, N. & Eggers, H. J. (1969) *Virology* **37**, 632–641

Koff, W. C. & Knight, V. (1979) *J. Virol.* **31**, 261–263

Korant, B. D. (1981) in *Antiviral Chemotherapy: Design of Inhibitors of Viral Functions* (Gauri, K. K., ed.), pp. 37–47, Academic Press, New York, London, Toronto, Sydney and San Francisco

Larnicol, N., Augery, Y., Le Bousse-Kerdiles, C., Degiorgis, V., Chermann, J. C., Teze, A. & Jasmin, C. (1981) *J. Gen. Virol.* **55**, 17–23

Larsson, A. & Öberg, B. (1981) *Antiviral Res.* **1**, 55–62

Leavitt, R., Schlesinger, S. & Kornfeld, S. (1977) *J. Virol.* **21**, 375–385

Leinbach, S. S., Reno, J. M., Lee, L. F., Isbell, A. F. & Boezi, J. A. (1976) *Biochemistry* **15**, 426–430

Lopez, C., Watanabe, K. A. & Fox, J. J. (1980) *Antimicrob. Agents Chemother.* **17**, 803–806

Machida, H., Ichikawa, M., Kuninaka, A., Saneyoshi, M. & Yoshino, H. (1980) *Antimicrob. Agents Chemother.* **17**, 109–114

Matsukage, A., Ono, K., Ohashi, A., Takahashi, T., Nakayama, C. & Saneyoshi, M. (1978) *Cancer Res.* **38**, 3076–3079

Maudgal, P. C., Missotten, L., De Clercq, E., Descamps, J. & De Meuter, E. (1981) *Albrecht von Graefes Arch. Klin. Opththalmol.* **216**, 261–268

McSharry, J. J., Caliguiri, L. A. & Eggers, H. J. (1979) *Virology* **97**, 307–315

Miller, W. H. & Miller, R. L. (1980) *J. Biol. Chem.* **255**, 7204–7207

Mitchell, C. D., Bean, B., Gentry, S. R., Groth, K. E., Boen, J. R. & Balfour, H. H., Jr. (1981) *Lancet* **i**, 1389–1392

Morrison, T. G., McQuain, C. O. & Simpson, D. (1978) *J. Virol.* **28**, 368–374

Müller, W. E. G., Zahn, R. K., Arendes, J. & Falke, D. (1979) *J. Gen. Virol.* **43**, 261–271

Norrby, E. (1971) *Virology* **44**, 599–608

Pancic, F., Steinberg, B. A., Diana, G. D., Carabateas, P. M., Gorman, W. G. & Came, P. E. (1981) *Antimicrob. Agents Chemother.* **19**, 470–476

Pelling, J. C., Drach, J. C. & Shipman, C., Jr. (1981) *Virology* **109**, 323–335
Peterslund, N. A., Seyer-Hansen, K., Ipsen, J., Esmann, V., Schonheyder, H. & Juhl, H. (1981) *Lancet* **ii**, 827–830
Plotch, S. J., Bouloy, M. & Krug, R. M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1618–1622
Prusoff, W. H. & Goz, B. (1973) *Fed. Proc. Fed. Am.Soc. Exp. Biol.* **32**, 1679–1687
Radsak, K. D. & Weder, D. (1981) *J. Gen. Virol.* **57**, 33–42
Richardson, C. D., Scheid, A. & Choppin, P. W. (1980) *Virology* **105**, 205–222
Saral, R., Burns, W. H., Laskin, O. L., Santos, G. W. & Lietman, L. S. (1981) *N. Engl. J. Med.* **305**, 63–67
Schildkraut, I., Cooper, G. M. & Greer, S. (1975) *Mol. Pharmacol.* **11**, 153–158
Selby, P. J., Powles, R. L., Jameson, B., Kay, H. E. M., Watson, J. G., Thornton, R., Morgenstern, G., Clink, H. M., McElwain, T. J., Prentice, H. G., Corringham, R., Ross, M. G., Hoffbrand, A. V. & Brigden, D. (1979) *Lancet* **ii**, 1267–1270
Skehel, J. J., Hay, A. J. & Armstrong, J. A. (1978) *J. Gen. Virol.* **38**, 97–110
Smith, C. B., Charette, R. P., Fox, J. P., Cooney, M. K. & Hall, C. E. (1980) *J. Infect. Dis.* **141**, 548–554
Smith, R. A. & Kirkpatrick, W. (1980) *Ribavirin: A Broad Spectrum Antiviral Agent*, p. 237, Academic Press, New York
Stoltzfus, C. M. & Montgomery, J. A. (1981) *J. Virol.* **38**, 173–183
Streeter, D. G., Witkowski, J. T., Khare, G. P., Sidwell, R. W., Bauer, R. J., Robins, R. K. & Simon, L. N. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1174–1178
Stridh, S., Öberg, B., Chattopadhyaya, J. & Josephson, S. (1981) *Antiviral Res.* **1**, 97–105
Sundquist, B. & Öberg, B. (1979) *J. Gen. Virol.* **45**, 273–281
Tamm, I., Eggers, H. J., Bablanian, R., Wagner, A. F. & Folkers, K. (1969) *Nature (London)* **223**, 785–788
Tidwell, R. R., Geratz, J. D., Dann, O., Volz, G., Zeh, D. & Loewe, H. (1978) *J. Med. Chem.* **21**, 613–623
Votruba, I. & Holy, A. (1980) *Collect. Czech. Chem. Commun.* **45**, 3039–3044
Watanabe, K. A., Reichman, U., Hirota, K., Lopez, C. & Fox, J. J. (1979) *J. Med. Chem.* **22**, 21–24
Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 280–284