Antiviral agents—some current developments

D. Shugar

Institute of Biochemistry & Biophysics, Academy of Sciences, 02—532 Warszawa; and Department of Biophysics, Institute of Experimental Physics, University of Warsaw, 02—089 Warszawa, Poland.

Abstract - The following is a brief overview of the present stage of development of chemotherapy of viral diseases, based on the use of both natural and synthetic products which selectively or preferentially interfere with viral replication in the host cell. Particular attention is devoted to the use of nucleoside analogues, many of which are known to inhibit defined step(s) in the biosynthetic pathways of nucleic acids. Illustrative examples include several off-the-shelf compounds like pyrophosphate analogues and amantadine, natural products from marine sources, the interferon (2—5)A system, nucleoside analogues such as ribavirin, araA, 5-bromovinyl-2'-deoxyuridine, and various acyclonucleoside analogues. Included are descriptions of what is known about the mode of action of some of these, the relevant enzymes involved, and perspectives for further research.

INTRODUCTION

The important strides of the past few years have firmly established the feasibility of chemotherapy of viral diseases, and the major complaint now is that progress is slow. But the long and tortuous pathway from the laboratory to the clinic applies to all new drugs, due at least in part to public insistence on "totally safe drugs". The resulting regulations have been concisely analyzed in a recent article under the title "An end to the search for new drugs?" (1). Amongst the numerous reviews on the subject of antiviral agents, that of Bucknall and Rutty (2) most systematically describes the problems that must be coped with.

There are presently 5 drugs licensed for viral chemotherapy in the U.S.A, and several more in other countries. Others, potentially superior to those now in use, are in trials. What is most encouraging and stimulating is that current research is accompanied by serious efforts, at least partially successful, to delineate mechanism(s) of action. In contrast to research in the field of tumour chemotherapy, where it is still difficult to pin-point targets which differentiate a neoplastic from a normal cell, the molecular biology of viral replication has made phenomenal progress, so that one may now envisage the selection of appropriate targets for interfering with the cycle of viral replication with minimal toxicity to the host cells. To a considerable extent, this is based on a consideration of the enzymes involved in the disease process.

One of the most striking, indeed fascinating, recent discoveries (see ref. 3 for review) relates to influenza virus, the RNA transcription of which exhibits an absolute dependence on a primer consisting of a 5'-terminal capped fragment 10—13 residues in length derived from newly-synthesized host cell mRNA. This explains why viral RNA replication is dependent on the functioning of host-cell nuclear RNA polymerase II. The virion contains an endonuclease which recognizes and cleaves these fragments from cellular mRNA. Characterization of this nuclease, and its mechanism of action (see ref. 4), may prove useful in the design of an anti-influenza drug.

A similar approach is being widely applied in parasitic chemotherapy, where at least 10 potential targets are now known (5). One of these is based on the fact that some parasites are deficient in de novo synthesis of purine nucleotides, so that purine salvage pathways become essential for survival.
and growth. Among the Leishmania spp., one unique salvage enzyme is a purine nucleoside phosphotransferase which transfers phosphate from a variety of donors to the 5' of purine nucleosides. This enzyme, which may be considered a formal counterpart of herpes virus pyrimidine deoxynucleoside kinase (see below), readily phosphorylates exogenously administered nucleoside analogues such as the ribosides of allopurinol and thiopurinol, and formycin B. These, in turn, are either converted intracellularly to the triphosphates to become incorporated into nucleic acids of the parasite, or are inhibitors of other essential enzymes in parasite purine metabolism. The foregoing three nucleoside analogues, the more interesting in that they are relatively nontoxic to mammalian hosts (which lack the same enzyme), are potent antileishmanial agents both in vitro and in vivo. It is worth noting that such phosphotransferases, also available from plants and microorganisms, furnish a convenient tool for phosphorylation of nucleosides, particularly in those instances where chemical methods pose difficulties.

INITIAL SCREENING

The search for antiviral agents is based, as for other drugs, on initial screening, in a suitable in vitro test system, of "selected" products. The selection systems include: (a) products of natural origin, (b) "off-the-shelf" chemicals, and, now more widely applied (c) compounds which may be logically anticipated to interfere with some key metabolic pathway(s). Once a compound with some activity has been located, it may be chemically modified and the resulting analogues compared for activity, cytotoxicity, etc. (structure-activity relationships, SAR). Screening procedures have become relatively rapid and efficient and while procedure (c) may appeal to the researcher esthetically more rational and satisfying, both (a) and, especially, (b) have many proponents. Two such "off-the-shelf" antiviral agents are amantadine, now licensed for use against influenza A, and phosphonoacetate (PAA) which led to phosphonoformate (PFA), a promising antitherpes agent undergoing clinical trials. Procedure (c) led to the multitude of promising nucleoside analogues presently under intensive study, several of which are now licensed for clinical use, 5-ido-2'-deoxyuridine, 5-trifluoro-2'-deoxyuridine, araA and acyclovir (acyclovir) in the USA, and several others, including 5-ethyl-2'-deoxyuridine, in Europe.

PRODUCTS OF NATURAL ORIGIN

In contrast to the situation prevailing in the field of tumour chemotherapy, products of natural origin, reviewed by Swallow (7) and Becker (8), have hitherto not played an important role in the development of antiviral agents (but see below, for interferons). A possible exception is araA, presently licensed for systemic use in proven cases of herpes encephalitis. Originally synthesized chemically as a potential antitumour agent, like 5-ido-2'-deoxyuridine (IUdR), it was found to exhibit significant antitherpes activity. The difficulties associated with the large-scale chemical synthesis of this compound were subsequently circumvented by development of a fermentation procedure (9) based on the ability of Streptomyces antibioticus to convert exogenous adenosine to araA, presumably via a 2'-epimerase; such an adenosine 2'-epimerase, which converts adenosine to araA in vitro, has now been isolated (Suhadolnik et al., cited in ref. 10). Other, less effective, fermentation processes have been described.

Enzymatic synthesis of D-arabinonucleosides

A general procedure (11) for the in vitro enzymatic synthesis of purine D-arabinonucleosides, including araA, with the required purine base and the readily available 2',2'-anhydro-arach, as starting materials, is based on the following sequence of reactions: (a) alkaline hydrolysis of 2',2'-anhydro-araC to arach, (b) deamination of arach to arach with cytidine deaminase, (c) phosphorolysis of arach with uridine phosphorylase to liberate α-D-arabinofuranosyl-β-phosphate, (d) reaction of the sugar phosphate with the required purine base, catalysed by purine nucleoside phosphorylase. The overall yield, for araA, was 80%, but the procedure requires three purified enzymes from E. coli, and is probably not a serious competitor of S. antibioticus. It is, however, a useful laboratory-scale method for other biologically important, but not readily accessible, analogues, e.g. the D-arabinoside of 2,6-diaminopurine, obtained in 60% yield. Furthermore, the procedure may be simplified by elimination of the first enzymatic step since, in acid medium, arach undergoes deamination at a rate 50-fold higher than that for
cytidine, due to intramolecular catalysis by the "up" 2'-OH, via formation of a cyclic intermediate with C(6). This has been profited from to quantitatively deaminate a variety of araC analogues with a free 2'-OH in acetic acid at elevated temperature (12). The foregoing may, however, become outdated by a new general procedure for the chemical synthesis of xylofuranosyl and arabinofuranosyl nucleosides via selective protection and oxidation of the ribose ring of ribonucleosides (13).

Natural products from marine sources
Rinehart et al. (14) have presented a systematic account of the results of two expeditions designed to examine extracts of marine species, from Baja California and the Caribbean, for various biological activities. Initial tests, performed directly on shipboard, revealed a surprisingly large number of extracts with significant antiviral activities. Continuing studies on shore, still in progress (15,16), led to isolation and identification, in a Caribbean tunicate of the family Didemnidae, of a class of depsipeptides, shown in Fig. 1, with significant activities against a variety of RNA and DNA viruses. The structures of the peptides were established by means of hydrolysis, NMR spectroscopy and gas chromatography/mass spectrometry (14). In in vitro tests, didemnins A and B inhibited growth of HSV-1 and HSV-2 at concentrations of 1.0 and 0.5 μM, respectively; and both conferred protection on mice infected with Rift Valley fever virus, albeit with a low therapeutic index, B being several times more effective than A, in agreement with the in vitro results with HSV. It was pointed out that the difference in activities between A and B, combined with their structural complexities, provides opportunities for chemical modifications. Both A and B were only moderately effective against cutaneous HSV infections in mice, and inactive against Semliki Forest virus. Further reports on this interesting class of compounds are anticipated. A more recent study describes the isolation, from the Caribbean tunicate Eudistoma olivaceum, of another class of compounds containing the hitherto unreported condensed oxathiazepine ring system, two of which were potent in vitro inhibitors of HSV-1 (17).

Didemnin A: \( R = H \)

Didemnin B: \( R = CH_2CHOHCO \)

Didemnin C: \( R = CH_3CHOHCO \)

Fig. 1. The didemnins, a class of depsipeptides with antiviral activity isolated from a Caribbean Tridemnium species.

Pokeweed antiviral protein
Many plants contain proteins, with ability to inactivate ribosomes, and associated with the A-chain of plant toxins, but lacking the B-chain, so that they do not bind to the surface and of mammalian cells and are therefore not potent toxins. The first such protein to be detected, discovered by virtue of its ability to reduce infectivity of tobacco mosaic virus, was isolated from Phytolacca americana (pokeweed) and is referred to as pokeweed antiviral protein. Its molecular weight is about 28,000 and it is slightly basic in nature (pI = 8.1). Its mechanism of action against RNA viruses, such as influenza and polio, appears to be based on ability to penetrate infected cells and to inactivate 60S ribosomes, thus inhibiting viral protein synthesis. In HSV-infected cells, the mechanism of action is more complex and includes inhibition of viral DNA synthesis. Proteins from a number of other plant species have been isolated and found to display similar properties (see ref. 18 for review). No in vivo trials appear to have been conducted to date.
Interferons

Undoubtedly the best-known natural products with antiviral activity are the interferons, of which there are three major classes, \( \alpha \) (leucocyte), \( \beta \) (fibroblast) and \( \gamma \) (immune). Until comparatively recently these were available only in rather impure form, and in extremely limited quantities, and it is, therefore, not surprising that reported results on clinical efficacy were frequently conflicting. In the past 3-4 years marked amelioration of classical isolation techniques, and the development of successful recombination methods, have made available adequate quantities of these interferons and some of their sub-classes for both fundamental investigations and clinical trials, both as potential antiviral and antitumour agents. However, while the "great expectations" presently prevailing for the therapeutic uses of interferon do have a sound theoretical basis, there are as yet no unequivocal results forthcoming from clinical trials. Some findings are, indeed, promising, e.g. \( \alpha \)-interferon prophylaxis against cytomegalovirus syndromes in high-risk patients (19), but even these still require independent confirmation. A useful innovation is the organization, by the WHO, of an international committee for establishment of interferon standards, methods of assay, and reporting of results so as to permit of direct comparisons of findings from different laboratories (20).

The (2-5)A system. The multitude of biochemical pathways of interferon action, which vary for different viruses, have been most recently reviewed by Sen (21). A development of particular interest to chemists and biochemists was the discovery by Kerr et al. (22) that interferon treatment of cells in culture induces, along with the antiviral state, a number of proteins, one of which is the so-called (2-5)A synthetase. This is an enzyme which strongly binds, and is activated by, double-stranded RNA to synthesize from ATP a unique 2',5'-linked oligoadenylate, ppp(A2'p)nA (n = 2, with high activity for n = 3). This, in turn, activates a latent endoribonuclease (RNase L) that effectively degrades single-stranded mRNA, leading to potent inhibition of protein biosynthesis at nanomolar concentrations (22), as shown in Fig. 2. The kinetics of appearance and decay of the synthetase

![Diagram of the (2-5)A system](image)

parallel the development and decay of the antiviral state, although there is also evidence that the (2-5)A system is equally involved, like interferon, in other cellular processes such as regulation and differentiation (22).
The foregoing points to the possibility of profiting from (2-5)A, and/or chemical and structural analogues, to achieve antiviral activity without use of interferon. Various (2-5)A analogues have now been synthesized and their activities examined (see, e.g. ref. 23). However, a proper assessment of results in cellular systems must take account of the relative impermeability of the cell membrane to the highly charged (2-5)A molecule, as well as its susceptibility to degradation by hydrolysis of the internucleotide linkages by phosphodiesterase(s), and of the terminal 5'-triphosphate by dinucleoside polyphosphatases.

The adenosine moiety appears to be an important requirement for activity. The unique 2',5' phosphodiester linkages are essential for binding to RNase L, and accompanying inhibition of protein synthesis. Replacement of only one of these linkages by a 3',5' bond led to a decrease in activity of an order of magnitude, and the (3-5)A analogue exhibited 10-5 the activity of (2-5)A (24). It is also of interest that (2-5)A is a relatively potent inhibitor of both vaccinia virus and cellular mRNA (guanine-7)methyltransferases, the enzyme which methylates the GMP cap of mRNA, albeit only at μM concentrations. But 3'-O-methyl analogues, methylated at the terminal 3'-OH or at all three 3'-hydroxyls of pppA2'p5'A2'p5'A, and with a varying number of terminal 5'-phosphate groups, were not only 10-fold more active than the parent (2-5)A, but selectively inhibited the viral enzyme. e.g. pppAAAM inhibited the viral enzyme by 60% at a concentration which was without effect on the cellular enzyme (25). Relevant to the foregoing is the fact that the 5'-triphosphate of the triazole ribonucleoside ribavirin is an inhibitor of vaccinia virus mRNA guanylyltransferase (see below).

It has been found that modifications of the 2'-terminus are relatively well tolerated. This was profitably to convert the 2'-terminal ribose of (2-5)A-tetramer to an N-hexylmorpholine ring (by periodate oxidation to a dialdehyde, followed by reaction with hexylamine and reduction). The resulting analogue was 5 to 10-fold more effective as an inhibitor of translational activity, presumably because of its more pronounced resistance to enzymatic degradation (23). However, this analogue still does not readily traverse the cellular membrane.

An additional interesting activity of (2-5)A synthetase, at least in vitro, is its ability to add 5'-AMP in 2',5' linkages to such important metabolites as ADP-ribose, NAD+ and A5'p5'5'A. The product with the latter is A5'p4'5'A2'p5'5'A2'p5'A, or adenosine "capped" (2-5)A (26). A series of such A-capped analogues of (2-5)A, synthesized chemically, turned out to be relatively stable under conditions of the protein synthesis assay, while casting did not appear to adversely affect binding to RNase L, but did not lead to activation of the latter (27). Perhaps the most significant finding was that p5'5'A2'p5'5'A2'p5'A was stable in human Nalma 71 cell extracts at a concentration which was rapidly hydrolysed by human Nalma 71 cell extracts to p5'5'A2'p5'5'A2'p5'A and ppp5'5'A2'p5'5'A2'p5'A. This points to the possible use of A-capped (2-5)A as a depot form of the latter. Furthermore, the formation of only two products in the decapping reaction in Nalma cells suggests that the enzyme involved is one of a class of dinucleoside polyphosphatases, in this case the specific dinucleoside tetraphosphatase identified in a variety of cells, the products of which are a nucleoside 5'-phosphate and a nucleoside 5'-triphosphate (28). A similar enzyme has been purified from higher plants (29). The specificity of potato tuber nucleotide pyrophosphatase, which can decap intact mRNA, towards various dinucleoside polyphosphates has been recently examined (30).

Removal of the highly charged 5'-terminal triphosphate from (2-5)A, gives (A2'p)2A, referred to as (2-5)A-core, which should theoretically more readily penetrate intact cells. While some biological activity has been reported (2-5)A-core, including inhibition of DNA synthesis, attempts to demonstrate antiviral activity have been negative. It was subsequently found that the xyloadenosine analogue of (2-5)A-core, (XyloA2'p)xyloA, exhibits antiviral activity vs HSV types 1 and 2, a finding originally ascribed to its higher resistance to enzymatic degradation than (2-5)A-core, but subsequently found to be due to slow degradation, with release of xyloAMP, which, because of its resistance to deamination by adenosine deaminase, is more effective than xyloA (31). The foregoing also suggests that the core penetrates at least viral-infected cells. A somewhat analogous situation may prevail for the cordycepin (3'-deoxyadenosine) analogue of (2-5)A-core, initially reported to inhibit transformation by Epstein-Barr virus, and to inhibit DNA synthesis in mouse 3T3 fibroblasts more effectively than...
(2-5)A-core. It now appears that these activities are due to a sustained enzymatic release of cordycepin nucleotides (32).

**Vaccines**

These do not formally fall within the scope of this presentation, since vaccines are prepared for prophylactic purposes. Effective chemotherapy may eliminate the need for a given vaccine, and this is obviously a desirable goal. It should, however, be noted that there are instances where development of a vaccine poses formidable, if not in some cases insurmountable, problems. A case in point are the rhinoviruses responsible for the common cold, where the number of serotypes is so large, and identification of each type frequently so difficult, as to require a considerable number of vaccines to cope with the specificity of the immune reaction. Furthermore, the development of new vaccines by classical methods has become increasingly arduous and costly. A new vaccine was approved in 1981 by the FDA in the USA against hepatitis B. But the cost of production of this vaccine, from hepatitis B surface antigens isolated from the blood of carriers, is so high that its use is very limited, only to those at high risk. And last, but by no means least, this was the first new clinically approved vaccine in 10 years.

A new exciting development, of major interest to the natural products chemist, is the finding that antigenic determinants in a protein may be mimicked by short carrier-linked peptides, and that antibodies against such peptides recognize the corresponding sequence of amino acids with either the native or denatured protein. Hence, with only a restricted number of sites on the viral surface of importance as targets for neutralizing antibodies, it becomes feasible to produce synthetic analogues of these regions (a) by expression of viral proteins in recombinant heterologous cells, or (b) by chemical synthesis of the required short peptide sequences. Both of these approaches are now being intensively investigated. In particular, two out of seven chemically synthesized peptides, spanning the regions 141-160 and 200-213 of the VP1 polypeptide of foot-and-mouth disease virus, produced high levels of neutralizing antibodies on multiple inoculation into cattle and rabbits. Subsequent single inoculations in guinea pigs demonstrated that peptide 141-160, at levels as low as 200 ug, conferred complete protection against infection (33). While there are numerous associated problems to be resolved before the use of such vaccines in humans (34), such as the present need for a carrier, and of adjuvants, widespread efforts are already under way to produce synthetic vaccines against other viruses such as polio and even herpes, with very recent impressive results reported for hepatitis B (35), where a peptide sequence of 26 amino acids was effective. In fact, the resulting antibodies elicited by this vaccine are already being distributed for utilization for diagnostic tests. Furthermore, with present developments in the design and synthesis of biologically active polypeptides (36), one may envisage chemical modifications of synthetic antigens with the aim of modifying or enhancing their activities.

**AMANTADINE**

This tricyclic primary amine (1-aminoadamante.HCl, amantadine.HCl, Symmetrel, see Fig. 3) is an off-the-shelf compound uncovered during random screening as far back as 1963. It has had a chequered career regarding its efficacy in prophylaxis and therapy of influenza A infections. It was initially licensed in the USA in 1966, but for use only vs the Asian H2N2 strain, and subsequently against all A strains; and was early widely tested
and employed in the USSR (37). Many structural analogues have been synthesized and tested for improved activity (see ref. 38 for review), the most promising being rimantidine (\(\alpha\)-methyl-7-amino adamantane \(\cdot\) HCl).

Amantadine is by no means an ideal drug against influenza. However, in 1979 an exhaustive examination of available data by an international panel convened by NIH led to a recommendation for its widespread use for prophylaxis and therapy against all strains of influenza A (it is inactive against B strains). Also underlined was the necessity of accelerated research on amantadine analogues, in particular rimantidine. The latter now appears likely to replace amantadine because of fewer side effects, particularly lower CNS toxicity, most pronounced in older patients particularly susceptible to respiratory diseases.

Although extensively investigated, the mechanism of action of amantadine remains to be clarified. It is apparently one of the few antiviral drugs which is not metabolized in the cell, and acts as such. There is also some evidence that it interferes with the process of penetration through the cell membrane. However, under conditions where penetration has been established (by electron microscopy), synthesis of virus-specific RNA and early polypeptides could not be detected, suggesting that its site of action is at the stage of viral uncoating, or shortly thereafter (38). It is, indeed, surprising that more effort has not been devoted to this aspect, following its approval for widespread use in 1979. These aspects, and problems involved in clinical applications, were most recently reviewed by Oxford (39).

There are, at the moment, no better candidates against influenza, but clinical trials are under way with ribavirin (see below).

**PYROPHOSPHATE ANALOGUES**

It was long ago noted that some pyrophosphate analogues exhibit antiviral activity, the most active being phosphonoacetate (PAA, Fig. 4). However, because it exhibits pronounced dermal toxicity and is also deposited in the bone, it has not progressed beyond animal trials (40). It was subsequently found that another analogue, phosphonoacetate (PFA), is equally or even more active against some viruses, and much less toxic, and it is presently undergoing clinical trials for topical herpes infections (reviewed in ref. 41). Its potential systemic use for herpes and other viral diseases is in abeyance pending clarification of the effects of deposition in bone.

Both PAA and PFA are almost unique in that they act as such without being metabolized in the cell, their mode of action involving preferential inhibition of viral DNA synthesis in infected cells. In vitro studies have shown that these compounds interact with DNA (and, to a lesser extent, RNA) polymerases at the pyrophosphate binding site, and are non-competitive inhibitors with respect to nucleoside triphosphates, and competitive inhibitors of the pyrophosphate exchange reaction. Host cell DNA polymerase \(\alpha\), but not polymerases \(\beta\) and \(\gamma\), is also affected, but to a much lesser extent, the \(K_i\) for inhibition being up to 30-fold lower for herpes and some other viral polymerases. These findings have been further substantiated by genetic evidence (ref. 42 and references therein). It would nonetheless be of interest to examine the potential role of PAA and PFA in at least a few of the multitude of other biochemical reactions which involve pyrophosphate intermediates. It should be noted that phosphonates are fairly widespread in living cells and are the subject of growing interest (44). PFA has been reported to be a moderate inhibitor of influenza RNA polymerases (41).
PAA and PFA are relatively highly charged molecules at physiological pH, and one might therefore expect that they would not readily traverse the cell membrane (43). Their activities in vitro and in vivo indicate that they do, and it would be of obvious interest to establish to what extent they are taken up by normal and infected cells. No such data are presently available. It should be noted that, for PAA, \( pK_1 (P-OH) = 2.30 \), \( pK_2 (COOH) = 5.40 \), and \( pK_3 (P-OH) = 8.60 \) (40), whereas for PFA the corresponding values are \( pK_1 (P-OH) = 0.49 \), \( pK_2 (COOH) = 3.41 \), \( pK_3 (P-OH) = 7.27 \) (41). Hence at physiological pH PAA is appreciably less charged than PFA (in fact PAA is almost entirely in the form of the disodium salt, whereas 50% of PFA is the trisodium salt), and this may account in part for the observed differences in activity between the two. Aliphatic and aromatic mono-, di- and triesters of PFA have been prepared and their activities examined (45). None of them inhibited HSV-1 DNA polymerase in vitro. However, several exhibited effective antiviral activity. It follows that the esters undergo hydrolysis (probably by non-specific esterases) following entry to the cell. Presumably they would more readily traverse the cell membrane, followed by enzymatic release of PFA, but none of them was more effective than the parent PFA (45). It had previously been found that phosphonates and alkyl esters of nucleotides were usually no more active than the parent nucleosides, and in some instances less so (for review, see ref. 43).

**BENZIMIDAZOLE ANALOGUES**

Enviroxime, the syn and anti isomers of which are shown in Fig. 5, is cited here as a striking example of the problems that are encountered on the pathway from the lab to the clinic. Antiviral activity of benzimidazole analogues was noted more than 30 years ago. From a large number of synthetic analogues, one of the better known, 2-(α-hydroxybenzyl)benzimidazole was shown to be a specific inhibitor of picornaviruses from its ability to induce or select resistant mutants (46).

![Fig. 5. Syn and anti isomers of Enviroxime, 6-[(hydroxyimino)phenyl]methyl]-1-[(1-methylethyl)sulfonyl]-1H-benzimidazol-2-amine.](image)

Enviroxime, the syn and anti isomers of which are shown in Fig. 5, is cited here as a striking example of the problems that are encountered on the pathway from the lab to the clinic. Antiviral activity of benzimidazole analogues was noted more than 30 years ago. From a large number of synthetic analogues, one of the better known, 2-(α-hydroxybenzyl)benzimidazole was shown to be a specific inhibitor of picornaviruses from its ability to induce or select resistant mutants (46).

A highly organized and concerted effort by DeLong's group at Eli Lilly led to the two isomers of enviroxime (47). The significance of this new analogue resides in its high in vitro activity against all picornaviruses tested and, in particular, against all 43 of 43 rhinovirus types (subsequently extended to over 65), the anti isomer being about 10-fold more effective. The interest associated with this finding may be gauged from the difficulties accompanying the clinical diagnosis of type and strain of rhinovirus, and the consequent attendant problems involved in the development of a vaccine against the common cold. Tests on blood and lung levels in mice and dogs, and activity in rhinovirus-infected human organ cultures, all appeared promising. But there has been a sparsity of data on therapeutic indexes. It now appears that this promising compound may not be effective in clinical trials, although final reports must be awaited.

**NUCLEOSIDE ANALOGUES**

The high priority presently accorded to nucleoside analogues as antiviral (and, to some extent, antitumour and anti-parasitic) agents is based on the rationale, which can be traced back to Hitchings in the 1940's, of using purine and pyrimidine analogues for possible interference with nucleic acid metabolism. Pathways for incorporation of purines and pyrimidines into nucleic acids were little known at the time. But Hitchings selected this...
approach, at least in part, as "an appropriate quiet backwater where a small group might work relatively undisturbed by the pressures of intensive competition" (see ref. 48). We no longer have the "quiet backwater", but we do possess considerable knowledge of the biosynthetic steps involved in the synthesis of nucleic acids, including viral nucleic acids, as well as a multitude of nucleoside and nucleotide analogues which can interfere in known manners at various stages of these pathways.

It should be noted that nucleosides in general readily traverse cell membranes. Furthermore, as will be seen from what follows, antiviral activity of nucleoside analogues is frequently dependent on their prior intracellular "activation" or phosphorylation, by cellular and/or viral kinases, and frequent reference will be made to virus specific kinases which, in a number of instances, are decisive in determining the activity of a given analogue.

The multitude of nucleoside analogues hitherto synthesized and tested for antiviral (and frequently for antitumour) activities is too extensive to even summarize here. The following discussion is limited to several illustrative examples, with emphasis on chemical and biochemical aspects (bearing in mind that clinical aspects are the subject of another session at this Symposium). Fig. 6, which exhibits four types of nucleoside analogues with high antiviral activity, and which are presently the centre of interest, also illustrates some of the modifications introduced by synthetic methods. Reference may be made to specialized reviews, e.g. a recent one on 5-substituted pyrimidine nucleoside analogues (49).

Fig. 6. Four classes of nucleoside analogues with high antiviral activities (from left to right): Vidarabine (araA), Acycloguanosine (AcycloG, ACG, Acyclovir), 2'-fluoro-5'-iodo-araC (FIAC), (E)-5-(2-bromovinyl)-2'dUrd (BVDU).

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

This thymidine analogue is the most effective of a class of 5-(2-halogeno- vinyl)-2'-deoxyuridine derivatives (for review see ref. 50) with antiviral activities. It is a potent inhibitor of HSV-1 (but less so of HSV-2) and other herpesviruses, including varicella zoster, pseudorabies, bovine herpes 1, and is presently undergoing clinical trials. Its presumed mode of action was originally ascribed to: (a) specific phosphorylation to the 5'-phosphate by in HSV-1 and HSV-2 infected cells by the viral thymidine kinase; (b) subsequent phosphorylation to the 5'-pyrophosphate by the dTMP kinase activity associated with the thymidine kinase of HSV-1, but not HSV-2 (perhaps accounting for the lower activity of the analogue against HSV-2); (c) further phosphorylation by cellular kinase(s) to the 5'-triphosphate; (d) marked preferential inhibition of HSV DNA polymerase relative to cellular polymerases, as shown in Fig. 7, below.

It was subsequently found, in cell culture studies, that BVDUTP is incorporated into viral DNA, that such incorporation is accompanied by increased lability of the DNA, placed in evidence by appearance of single-strand breaks, and that the extent of incorporation correlates well with the level of antiviral activity (51). Incorporation of the analogue into cellular DNA occurs only at much higher concentrations of the drug, suggesting that incorporation into viral DNA may significantly contribute to the mode of action.

Similar incorporation of BVDUTP has been observed in viral DNA in bovine herpesvirus 1 (BHV-1)-infected cells, but apparently unaccompanied by lability of the DNA (52). By contrast, BVDU-treated cells released relatively little virus into the supernatant, and closer examination revealed that
glycosylation of BHV-1 glycoproteins was inhibited. This inhibition was dependent on prior phosphorylation of BVdU, at least (but not necessarily exclusively) to the 5'-monophosphate, by the viral thymidine kinase, since the glycoproteins of a thymidine kinase-deficient mutant of BHV-1 were unaffected. Subsequent to the foregoing, it was shown that BVdU also interferes with the glycosylation of HSV-1 late polypeptides (53). The mechanism of this inhibition remains to be clarified, but could conceivably be mediated via interference with the nucleotide coenzymes involved in carbohydrate synthesis.

Relevant to the foregoing is the findings of Barr et al. (54) that BV-dUMP, the initial product of phosphorylation of BVdU is an excellent "substrate" for thymidylate synthetase, which catalyzes conversion of the nucleotide to three diastereomeric products which were isolated and characterized. This reaction may not be of particular significance in HSV-1 infected cells, in which the viral kinase phosphorylates BVdU directly to the 5'-pyrophosphate. However, in HSV-2 infected cells the viral kinase phosphorylates the nucleoside only to the 5'-monophosphate, which may then be subject to attack by cellular thymidylate kinase before it can be further phosphorylated by cellular kinase(s). This may partially account for the lower potency of BVdU vs HSV-2. Furthermore the products of conversion of BVdUMP by thymidylate synthetase, which are all 5-substituted 2'-deoxyuridines, may conceivably also exhibit antimetabolite properties.

A new, not entirely unexpected, finding is that BVdU and other 5-vinyl-2'-deoxyuridine analogues are effective substrates for thymidine and uridine phosphoribosyltransferases. The resulting cleavage of the glycosidic bond in vivo is accompanied by liberation of the free base. The phosphorolytic reaction is reversible, so that addition of a deoxyribose donor like thymidine will regenerate BVdU in the plasma of patients (50). But the eventual therapeutic implications of these findings will have to be clarified.

**Fig. 7.** General mechanism of antiviral action of BVdU in herpes simplex type 1 infected cells. TK is thymidine kinase. See text, above, for additional details.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). This triazole nucleoside bears a formal structural resemblance to adenosine or guanosine (and even more so inosine), depend on the orientation of the carboxamide group at the ring C(3), as shown in Fig. 8. It has been the centre of attention for some time, and has undergone clinical trials in various countries (see refs. 55,56 for reviews), but with conflicting reports. Its continued interest stems from two facts: (a) its unusually broad spectrum of in vitro activity, embracing both RNA and DNA viruses, and (b) its potential utility against both influenza A and B, either alone or in combination with other drugs such as amantadine or rimantadine, and respiratory syncytial virus, by continuous aerosol delivery (e.g. ref. 57).

The possible mechanism of action of this compound still is to elucidated, but undoubtedly is not the same for all the viruses it is capable of inhibiting in vitro. Following entry into the cell, it is initially phosphorylated to the 5'-phosphate by adenosine kinase, but it is possible that other kinases may be involved as well. It is subsequently phosphorylated by cell-
ular kinases to the 5'-pyrophosphate and the 5'-triphosphate. Following the finding that the 5'-monophosphate is a potent inhibitor of IMP dehydrogenase, it was for a while considered that this activity, which would lead to a decrease in the GMP pool (IMP $\overset{\rightarrow}{\rightarrow}$ XMP $\overset{\rightarrow}{\rightarrow}$ GMP), accounted for inhibition of viral replication. However, it is unlikely that inhibition of this enzyme could be specific enough relative to cellular metabolism; and it was subsequently shown to be untenable by the finding that a related thiazole analogue, which equally inhibits this enzyme, exhibits only minimal antiviral activity (58).

It was subsequently found that the 5'-triphosphate of ribavirin is a reasonably good inhibitor of vaccinia virus mRNA guanyltransferase (Km $\approx 30 \mu M$), i.e. the initial step in capping of mRNA, by transfer of GMP from GTP to the 5'-terminus of viral mRNA (59). Again, however, there is no evidence that this inhibition is selective, or even preferential, for viral mRNA as compared to cellular mRNA. This finding clearly warrants further study with additional viral systems. By contrast, the 5'-triphosphate has been reported to be a selective inhibitor of influenza RNA polymerase (60); while the concentration required for 50% inhibition is rather high ( $\sim 100 \mu M$), such inhibition is in line with the reported activity of ribavirin against influenza virus (see above).

Particularly interesting was the observation that ribavirin inhibits assembly of mature infectious particles in Sindbis virus-infected Aedes albopictus cells (61). Infectious RNA synthesis proceeded normally, nucleocapsids were assembled, and envelope proteins synthesized and normally glycosylated. But no assembly of infectious virions occurred. Similar results were found with vaccinia, a DNA virus. This is one of the very few, if not only, instance of inhibition of assembly, and it is indeed surprising that it has not been further investigated. Relevant to this is the subsequent finding that ribavirin inhibits polyprotein synthesis of Simian (SA11) rotavirus in vitro and of murine rotavirus gastroenteritis in vivo (62).

Like chloramphenicol, 3-amino-1,2,4-triazole inhibits protein synthesis on mitochondrial ribosomes. Since the aglycone of ribavirin, 1,2,4-triazole-3-carboxamide (hence an analogue of 3-amino-1,2,4-triazole) also exhibits some antiviral activity (perhaps by conversion to the nucleoside by a nucleoside phosphorylase, followed by phosphorylation to the nucleotide), it is conceivable that it is active as such by inhibition of protein synthesis. The foregoing by no means exhausts all the possible mechanisms of action of ribavirin, and undoubtedly accounts in part for its broad spectrum of antiviral activity. Furthermore, it would be of interest to establish whether the 5'-triphosphate of ribavirin is a substrate for RNA polymerase and whether it undergoes incorporation into RNA.

\[\text{Fig. 8. Ribavirin (above) in two of its possible conformations, corresponding to the two rotamers of the 3-carboxamide group, and its resultant resemblance to adenosine (below, left) and guanosine (or inosine, below right).}\]
Vidarabine (9-β-D-arabinofuranosyladenine, araA)

On the basis of results of clinical trials, this compound has been approved for topical treatment of herpes keratitis. It is also the first antiviral agent to be licensed for systemic use, in treatment of proven cases of herpes encephalitis, varicella zoster virus infection in immunocompromized patients, and is under consideration for approval for therapy of neonatal herpes.

Notwithstanding the foregoing, the precise mechanism of action of this analogue is not clear. Intracellularly it is phosphorylated stepwise to the triphosphate, principally by cellular kinases. The extent of involvement of viral kinases, if at all, remains to be established. The 5'-triphosphate is a preferential inhibitor of HSV DNA polymerase, but is incorporated into both viral and cellular DNA. It is also an inhibitor of ribonucleoside diphosphate reductase, of RNA-dependent RNA polymerase, and several other enzyme systems (reviewed in ref. 64).

Although araATP slows DNA elongation, it is now reasonably well established that it is incorporated internally into DNA, but not RNA, and that such DNA is inherently more alkali labile than normal DNA, as a result of strand scission at the 3'-carbon of the incorporated arabinosyl sugar without degradation of the araA moiety itself (65). It is conceivable that such incorporation, and accompanying lability, may be more pronounced with viral, relative to cellular, DNA. In general, it is somewhat disconcerting that the mode of action of one of the most widely approved antiviral agents is presently so ill-defined, and represents a real challenge to further progress along rational lines (66).

Indeed, if a quantitative comparison is made between different nucleosides for in vitro antiviral activity and cytotoxicity (Table 1), it will be seen that araA appears the least promising against HSV-1 and HSV-2, although araA is more effective against vaccinia virus (67). However, the host cell system employed may, in many instances, appreciably affect the validity of such comparisons, and examples have been cited above where there are apparent differences in mechanism(s) of action in different host cells. Note, from Table 1, the high activities of BVdU, FIAC and acycloG (Fig. 6) and their low cytotoxicities. Attention should also be drawn to one of the earliest and simplest of thymidine analogues, 5-ethyl-2'-deoxyuridine (68,69), which is still a reasonable candidate against HSV-1 and HSV-2, and is in fact licensed for use in several European countries.
Of the multitude of nucleoside analogues hitherto examined for antiviral activity, none has aroused as much interest as the class of acyclonucleosides shown in Fig. 9, initiated in 1977 by the report of Elion et al. (70) that 9-(2-hydroxyethoxyethyl)guanine (acycloG, acyclovir) is a potent agent vs HSV-1 and HSV-2 in vitro. This finding was rapidly extended to include animal tests and pharmacological studies, with the result that acyclovir acquired the unusual distinction of being licensed for clinical use in 1982, only 5 years after the first laboratory report. It is available in different forms for treatment of ocular keratitis, genital herpes and varicella zoster lesions. This event has stimulated the synthesis, and tests for antimetabolic activities, of an impressive number of such acyclonucleoside analogues with purine, pyrimidine and other heterocyclic bases. A symposium was recently devoted to clinical applications of acyclovir (71).

Mechanism of action of acyclovir
During its short course from the lab bench to the clinic, significant progress was made in elucidation of the mode of action of acyclovir, viz. (a) phosphorylation to the monophosphate, in infected cells only, by the HSV-coded thymidine/deoxycytidine kinase; (b) subsequent phosphorylation to the pyrophosphate by cellular GMP kinase; (c) further phosphorylation to the triphosphate by several cellular, principally glycerophosphate, kinases (72); (d) preferential inhibition by acycloGTP of the viral-coded, relative to cellular, DNA polymerase; (e) incorporation into, and chain termination of, oligonucleotides which are also potent inhibitors of the viral polymerase (73).

Role of viral kinase. The key role of the viral thymidine/deoxycytidine kinase is underlined by the fact that acyclovir is inactive against pseudorabies (a member of the herpes group) and vaccinia viruses. However, in host cells biochemically transformed so that they express the HSV-1 kinase gene, the drug is equally effective against both these viruses. It follows that the kinase activities of pseudorabies and vaccinia viruses, which differ from each other and from the corresponding cellular kinases (74), also differ distinctly from HSV kinase; and that the triphosphate of acyclovir is an effective inhibitor of pseudorabies and vaccinia DNA polymerases. The differences in specificities of the viral kinases also account for the narrow spectrum of antiviral activity of acyclovir. While at first sight unusual that a viral thymidine/deoxycytidine kinase phosphorylates a nucleoside analogue with a purine base, it should be noted that the so-called deoxycytidine kinase of calf thymus also phosphorylates deoxyadenosine and deoxyguanosine (75). The adenine analogue of acycloG, and acyclonucleosides with pyrimidine bases, are very poor substrates for HSV kinase (72).

9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG)
Extensive structure-activity relationships led to the almost simultaneous synthesis in several laboratories of DHPG (76,77,78), depicted in Fig. 9.
This compound is reported to be a much more effective substrate for HSV-1 thymidine kinase, with a value of $V_{\text{max}}/K_m$ 50-fold higher than for acyclovir, while the monophosphate is a more effective substrate for cellular GMP kinase, the $V_{\text{max}}/K_m$ being almost 500-fold that for acycloGMP (78). The result is more rapid formation, in infected cells, of the triphosphate, which is an effective and inhibitory of the viral polymerase as acycloGMP. In vitro results have been forthcoming at a rapid pace, and are all consistently promising. The analogue is as effective, or more so, against HSV-1, HSV-2 and VSV, than the parent acyclovir, and also exhibits a broader spectrum of activity in that it is also effective against human cytomegalovirus and Epstein-Barr virus at concentrations that do not affect cell growth (79). Results of animal tests from two different laboratories underline its low cytotoxicity in mice and up to a 50-fold higher efficacy in treatment of systemic or local HSV-1 infections or HSV-2 intravaginal infections.

More detailed in vitro studies showed that activity vs HSV-1 is dependent on induction and properties of the viral thymidine kinase. Virus variants with altered kinase, resistant to acyclovir, retained their sensitivity to DHPG, which was also active against 5 different HSV variants with altered DNA polymerase activities and resistance to acyclovir. Particularly interesting was the finding that DHPG was incorporated internally into DNA strands (80). In cytomegalovirus-infected cells, DHPG, at concentrations of 2-4 $\mu$M, not only suppressed viral DNA synthesis, but also inhibited the synthesis of 6 virus-specific polypeptides, whereas concentrations up to 100 $\mu$M did not affect uninfected cell macromolecular synthesis or cell growth (79). Since there are still doubts as to whether human cytomegalovirus encodes a specific thymidine kinase, the mechanism of action of DHPG may differ from that against HSV. While it is too early to predict the clinical utility of DHPG in HSV infections, its potential use against cytomegalovirus infections is at the moment under investigation.

3'-Branched and $\alpha$-anomeric nucleosides. The surprisingly more rapid rate of phosphorylation of DHPG, relative to acyclovir, by the viral kinase is possibly relevant to the behaviour of 3'-branched analogues of the antitumour compound 6-thio-2' deoxyguanosine (TGdR) described by Acton et al. (81). From the observation that $\alpha$-TGdR exhibits antitumour activity, is phosphorylated in tumour cells (but not in bone marrow cells, hence its low toxicity), and is eventually incorporated into short DNA chains as a terminator, it was reasoned, as earlier proposed by Horton and Sakota (82), that the OH functions at the 3' and 5' of 2'-deoxyribosides are essentially interchangeable, and that the furanose ring O and the 2'-CH$_2$ are also interchangeable. From this, it was concluded that the OH functions should be improved if a primary CH$_2$OH were present at both 5' and 3'. This led to the synthesis of the 3'-CH$_2$OH derivatives of $\alpha$- and $\beta$-TGdR (Fig. 10). Both of these were, in fact, significantly and equally more active, and were phosphorylated and incorporated into DNA (as chain terminators) 3-fold more rapidly than $\alpha$-TGdR. Furthermore, nucleotides of the three foregoing were totally cleaved by 5'-nucleotidase, an enzyme system for which some conformational requirements have been reported (83). Although it was subsequently found that the 3'-branched $\alpha$-TGdR is too toxic (because it is phosphorylated by bone marrow kinases), this problem is worthy of further investigation, particularly from an enzymological point of view, as pointed out elsewhere (84). 3'-Branched nucleosides are usually obtained by prior preparation of the branched sugar and condensation with the appropriate base (85); but Shuto et al. (85) have described a synthesis of the two epimers of 3'-hydroxymethyl-2'-deoxyuridine from uridine by reactions involving

![Fig. 10](image-url)
ring-expansion and reconstitution of the ribose moiety, probably applicable to other nucleosides. Furthermore, the foregoing underlines once again the utility of examining the potential antimitabolic properties of the α anomers of nucleosides, pointed out some years ago (86), and now being actively pursued in some laboratories (e.g., ref. 87).

9-Dihydroxypropyladenine and 9-dihydroxybutylguanine
In contrast to acycloG and DHFG, these acyclonucleoside analogues retain the lower portion of the pentose ring (see Fig. 10). (9) 9-(2,3-dihydroxypropyl)adenine, but not the R enantiomer, is active against a number of RNA and DNA viruses, but at considerably higher concentrations than acycloG or DHFG. It was the only one, out of 68 analogues with various heterocyclic bases and with various modifications of the acyclic moiety, to exhibit such activity (86). Its mode of action has not been elucidated, but it must obviously differ from those of ACG and DHFG. It has hitherto not been considered a candidate for clinical trials. By contrast, modification and extension of the acyclic moiety to include a terminal primary hydroxyl, with guanine as the base residue, led to 9-(3,4-dihydroxybutyl)guanine, which proved a reasonably good in vitro inhibitor of HSV-1 and 2, and reasonably effective in animal trials, the R enantiomer being more inhibitory than the S (89). Its mode of action, like that of ACG, appears to involve phosphorylation, initially by the HSV kinase, and subsequent inhibition of viral DNA synthesis. It should be noted that it had previously been found that 9-[(R)-3,4-dihydroxybutyl] adenine is inactive (86), most likely because it was not a substrate for the viral kinase.

Additional activities of acyclonucleosides
In addition to antiviral activity, acyclonucleosides and nucleotides have been found to exhibit some interesting properties in other enzyme systems. The classic example is the work of Schaeffer and coworkers (see ref. 90) on the development of an adenosine deaminase inhibitor, in what constitutes one of the best examples of the use of structure-activity relationships with a specific goal, and culminating in the synthesis of the potent inhibitor EHNA, a racemic mixture of erythro-(-)-9-(2R-hydroxy-3S-nonyl)adenine and its (2S,3R) epimer, with a K of 10^-9 M. Although two better inhibitors are now known, coformycin and 2'-deoxycoformycin, EHNA possesses two advantages over these, its more ready reversibility of inhibition and its lower cytotoxicity. It has been advocated as the inhibitor of choice for use with antiviral agents which are readily deaminated intracellularly, such as araA (91,92). Furthermore EHNA itself exhibits potent antiviral activity (91), although its mechanism of action is not known.

More recently it was found that 1-(2-hydroxyethoxymethyl) derivatives of uracil and some 5-substituted uracils are fairly good inhibitors of uridine phosphorylase, the most potent being acyclothymine, with a K of 3 μM for inhibition of phosphorolysis (the reverse synthetic reaction was not studied). By combining this property with the known inhibitory properties of 5-benzyluracil and 5-benzoylxybenzyluracil, Niedzwicki et al. (93) were led to the 1-(2-hydroxyethoxymethyl) analogue of 5-benzoylxybenzyluracil, presently the most potent inhibitor of uridine phosphorylase (K = 30 μM), at least for the phosphorolytic reaction. The inhibition is fairly specific inasmuch as there was no significant effect on thymidine phosphorylase. It would be of interest to examine the effects of these compounds on uridine-thymidine phosphorylase. In a study still in progress, we have both confirmed and extended the foregoing results with a highly purified uridine phosphorylase from E. coli (A. Drabikowska et al., in preparation). The inhibitory properties of the foregoing analogues are of considerable interest in relation to their conformational properties on interaction with the enzyme, since it has been shown that phosphorolysis of uridine, and 5-substituted uridines, proceeds via an intermediate state in the syn conformation about the glycosidic bond (94).

It has also been reported (95) that acycloG is a weak inhibitor of purine nucleoside phosphorylase (K = 90 μM), but only some of its minor intracellular metabolites, 6-hydroxy-acycloG, is much more potent (K = 5 μM). By contrast, acycloG's 5'-phosphate (physiological concentration), is reduced to 10 μM, hence the most potent inhibitor of this enzyme presently known. From the measured levels of phosphates of acycloG in different tissues in vivo, it appeared unlikely that the mode of action of acycloG involves purine nucleoside phosphorylase (PNP). Since one of the major goals in the search for PNP inhibitors is the fact that a genetic deficiency of this enzyme is accomp-
enied by impaired cellular, but not humoral, immunity, it was noted that the use of doses 10-40-fold higher than that recommended for systemic use as an antiviral agent apparently has little or no effect on a variety of immune-related functions in vivo (95). In a study currently under way in our laboratories, with the aid of a new continuous fluorimetric assay for PNP (E. Kulikowska et al., in preparation), it has been found that DHPG is about 3-fold more effective an inhibitor than acyclovir, and 2',3'-seca guanosine equally or slightly more effective.

Conformational aspects. The acyclonucleosides exhibited in Fig. 10 are depicted in such a manner as to illustrate the possible resemblance of the acyclic chain to the pentose ring. For each of the analogues shown this is, obviously, only one of a multitude of possible equilibrium conformations in solution. It will clearly be of interest to establish the specific conformation adopted by each of these on interaction with an appropriate enzyme for which it is either a substrate or inhibitor, e.g. the HSV thymidine kinases. The development of a recombinant procedure for cloning HSV-1 thymidine kinase in E. coli (96) should furnish adequate quantities of enzyme for such investigations. Meanwhile, solid-state structures have been determined by means of X-ray diffraction for acyclovir (97), for DHDP, DHDP (R. L. Tolman, personal communication) and several other acyclonucleosides (see ref. 98 for review), and for the 2',3'-seca nucleoside of 5,6-dichlorobenzimidazole (G. I. Birmbaum et al., Foster Session, this Symposium). Solution conformations are now under investigation by NMR spectroscopy.

TARGETS FOR ANTIVIRAL ACTION

From the rather brief account presented above, it must be admitted that more satisfactory antiviral agents are still an important goal, and we are far from possessing a drug that even approaches the level of one of the common antibiotics. On the other hand, a good deal has been learnt about the steps required in further research, particularly about the nature of at least some of the "targets" which might be effectively attacked; and a recent symposium, devoted entirely to this subject (99), in which an overall survey of possible targets for viral chemotherapy (100). Although several compounds are now known which inhibit such processes as uncoating, assembly, etc. (see above), the major emphasis at the moment is on viral-encoded enzymes, a good example being PFA, which appears to act largely as a preferential inhibitor of herpes simplex DNA polymerase. It is therefore to be anticipated that isolation, and characterization of the properties of viral enzymes is one of the important requirements for further progress. Prior attempts to study currently reductases of HSV-1, HSV-2 and pseudorabies virus (101,102,103). Meanwhile there are already a number of examples of the use of highly sensitive enzyme assays for the rapid clinical diagnosis of virus infections, based on detection in physiological fluids of a specific viral enzyme, e.g. diagnosis of varicella-zoster virus infection by monitoring the level of viral thymidine kinase in serum and vesicle fluids (104). The above-mentioned ribonucleotide reductases are equally sufficiently different in various properties from the corresponding cellular enzymes as to permit of such applications, as are other enzymes referred to above, such as RNA and DNA polymerases.

Acknowledgment - The foregoing, and some of the results reported, were supported by the Polish National Cancer Research Program (PR-6) and the Polish Academy of Sciences (No. 09.1.3).

REFERENCES


28. B. Robson, No. 23, 239-244 (1980).


