PHARMACOLOGY OF VIRUSES[1]

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INTRODUCTION

Although each year numerous reports appear which describe new antiviral agents, only two have been approved by the FDA for therapy in man —idoxuridine (IUdR, IDU, 5-iodo-2′-deoxyuridine) and amantadine (adamantanamine·HCl, Symmetrel). An obvious concern is the relation between the great expenditure of time, effort, and financial resources and the paucity of practical results. A concomitant question is whether the approach that has been taken for either the synthesis of new compounds or the systems used to evaluate these compounds is wise and relevant. There is good precedence for random screening of compounds to produce efficacious drugs, since the majority of present day drugs in clinical use has been derived from just such an approach. Logic indicates that one cannot abandon a procedure that has been successful. The question posed is whether another direction, the so called "rational approach", is feasible today. Examples of the two contrasting methods for design of drugs that have been successful are the synthesis of effective antimalarials and of British Anti Lewisite (BAL). Through the combined efforts of British and American organic chemists thousands of potential antimalarials were synthesized, a feat that resulted successfully in the finding of efficacious compounds. In contrast with this herculean effort, BAL represented one of the first few compounds synthesized in an attempt to find an effective antidote to the lethal poison agent Lewisite. Why this difference in effort to achieve success? BAL was designed on a rational basis because appropriate effort went into elucidation of the biochemical mechanism for the toxicity of Lewisite. Thus, adequate molecular understanding resulted in appropriate or relevant synthesis. Similar biochemical understanding of the malarial parasite was not and is still not available. Therefore, one is left with random modification of known effective agents with the hope that compounds with improved antimalarial activity will evolve.

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Fortunately, a strong effort is being made today to attain understanding of animal virus formation and composition at the molecular level as well as an understanding of the biochemistry of the host-virus interrelationship. The hope, and as of today it still remains a hope, is that in time one will be able to duplicate the BAL success in the synthesis of clinically useful antiviral agents. What can be done will be discussed below.

SITES OF INHIBITION BY ANTIVIRAL AGENTS

Direct Inactivation

An agent that inactivates the virion present either extra- or intracellularly would serve as an effective prophylactic agent. Numerous compounds have been observed *in vitro* that exert their prime effect by direct inactivation of extracellular viruses. A compound that exerts its effect by direct inactivation of progeny virus prior to extrusion from the cell would also effectively prevent spread of the virus to other cells. No synthetic compound with acceptable host toxicity has been described however, with the possible exception of kethoxal (α keto-β-ethoxybutyraldehyde hydrate), a compound that has been reported to be effective against herpes simplex infection of the skin in hairless mice and baby rabbits (1). Previous studies (2) of kethoxal demonstrated potent direct inactivation of vaccinia virus and Newcastle disease virus but not of polio-2 virus. Underwood (1) proposed that this compound merits clinical evaluation in cutaneous herpes simplex infections in man. The problems concerned in the therapy of this disease with drugs are discussed below.

2-Thiouracil is a potent inhibitor of the reproduction of plant viruses [literature cited in (3)] and more recently Steele & Black (4) demonstrated that this compound caused a decrease in the infectivity of polio-virus. They observed that an oxidized form of 2-thiouracil reacted with the capsid sulfhydryl groups. This type of compound might have utility as a prophylactic agent and perhaps merits further study with other viruses for which an effective vaccine is not available.

Endogenous or exogenous antibodies are effective in preventing the penetration of the virus into the cell, and should prevent viral spread provided the virus has an extracellular period before transmission into a second cell. The role of antibody therapy has been reviewed recently (5).

Inhibition of Absorption and Penetration

Reproduction of a virus requires that the virus adsorb to the cell membrane and penetrate into the cell. Adsorption may necessitate either a physicochemical interaction with a specific portion or "receptor" of the cellular membrane or a nonspecific adsorption mechanism. What are the forces involved and how can they be interfered with? Are their specific binding sites on the cellular membrane? Of pertinence may be the studies of Choi & Kay (6), who observed that treatment of Ehrlich-Lettré-ascites carcinoma cells

with neuraminidase, phospholipase H, or pronase inhibits the uptake of protein. The uptake of protein is an energy-dependent process (7).

Amantadine (adamantanamine, Symmetrel).—has been reported to exert its effect by preventing penetration but not adsorption of influenza A-2 virus (8, 9). Kato & Eggers (10), however, believe the evidence provided for this mechanism is incomplete and, therefore, not conclusive. Studies by Kato & Eggers (10) of the inhibitory effect by amantadine on fowl plague virus reproduction provide evidence to support a hypothesis that this compound exerts its prime effect by inhibition of the uncoating of the virus. These studies open a new dimension in the elucidation of the site or mechanism of inhibition that may be of value in the design of appropriate derivatives.

In addition to the inhibition of influenza virus and fowl plague virus, amantadine demonstrates antiviral activity against two oncogenic viruses, Rous and ESH sarcoma virus (11) and the cytomegalo-virus (12).

This compound has been approved by the FDA for use in man for the prevention of illness caused by influenza A-2 virus. Immediate protection is provided which continues as long as the drug is ingested. It is a prophylactic agent and is not effective after clinical signs of infection appear. Reviews of the early studies have been presented (13).

There has been considerable controversy concerning the use of amantadine in man (14, 15), and recent clinical studies (16, 17) indicate that amantadine does not affect the infection rate but rather has a suppressing effect on many of the clinical symptoms. Both in experimental animals and man amantadine has decreased the anti-influenzal antibody and the question to be answered is whether this was caused by either an interference with replication of the virus or a direct effect on the ability to synthesize antibodies. The studies of Maciag & Hoffman (18) support the former mechanism.

The drug is well absorbed orally and is highly stable with 56 percent and 86 percent being excreted within 24 and 96 hours respectively. Because of this, accumulation of amantadine can occur with daily continued dosage and this may explain the problems encountered even on the recommended regimen of 200 mg daily. Doses of 300 to 400 mg per day may cause marked CNS disturbances similar to that seen in some patients receiving the recommended dose. This is not surprising to see with a drug that not only has a therapeutic dose which is ½ to ⅔ of a toxic dose but also has a rate of excretion that permits rapid accumulation.

Lavrov and coworkers (19) have attempted to retain or increase the antiviral prophylactic activity of amantadine with concomitant reduction in its dosage to decrease toxicity by combining it with interferon, a combination that demonstrated a synergistic effect in cell culture. They suggest a similar approach should be investigated in man. If adamantadine were to be used on a mass scale, an approach of this type is clearly indicated.

Adamantadine derivatives.—Various derivatives of adamantadine have been prepared and of these rimantadine hydrochloride (α methyl-1-adamantanemethylamine-HCl) is in clinical trial. This compound is a more

effective antiviral agent than amantadine in tissue culture (*20*), and in mice (*21*). Rimantadine, like amantadine, is effective in producing prophylaxis in man (*22*). Additional studies will be required to evaluate properly the future role of this derivative of amantadine in man. The hope for the future with this type of compound is the synthesis of one with a greater therapeutic index and with less selectivity for the various strains of influenza virus that nature is unfortunately so capable of rapidly evolving.

1-Adamantylguanidine is a derivative of amantadine that has enhanced basicity because of replacement of the amino group by a guanidino group (*23*). Although the activity of this compound *in vitro* was comparable with amantadine, its activity *in vivo* was less.

Inhibition of an Intracellular Event

Following penetration of the virus into the cell and subsequent release of its nucleic acid, a vast array of events occur for which only partially sophisticated knowledge for a relatively few viruses exists. Although it is not the purpose of this review to explore the present status of our understanding of viral biochemistry, aspects relevant to an understanding of sites of inhibition of present antiviral agents as well as for development of potential antiviral agents will be discussed. A few representative references concerned with the biology and chemistry of viruses are listed (*24–26*).

Halogenated pyrimidinedeoxyribonucleosides.—(a) molecular and in vitro *studies of IUdR:* The chemistry, biochemistry, and clinical applications of these compounds appeared in a number of reviews (*13, 27–29*). Of the various halogenated derivatives, that substituted with iodine in the 5-position of the pyrimidine moiety has been most effective clinically as an antiviral agent, whereas the corresponding fluoro-derivative is most valuable as an antineoplastic agent. Such differences in biological activity may be understood from a knowledge of the chemistry of these compounds. Not only is there variation of the van der Waals radius of the 5-substituent, a factor that produces stereochemical effects, but also the distribution of electrons within the molecule is affected to varying extents, thereby resulting in dramatic chemical and biological effects.

Among the various halogenated derivatives, IUdR has achieved the status of a drug permitted by the Food and Drug Administration to be sold in the United States for the therapy of herpetic keratitis in man. Therefore, most of the discussion in this section will be devoted to this compound.

Extensive biochemical investigations with IUdR have revealed three major areas where this agent or the appropriate phosphorylated derivative exert marked inhibitory effects: (*a*) competitive inhibition of several enzymes concerned with the biosynthesis of DNA-thymine (thymidine kinase, thymidylate kinase and DNA polymerase) due to the analogue or the appropriate phosphorylated derivative functioning as an alternate substrate (*30*); (*b*) allosteric or feedback inhibition by the triphosphate of IUdR, mimicking the normal regulatory activity of deoxythymidine triphosphate of

thymidine kinase (31), deoxycytidylate deaminase (32) and cytidine diphosphate reductase (33); (*c*) incorporation into DNA with subsequent effect in the expression of genetic information either during replication or transcription [for references see (3, 28, 34–37)].

The first two areas of inhibition were investigated in virus infected cells because of the possibility that the enzymes induced by the virus (either via the host or viral genome) were more susceptible to inhibition by IUdR or its phosphorylated derivatives. No differences were observed in susceptibility to inhibition by IUdR or its monophosphate derivative of thymidine kinase and thymidylic acid kinase of mammalian cells before and after infection with herpes simplex virus (28); similarly there was no difference in the susceptibility to inhibition by IUdR triphosphate of DNA polymerase derived from cells before and after infection with herpes simplex virus (38). No difference was observed in susceptibility to inhibition of thymidine kinase by deoxythymidine triphosphate in noninfected cells and in cells infected with adenovirus (39) or with SV40 or herpes simplex viruses (40). However, thymidine kinase specified by herpes simplex virus (41) and by vaccinia virus (42) was reported to be significantly less susceptible to inhibition by deoxythymidine triphosphate than the enzyme present in the uninfected host cell. Whether a difference in host cell or strain of herpes virus is responsible for the difference observed in the inhibition of thymidine kinase is not known. A comparison was made of the relative susceptibility of deoxycytidylate deaminase to inhibition by IUdR-triphosphate and by deoxythymidine triphosphate. Levels of the enzyme present in uninfected cells and in cells infected with herpes simplex virus showed that although the amount of enzymic activity increased almost threefold in infection, there was no alteration in the susceptibility of the "induced" enzyme by either of the triphosphate derivatives (43).

The inhibitory effects of IUdR or its mono- or triphosphate derivative on the various enzymes that have been investigated are of the competitive type and hence are readily reversible either by increase in the normal substrate or by metabolic conversion of the appropriate analogue. We believe that the primary site of inhibition by IUdR is in an event that is subsequent to incorporation of the antimetabolite into the viral DNA in substitution of the thymidine moiety. A number of very important biochemical and physical effects result from such incorporation of halogenated deoxyribonucleosides into nucleic acid (3, 28, 34, 44, 45).

Although incorporation of halogenated deoxyribonucleosides into DNA has no effect on the specific transforming activity of the DNA, certain genetic markers are affected (46). Positive biological effects from such incorporation into DNA include: (*a*) increased rate of mutation, (*b*) increased number of errors in protein formation, (*c*) inhibition of cellular reproduction, and (*d*) increased sensitivity to X- and ultraviolet radiations, mitomycin C, and some radiomimetic alkylating agents.

Recent studies in our laboratory have been concerned with the biophysi-

cal and biological consequence of incorporation of IUdR into viral DNA (35–37, 47). Bacteriophage T4 has been used initially as a model system. Phage, in which 60 percent or more of the DNA-thymidine was replaced with IUdR, were evaluated for their ability to induce phage-specific enzymes in *E. coli* cultured in a medium not containing IUdR. The IUdR-substituted phage were unable to induce normal levels of activity of 5′-deoxycytidylate hydroxymethylase and lysozyme. Further, the appearance of these enzymic activities was delayed relative to that observed with normal phage. Dihydrofolate reductase activity followed a similar pattern. These effects were shown not to be caused by either poor adsorption of the phage, faulty injection of the phage DNA, or the production of an inhibitor. Presumably these events are a result of mispairing of bases in DNA with subsequent production of a "fraudulent" mRNA which either may be unable to direct protein synthesis or may result in the formation of an enzyme that has less or no activity.

DNA contains clusters of thymine of varying length and hence it is not unlikely that the different genes that control the synthesis of enyzmes, structural proteins, or other functions may be differentially affected dependent upon the extent of DNA-thymidine replacement by IUdR. Experiments by Goz & Prusoff (36, 37) indicate that a differential effect on the expression of the various genes of the phage T4 genome is indeed observed. These studies (36, 37), as well as those of Byrd & Prusoff (48), indicate that the presence of IUdR in the medium inoculated with unsubstituted phage does not affect the number of phage particles formed, but rather, most of the phage particles produced are unable to form plaques. Incorporation of halogenated uracil derivatives into the nucleic acids of animal viruses has been demonstrated [literature cited in (13)], and very marked inhibition of the formation of infectious virus particles has been observed with ratios of virus particles to infectious virus being as high as 10^7:1 (49).

Kaplan and co-workers (50, 51) have investigated the effect of IUdR on the biochemical events involved in the formation of pseudorabies virus and their observations support the concept that replacement of DNA-thymine by 5-iodouracil results either in an inability of the virus to form the protein coat or in the formation of an inadequate one. The noninfectivity of BUdR-containing virus was postulated to be a result of an inability to induce early enzymes or a failure to be adsorbed, to penetrate, or to uncoat because of a faulty protein coat (50, 52).

(*b*) *biological studies of IUdR:* Only those studies that have been performed since 1966 will be discussed because pertinent investigations reported up to that time have been reviewed adequately (13). It perhaps merits reiteration that Herrmann (53) observed originally that IUdR and BUdR inhibited the replication of several DNA-containing viruses *in vitro,* particularly vaccinia and herpes simplex, and that Kaufman and his collaborators [literature cited in (54)] demonstrated the efficacy of this compound in rabbits and more importantly in man. Numerous reports have confirmed

the efficacy of IUdR in the treatment of herpes simplex infections of the corneal epithelium in man, as well as its questionable merit in the deeper stromal infections [literature cited in (54, 55)]. The major question of concern here is whether the failure to affect the stromal infections is a result of an inability of the compound to penetrate to the cells where the virus is reproducing, or whether the compound is capable of penetration but is rapidly catabolized by these tissues before an effective antiviral concentration can be achieved. The approach to a solution of this problem is dependent upon acquisition of such knowledge. Penetration problems may be solved by the use of solvents that are not toxic to the eye tissues, whereas catabolic problems would require the use of one of several techniques to circumvent such destructive enzyme activity. Examples of two approaches that may be used will be described. The first approach involves the co-administration of IUdR and a compound such as deoxyglucosylthymine (1(3)-[2-deoxy-D-arabino-hexopyranosyl]-thymine) which has been reported by Langen & Etzold (56, 57) to inhibit uridine-deoxyuridine phosphorylase, an enzyme that catabolizes thymidine and IUdR. Unfortunately it does not inhibit a second enzyme, thymidine phosphorylase, which is present also in many tissues and can catabolize thymidine and IUdR. Whether both enzymes exist in the eye tissues of man should be investigated, since these workers have observed an enhancement of IUdR incorporation into the DNA of cat tissues *in vivo* by virtue of inhibition of uridine-deoxyuridine phosphorylase. The second approach involves the administration of IUdR as 5-iodo-2′-deoxycytidine-5′-phosphate salt-linked to the polycation, polyethyleneimine, which Woodman (58, 59) has demonstrated to result in an increased uptake of iodine into DNA of tumor tissue. It is postulated that such a complex enhanced transport specifically across tumor cell membranes, and the hope would be that a similarly enhanced uptake would occur in viral infected cells. Such a complex may have not only greater penetrability but also afford protection from enzyme degradation prior to transport into the infected cells.

Topical therapy of cutaneous herpes simplex infections is still in a controversial stage since previously reviewed (13). Positive claims have been made for a decrease in the time required for healing of acute infections, an increase in time between recurrences as well as prevention of recurrences. The major problem appears to be one of penetration of IUdR into the cells where the virus resides. The previous report by MacCallum & Juel-Jensen (60) in which successful treatment of herpes simplex infection of the skin in man by application of a 5 percent solution of IUdR in dimethylsulfoxide merits additional investigation.

Herpes zoster (shingles) has been treated in man by topical application of a 0.1 percent solution of IUdR but was not found to be effective (61). Therapy of herpes genitalis in man has been described and statistically valid beneficial effects were reported; however, considerably more controlled studies are required (62, 63).

Herpes simplex encephalitis was reported by Breeden and co-workers

(64) to be affected beneficially by an intravenous injection of IUdR after surgical decompression, and they suggested that further trials of the drug in this disease are indicated. There have been other reports describing the use of IUdR in this disease with generally encouraging results (65–71). Although most of these findings are encouraging, considerably more patients will be required for study before the role of IUdR in this disease entity is elucidated. It should be stressed that virological studies are required for definitive diagnosis if therapy with IUdR is to be meaningful, since herpes simplex etiology has been implicated in only about 10 percent of the established cases of encephalitis (72, 73).

Congenital cytomegalovirus infection has been treated with 5 fluoro-2′-deoxyuridine (74) and with IUdR (75) with definite clinical improvement in both cases. These provocative findings merit additional study. Recent studies have shown an inhibition of this virus by 6-azauridine (76).

Partridge & Millis (77) treated a newborn infant who developed systemic herpes simplex infection with IUdR and clinical improvement was observed. Five days after cessation of therapy the infant had a relapse and after an additional 5 days the baby died. No virus could be isolated from post-mortem tissues and it was believed that a secondary bacterial infection may have been the responsible agent for death. These investigators recommended that additional trials of IUdR in neonatal systemic herpes simplex is indicated.

Although methisazone can prevent smallpox in individuals exposed to patients with smallpox, it has no therapeutic value after onset of even the early manifestation of this disease. Preliminary evaluation of IUdR [see (13)] although provocative was far from conclusive because of the limited number of patients. Unfortunately, a clinical study of the potential usefulness of this compound has not appeared.

In cell culture the range of viruses whose reproduction is inhibited by IUdR has been widened to include infectious bovine rhinotracheitis virus (78), H-1 virus which is also inhibited by cytosine arabinoside (79), and Herpes virus simiae (B virus) which is responsible for a lethal myelitis in man when infected by monkey bites (80).

A prerequisite for successful therapy of viral diseases of the central nervous system as well as other tissues is attainment of an adequate concentration of the drug in the infected cells. The drug may be administered intravenously with the hope that either the compound equilibrates well with the cerebrospinal fluid or the infection in the CNS causes a breakdown in the "blood-brain barrier" with concomitant increased penetration of the drug into the CNS. The other more direct approach is by intrathecal injection, thereby bypassing the transport mechanism involved in the "blood-brain barrier." Clarkson, Oppelt & Byvoet (81) studied the distribution and metabolism of IUdR in the cerebrospinal fluid and plasma of normal dogs after intravenous and intracisternal injection. No significant amount of IUdR per se was observed in the cerebrospinal fluid after intravenous injection. Intra-

cisternal injection of IUdR also resulted in rapid catabolism of IUdR with concomitant appearance of 5-iodouracil and free iodide. Because IUdR was not degraded when incubated *in vitro* with cerebrospinal fluid, it is logically assumed that the enzymes involved in this metabolic conversion reside intracellularly. Although in early 1967 IUdR appeared to have limited utility in therapy of CNS viral infections because of rapid metabolic degradation of the compound, today sufficient provocative reports have appeared to justify further study in this area. The studies of Clarkson and co-workers (81) are an excellent description of the metabolic fate of trace or extremely small amounts of IUdR in the CNS; in therapy, however, amounts of IUdR several orders of magnitude greater are used. Such large amounts would be expected to saturate the nucleosidases and hence enable phosphorylation to occur intracellularly with subsequent incorporation into the viral DNA and resultant inactivation of the virus.

A problem of grave concern in therapy of any infection with a drug is the possible emergence of resistant organisms and this applies also to viral infections. Resistant forms of herpes simplex virus exist normally and the resistant population may be increased to 40 percent by serial passage [literature cited in (13)]. Jawetz and co-workers (82) observed that strains of herpes simplex virus that were sensitive to IUdR *in vitro* were resistant to therapy *in vivo* and postulated that this was because of a failure of IUdR to be transported into the deeper areas of the infected cornea. Coleman, Tsu & Jawetz (83) concluded on the basis of an extension of their original studies that "the use of IUdR in a large majority of patients with herpes simplex virus keratitis has had little impact to date on the emergence of IUdR-resistant herpes simplex virus strains." These investigators suggest that the dynamics of virus population may be for preferential domination of the susceptible strains, which if true is fortunate for man.

A question raised early in this article as to whether relevant systems are being used for evaluation of antiviral agents is perhaps brought into focus most sharply by the report of Tomlinson & MacCallum (84), who treated herpes simplex virus infections in guinea pig skin by either a topical application of a 9 percent solution of IUdR in a mixture of 90 percent dimethylsulphoxide—10 percent water or by systemic treatment with IUdR at 120 mg/kg. In both approaches healing was promoted but the virus was not eliminated. Thus, the previous observations by this group of investigators (60) demonstrated a better therapeutic effect in man than in the guinea pig.

Subacute sclerosing panencephalitis is a lethal disease caused by a viral infection of the central nervous system. Freeman (85) treated several patients with BUdR with and without pyrancopolymer and observed an arrest of the progression of the disease and some clinical improvement.

(*c*) *5-fluorouracil and derivatives:* 5-Fluorouracil and FUdR inhibit DNA-viruses in cell culture, but have little or no efficacy either *in vivo* (*29*) or in the agar plaque assay described by Herrmann (53). Representative studies

of the effect of these fluorinated compounds and 5-trifluoromethyl-2′-deoxyuridine (F_3TdR), on the host-virus relationship, as well as on the biochemistry of viral replication are included or referred to in the following references (86–89).

Pétursson & Weil (90) concluded that the inhibition by FUdR of capsid protein synthesis in polyoma virus is an indirect one and related not to the incorporation into RNA of fluorouracil derived from FUdR but rather from inhibition of thymidylate synthesis.

F_3TdR, first synthesized by Heidelberger *et al.* (91) has marked antiviral as well as antineoplastic activities. This compound suppresses herpetic keratitis in rabbits and acts against a strain of herpes simplex virus resistant to IUdR (92). A double-blind study of F_3TdR and methisazone in the therapy of adenovirus type III instilled in the lower conjunctival sac of man indicated that these compounds had no value in prevention or treatment of this adenovirus infection in man (93).

An interesting compound of potential value against DNA viruses is 5′-deoxy-5′-fluorothymidine, a compound synthesized by Langen & Kowollik (94), and shown to inhibit replication of neoplastic cells presumably by inhibition of the phosphorylation of thymidine-5′-monophosphate by thymidine-5′-monophosphate kinase. Thus, this compound is a metabolic analogue of a nucleotide rather than that of a nucleoside and hence should be able to bypass resistance, developed to compounds like IUdR, that is caused by a deficiency or lack of thymidine kinase.

D-Arabinosylnucleosides.—(*a*) *cytarabine* (*cytosine arabinoside*) The biochemistry and biological properties of this group of compounds have been reviewed as well as its role in the therapy of neoplastic disease in man and its potential as an antiviral agent (13, 95–98). Whereas cytosine arabinoside is quite inhibitory against DNA viruses, RNA viruses in general are resistant to the inhibitory effects of cytosine arabinoside; however, Rous sarcoma virus, an RNA virus, is sensitive. Although cytosine arabinoside is as effective as IUdR in inhibiting the replication of herpes simplex or vaccinia virus (99), cytosine arabinoside is significantly more cytotoxic (2, 100). This is unfortunate since strains of herpes simplex virus resistant to IUdR are sensitive to the inhibitory effects of cytosine arabinoside and appreciable resistance to this agent by viruses has not been observed.

Cytosine arabinoside, like many other metabolic analogues (e.g. IUdR), exerts an inhibitory effect at several biochemical sites and the question to be resolved is which event is causally related to the inhibitory effect observed. The literature concerned with this aspect of the problem has been reviewed recently by Sartorelli & Creasey (97).

Rabies virus, believed to be an RNA virus on the basis of response to various inhibitors, is inhibited by cytosine arabinoside (101, 102). Of the various pyrimidine arabinonucleosides (cytosine arabinoside, uracil arabinoside, 2-thiouracilarabinoside, 5-iodouracilarabinoside) only cytosine arabinoside markedly inhibited the production of rabies fluorescent antigen. The pre-

ious hypothesis of these investigators, that cytosine arabinoside exerted its inhibitory effect on rabies virus reproduction by affecting the formation of phospholipid essential for maturation of the virus, has been reconsidered because they observed that other lipid-containing RNA viruses were not affected by this analogue. Their present interpretation, based on the observation that cytosine arabinoside exerts an inhibitory effect on this virus within the first 3 hours post infection, is that the induction of a cellular protein is required for exertion of its inhibitory effect. No information has been obtained yet as to how this hypothetical inhibitory protein acts. Thus, an additional site and mechanism of inhibition is introduced to what has been described above.

Levitt & Becker (103) observed complete suppression of the formation of the mature herpes simplex virion by cytosine arabinoside only when this analogue was present prior to the synthesis of viral DNA. If this analogue was added during the period of viral DNA synthesis, additional formation of viral DNA was prevented; however, that DNA already made was coated and formed an infectious virus. The mechanism of viral DNA inhibition was not investigated. Hirschman and co-workers (104) investigated the effect of cytosine arabinoside on the replication of the Moloney sarcoma virus in 3T3 cell culture. The host cell synthesis of DNA but not of RNA was markedly inhibited by this analogue. This RNA virus, like Rous sarcoma virus, requires an uninhibited cellular DNA for replication. These workers demonstrated that the inhibition of Moloney sarcoma virus by cytosine arabinoside was mediated by a reversible inhibition of DNA synthesis. Of prime importance, is the eventual elucidation of the unique role that cellular DNA plays in the replication of RNA-containing murine and avian sarcoma and leukosis viruses.

Ben-Porat, Brown & Kaplan (105) have investigated the specificity of the inhibitory activity of cytosine arabinoside in rabbit kidney cells infected with herpes virus and concluded that this agent is not a specific antiviral substance. Their studies show that DNA synthesis in noninfected cells is inhibited to a greater degree than in cells infected with herpes simplex or pseudorabies virus. These results explain why, although IUdR and cytosine arabinoside are effective in herpes keratitis, cytosine arabinoside is more toxic to the host cells (100). Whereas the level of deoxycytidine kinase activity decreases in the infected cell (105), that of thymidine kinase increases (106) [see literature cited in (13)]. These conditions result in a greater inhibition by cytosine arabinoside of the uninfected cell rather than of the virus infected cell, and a greater uptake of IUdR into infected cells than in the noninfected cells. Thus, as Ben-Porat, Brown & Kaplan (105) indicated, advantage can be taken of the differential enzymic activity of the infected and noninfected cells to produce, as in the case of IUdR, a selective toxicity of the virus infected cells.

Therapeutically, cytosine arabinoside has been shown to be effective in treatment of herpes keratitis in man (106), in two cases of disseminated

varicellor infections in man (107), and in one case of vaccinial blepharokeratitis.

(*b*) *vidarabine* (*adenine arabinoside*) 9-β-D-Arabinofuranosyladenine (108), like IUdR (109), was first synthesized to be an anticancer agent, however, like IUdR, it appears to have its best promise as an antiviral agent (110–112). The antiviral properties and potential of adenine arabinoside have been reviewed recently by Schabel (112), who described a broad spectrum of activity against DNA viruses with minimal activity against RNA viruses in cell culture.

5-Ethyluracil-2′-Deoxyribonucleoside.—5-Ethyldeoxyuridine was synthesized by Shapira (113) and by Swierkowski & Shugar (114) and shown to be incorporated into phage DNA (114). The chemotherapeutic potential of this compound against herpes simplex virus (115) and against vaccinia virus in HeLa cells (116) has been reported. 5-Ethyl-2′-deoxyuridine was observed to be nonmutagenic in phage (116, 117) as well as in a strain of *Drosophilia melanogaster* (118). Of pertinence is the observation that this analogue not only has antiviral activity but also is nonmutagenic, and one might assume that this compound therefore does not constitute a genetic hazard to man. Despite impressive evidence in a bacterial virus, and in *Drosophilia melanogaster,* it would be hazardous if these results were projected to man. It is difficult to understand how a compound, capable of producing such profound effects in viruses, presumably by virtue of being incorporated into the DNA, can be expected not to have an effect on transmission of genetic information. Additional studies clarifying this point are strongly indicated.

Azapyrimidinenucleosides.—(*a*) *azauridine:* 6-Azauridine (6-azauracil ribonucleoside) has been investigated extensively as an antineoplastic agent and more recently in the treatment of psoriasis (119–121). In cell culture systems both RNA- and DNA-viruses are inhibited (122–128). Preliminary studies of the efficacy of 6-azauridine in the therapy of herpes simplex infection of the eye in man by Myška et al. (129) indicated a beneficial effect; however, as the investigators indicated, a much larger sample is required as well as longer-term studies in order to establish the role of this agent in this disease. Recent studies by Demidova and co-workers report inhibition of the replication of cytomegalo virus in man by 6-azauridine (76).

(*b*) *azathymidine:* 6-Azathymidine, the 2′-deoxyribonucleoside of 6-azathymine, inhibits the reproduction of herpes simplex virus *in vitro,* and this inhibition can be prevented completely by thymidine (130). The ability of relatively small amounts of thymidine to prevent the inhibitory effect of this analogue does not encourage animal experimentation; however, preconceived notions should not discourage the courageous.

Isatin-β-Thiosemicarbazones.—The antiviral properties of methisazone (N-methylisatin-β-thiosemicarbazide, Marboran) have been reviewed (13, 131) and in summary it has been shown to be an effective prophylactic agent in man exposed to smallpox (132) and of value in the therapy of vac-

cinia gangrenosa and disseminated vaccinia (131–135). The only significant toxicity that has been reported is vomiting; however, this does occur in about 25 percent of the patients.

The mode of action of methisazone has been investigated (136–138) and no effect was observed either in early RNA transcription, early enzyme synthesis, or the replication of viral DNA. Although the late mRNA that is formed is normal in size and is incorporated into polyribosomes, within a very short time after its transcription the late mRNA is reduced in size to about one-third and dissociates from ribosomes. Hence one observes a rapid degradation of polyribosomes and a concomitant inability to synthesize proteins necessary for encapsidation of the newly formed viral DNA. Isatin-β-thiosemicarbazide is postulated by Joklik (137, 138), because it exerts no effect when viral DNA replication is inhibited, to stimulate progeny DNA to produce a protein that either causes chain breaks in mRNA or causes dissociation of the polyribosomes. Why this compound specifically affects adversely "late" virus mRNA and not "early" virus mRNA or host cell mRNA is not known.

Benzimidazole derivatives and guanidine.—Various benzimidazole derivatives have been tested for antiviral activity. Dichloro- and trichloro-ribofuranosyl benzimidazoles can inhibit viral replication but the concentrations required also affect host metabolism (139). More recent studies by Bucknall (140) and those by Diwan et al. (141) with derivatives of benzimidazole deoxyribonucleoside are in agreement with these results.

Of greater interest, is the compound 2-(α-hydroxybenzyl)-benzimidazole (HBB) which selectively inhibits the replication of several members of the picornavirus group such as polio, ECHO, Coxsackie, and rhinoviruses (although exceptions have been reported) (142, 143). The literature on this compound as well as guanidine has been extensively reviewed recently (144, 145).

The HBB derivative, 1-propyl-HBB, has been reported to be even more active against certain viruses than HBB (146), but with less development of resistance, a factor that is a major drawback to the use of HBB as well as guanidine.

Guanidine has a similar selective inhibitory action on certain picornaviruses, but, as indicated by Eggers, Ikegami & Tamm (147) the sites of action are probably different. Recent experiments by Caliguiri & Tamm (148, 149) indicate that the primary block of inhibition is the initiation of new viral RNA chains with protein synthesis being affected secondarily.

The mechanism of action for HBB has not been studied to the same extent as guanidine but it is known that inhibition of protein synthesis in ECHO type 12 virus-infected cells is secondary to inhibition of RNA synthesis as indicated by limited but continued synthesis of empty capsids after arrest of RNA synthesis (150).

Rifampicin.—Rifampicin (151) is a new and potentially valuable antiviral agent. Two groups of investigators (152, 153), reporting similar re-

sults, have found marked inhibition of vaccinia virus. Heller et al. (152) also reported that vesicular stomatitis virus, an RNA-containing virus, was not inhibited. A more complete study was performed by Subak-Sharpe, Timbury & Williams (153) who tested rifampicin against 19 different viruses and observed inhibition against three, vaccinia, cowpox, and adenovirus 1. Rifampicin alone, of the various derivatives of the antibiotic rifamycin evaluated, has thus far been proven effective (154). Radioautographic studies of utilization of labelled thymidine or uridine by vaccinia-infected BHK21 C13 cells indicated inhibition of both DNA and RNA synthesis. However, DNA or RNA synthesis was not inhibited in a rifampicin-resistant mutant of vaccinia.

Although the mechanism of the antiviral activity of rifampicin is not known, a good candidate is the virally associated DNA-dependent RNA polymerase. It is known that rifampicin inhibits RNA synthesis *in vivo* (155) and *in vitro* (156–158) in *E. coli* by binding to this enzyme (159). It does not inhibit the mammalian enzyme at similar concentrations (157). Rifampicin-resistant mutants of *E. coli* possess a DNA-dependent RNA polymerase which is resistant to rifampicin when assayed *in vitro* (160–163). Even more recently, di Mauro et al. (164), keeping pace with the increased knowledge concerning DNA-dependent RNA polymerase, have shown that rifampicin blocks the step in RNA synthesis associated with the PC (phosphocellulose) component but not the sigma factor reported by Burgess et al. (165).

Interferon.—The number of papers published on interferon since its discovery by Isaacs & Lindenmann in 1957 (166) attests to the excitement, complexity, and hopefully the value of this substance. At present, there is no practical procedure for making large enough quantities of sufficiently pure interferon for therapeutic use. Accordingly, interest has focused upon finding the most effective inducers of interferon so that the infected host can block viral proliferation by elaboration of endogenous interferon.

(*a*) *interferon inducers:* The inducers of interferon may be broadly classified as viral and nonviral. Interferon may be induced in appropriate hosts by both DNA and RNA viruses from all the major groups (167). Of prime importance is the demonstration of interferon in humans after viral infection. These investigations have been reviewed recently (168).

Of the nonviral inducers, the double-stranded complex polyriboinosinic acid : polyribocytidylic acid (poly I : C) has aroused the greatest interest recently. Field et al. (169) first reported the interferon-inducing capacity of poly I:C. Dianzani et al. (170) have reported that diethylaminoethyl dextran (DEAE-dx) increased interferon formation due to poly I:C stimulation up to 100-fold in L cells as compared to the low levels found after adding poly I:C alone. DEAE-dx injected with poly I:C into mice increased interferon production up to 30-fold (171). Others have reported similar results (172, 173).

Double-strandedness of RNA and polynucleotides seems to be a prerequisite for interferon inducing capability (169, 174–176). In a recent commu-

nication De Clercq & Merigan (177) have critically examined this issue. They conclude that double- or more strandedness is necessary for interferon induction and that double-strandedness increases the stability of the polynucleotide secondary structure. They also conclude that a correlation exists between increasing melting temperature, used as an indicator of secondary structure stability, and interferon inducing capability. Colby & Chamberlin (178) in contradistinction, reported no correlation, in their judgment, between the efficiency of interferon induction and thermal stability of the various polynucleotides tested.

Most evidence thus far implicates double-stranded RNA molecules as the most likely cellular entity involved in interferon production. It is known that infection by RNA viruses results in synthesis of double-stranded forms of RNA but it has been reported only recently that infection by a DNA virus will cause the synthesis of double-stranded RNA. Colby & Duesberg (179) sought and found an apparently double-stranded RNA in vaccinia virus-infected chick embryo cells. If these findings are confirmed, and if the double-strandedness is not just an artifact of the isolation procedure, or a property peculiar to vaccinia virus infection, then an important advance has been made in our understanding of the induction of interferon by DNA viruses in addition to the finding of a new factor in viral replication. These workers also found a small but measurable quantity of double-stranded RNA in uninfected cells, an observation that confirms a previous report by Montagnier (180). Perhaps this double-stranded RNA in uninfected cells is somehow related to the preformed interferon (discussed in a later section) reported by some laboratories, which is released by appropriate stimuli.

It is of interest that statalon, obtained from the mold *Penicillium stoloniferum* (181) and helenine from the mold *P. funiculosum* (182), originally thought to be nonviral inducers of interferon, have recently been shown to owe their inducing activity to the double-stranded RNA contained in viruses present in those preparations (174, 183).

The substances mentioned above represent a small portion of the list of known inducers of interferon. A comprehensive elaboration of interferon inducer may be found in the excellent monograph on interferon by Vilček (184).

(*b*) *mechanism of interferon production:* The means by which interferon production is promoted in the cell is at present not fully understood. It is not clear whether all the inducers act in a similar fashion. There are at least two possible subdivisions of interferon inducers; those that appear to stimulate *de novo* synthesis of interferon and those that may cause release or unmasking of previously synthesized interferon. The term "induce" as generally used in this review is meant to include both possible situations and does not imply that a substance causes *de novo* synthesis. This issue will be dealt with in a later portion.

Interferon is largely and possibly entirely a protein (185) and as such is a product coded for by either the cellular or the infecting virus genome. Ev-

idence accumulated thus far indicates that it is the cellular DNA that directs the synthesis of interferon upon viral infection. The main proof for this is that cells infected with RNA viruses such as Chikungunya (186), influenza, and Newcastle disease virus (187) failed to produce interferon if actinomycin D was added to the medium. Actinomycin D is known to inhibit DNA-dependent RNA synthesis (and thus mRNA synthesis) by complexing with DNA (188). RNA viruses presumably do not depend upon DNA-dependent RNA synthesis (189) but code directly for proteins and therefore should still be capable of directing the synthesis of interferon in the presence of actinomycin D if such were the case. The most plausible explanation of these experiments is that the cell DNA directs the synthesis of interferon and that actinomycin D prevents this by blocking transcription of cellular DNA. The main reservation to the experiments with actinomycin D is the possibility that it may be inhibiting a function other than RNA synthesis. The experimental support for actinomycin D affecting other processes has recently been discussed by Hendler (190).

Since interferon is predominantly or entirely protein it is not surprising that inhibitors of protein synthesis can inhibit synthesis of interferon (191–193). Puromycin and cycloheximide, both inhibitors of protein synthesis, have also been used to determine whether an agent that causes the appearance of interferon does so by inducing new synthesis or by releasing preformed stores of interferon. Thus, Youngner and his associates (194–196) concluded that the interferon inducers endotoxin, statalon, and poly I:C, when injected into mice, caused release of preformed interferon and not new synthesis. Surprisingly, cycloheximide, injected alone, was able to release interferon in mice (196).

These findings appear contrary in part to the results of Field et al. (197) and Vilček, Ng, Friedman-Kien & Krawciw (173) who found that interferon induction in tissue culture by poly I:C was inhibited by actinomycin D, implying that new mRNA and protein synthesis is necessary for the appearance of interferon after poly I:C treatment. Finkelstein, Bausek & Merigan (198) also reported that actinomycin D would prevent the induction of interferon by poly I:C when they used human skin fibroblasts. However, it was necessary to use a concentration of 1 μg/ml actinomycin D, which the authors found caused observable cytotoxicity 18 hours after a 90 minute exposure to the drug. When Newcastle disease virus or statalon were used as the inducer only 0.2 μg/ml actinomycin D was required. In addition 0.25 μg/ml puromycin markedly reduced the level of interferon produced by Newcastle disease virus, but a concentration of 2.5 μg/ml had no apparent effect on interferon levels when poly I:C was used.

An interesting and provocative alternative analysis of the issue of preformed versus newly synthesized interferon has been suggested by Vilček (199), who has proposed that in rabbit kidney cells an inhibitor protein is made which shuts off release of interferon at about 4 hours after poly I:C treatment.

How an infecting virus evokes synthesis of interferon has also been studied through the use of temperature-sensitive mutants of viruses. Skehel & Burke (200), using the RNA virus Semliki Forest virus, infected chick cells for 1 hour at 36° and then incubated the infected cells thereafter at 42°. Interferon was produced but not virus. Under these conditions viral RNA was synthesized. If infection as well as subsequent incubation was done at 42° neither interferon nor virus were made. These conditions produced no observable RNA synthesis. A portion of the RNA formed under the former conditions was double-stranded. On the basis of this and other evidence, together with the findings of the superiority of double-stranded RNA as an inducer of interferon, the authors postulated that the first step in interferon induction by single-stranded RNA viruses is the formation of double-stranded RNA.

In another study of this type, Lockart et al. (201) made use of temperature-sensitive mutants of Sindbis virus which also contains single-stranded RNA. The wild type, nontemperature-sensitive, virus produced equivalent quantities of interferon at 29° or 42°, whereas a mutant incapable of synthesizing RNA at 42° produced negligible quantities of interferon at the higher temperature. However, synthesis of double-stranded RNA per se was insufficient to induce interferon, as three other temperature sensitive mutants, defective in three other genes, were able to make viral RNA but could not induce interferon at the nonpermissive temperature. The inference to be drawn from these experiments is that synthesis of viral protein is required for interferon induction. The proteins formed by the three mutants have been tentatively classified as a nucleocapsid protein, a membrane protein, and a minor structural protein or maturation protein involved in assembly of the virus (202, 203). It is puzzling how well three of these proteins could be requisite for interferon induction.

(*c*) *physicochemical and biological properties:* Interferon has not yet been completely purified. Fantes (204) has reviewed recently the progress to date in this direction as well as the physicochemical properties of interferon. Two hallmark characteristics of interferon are stability to heat [no loss of activity after 1 hour at 70° (205)] and no loss of activity after incubation at pH 2 (206).

Interferon is at least in part, a protein. The basis for this assumption as reviewed by Fantes (204) rests upon the finding that proteolytic enzymes and amino acid specific reagents abolish the antiviral activity of interferon. Failure, thus far, to obtain pure interferon makes the reliability of analytic data concerning the constituents of interferon of doubtful value.

A variety of molecular weights have been reported for interferon, and an example of the complexity of this parameter is afforded by the report of Hallum, Youngner & Merigan (207). Mice were injected with Newcastle disease virus or *E. coli* endotoxin, and measurement of the molecular weight of the interferon produced was made by gel filtration with Sephadex G-100. Over a 2 year period the molecular weights of interferon made in response

to the virus varied from 28,000 to 74,000 while over the same period endotoxin-stimulated interferon stayed at 90,000.

An important property of interferon is species specificity. In general, interferon is inactive against heterologous cell types. For example, chick interferon is not active in mouse cells (208) and mouse interferon affords no protection for chick cells. However, some heterologous activity has been reported, as for the case of cross-reactivity of human and monkey interferons (209).

Interferon is active against a wide range of viruses and in numerous hosts. Different cell types vary in sensitivity to interferon and within any one sensitive cell type the inhibitory effect varies relative to the virus (210–212).

(*d*) *mechanism of interferon action:* The use of various inhibitors of cellular processes gave important clues as to how interferon was acting. Taylor (213, 214), using the RNA-containing virus, Semliki Forest virus, and chick embryo fibroblasts, found that actinomycin D, added to cells before or at the same time as the addition of interferon, effectively prevented most of the antiviral activity of interferon. These results were interpreted as an indication that cellular, DNA-dependent RNA synthesis is necessary for the antiviral action of interferon. Taylor hypothesized (213, 214) that interferon was acting as a derepressor for a cell-specified protein which inhibits viral replication. Other investigators have since confirmed these findings and extended them to other viruses such as Western equine encephalomyelitis virus (215, 216) and Sindbis virus (217).

A logical extension of these experiments was the demonstration that protein synthesis is needed for the action of interferon. Lockart (215) and Levine (216), both working with Western equine encephalomyelitis virus, showed that puromycin added with interferon could prevent, although not completely, the action of interferon. Friedman & Sonnabend (218) showed a similar effect with DL-fluorophenylalanine using Semliki forest virus as the test virus. Paradoxically, according to Dianzani, Baron & Buckler (219) cycloheximide, which is also an inhibitor of protein synthesis, does not prevent interferon from reducing the yield of vesicular stomatitis virus in mouse embryo cells. Their explanation is that in the presence of interferon and cycloheximide, the mRNA for an antiviral protein is transcribed, which is then rapidly translated after removal of interferon and cycloheximide.

Several groups have concerned themselves with the site and mechanism of action of interferon. Joklik & Merigan (220), working with vaccinia virus-infected L cells, proposed from their data that vaccinia mRNA does not form polyribosomes in cells pretreated with interferon. After infection with vaccinia (a DNA virus) the polyribosomes in interferon treated cells were rapidly disaggregated. They also concluded that interferon was preventing formation of polyribosomes with vaccinia mRNA rather than disaggregating formed polyribosomes. Interferon had no effect on polyribosomes in uninfected cells. No conclusion could be drawn from the data as to whether the effect of interferon was on the ribosomes or the vaccinia mRNA.

Marcus & Salb (221) approached the problem in a somewhat different fashion, studying the effect of interferon on infection of chick embryo cells by the RNA-containing Sindbis virus. Ribosomes from normal or interferon-treated cells were reacted at 0° with 40s RNA from purified Sindbis virus. Sedimentation analysis on a sucrose gradient revealed that the ribosomes from untreated cells combined with the viral RNA to form what appeared to be polyribosomes. Ribosomes from interferon-treated cells also formed what appeared to be polyribosomes but to a lesser extent as might be expected in view of the results of Joklik & Merigan (220).

A more pronounced difference between ribosomes from treated and untreated cells was apparent when the polyribosomes formed at 0° were incubated at 37° with a cell-free amino acid incorporating system. The polyribosomes formed from ribosomes derived from untreated cells broke down during the incubation and concomitantly there was amino acid incorporation. The polyribosomes formed from ribosomes of interferon-treated cells failed to break down during the 37° incubation and failed to incorporate significant amounts of amino acids. On the basis of their own and other data, Marcus & Salb (221) have postulated that interferon causes the synthesis of a cell-specified translation inhibitory protein which is associated with the ribsomes in some fashion.

Levy & Carter, who recently summarized their findings (222), found similar evidence with Mengo virus and mouse L cells. They demonstrated that 2.5 hrs after infection the quantity of polyribosomes in interferon-treated cells was 55 to 70 percent of that in untreated cells. In addition, pulse labelling experiments showed no newly synthesized protein or polyribosomes from infected, interferon-treated cells. Experiments with ribosomes from treated or untreated cells showed that each type of ribosome supported amino acid incorporation equally if endogenous mRNA or poly U was used. However, no amino acid incorporation occurred with ribosomes from interferon-treated cells if Mengo virus RNA was used.

Preliminary evidence for information of what may prove to be an antiviral or translation inhibitory protein has been published recently (223–225). With the aid of double-labelling methods an increased labelling of the nuclear fraction from interferon-treated chick cells was demonstrated. Isolated nuclei from chick cells when incubated with interferon had augmented incorporation of labelled uridine and leucine. A slight increase in labelling of ribosomal protein from interferon-treated cells in the 40s region, was also found by double-labelling experiments. Analysis by polyacrylamide gel electrophoresis of the protein extracted from the ribosomes showed a band of fast moving protein and two other regions that were made only by cells incubated with interferon.

The fact that cellular mRNA can bind to ribosomes from interferon-treated cells and be successfully translated, whereas viral mRNA is poorly bound and not translated, implies that there is some sort of recognition system whereby the ribosomes of interferon-treated cells distinguish between viral and cellular mRNA. In this regard, Oxman et al. (226, 227) and To-

daro & Green (228) have reported on the effects of interferon relative to transformation by SV40. Transformation of cells by SV40 leads to the synthesis of a new, specific antigen, SV40 T antigen, which is believed to be coded for by the virus and which is maintained in the transformed cells upon subsequent replication. If the uninfected cells are pretreated with interferon, the synthesis of SV40 T antigen is markedly inhibited after SV40 infection. However, once the transformed cell line has been established, and, therefore, SV40 T antigen synthesized, continuous passage of the cells in the presence of interferon fails to reduce the level of this antigen in the transformed cells. The implications and speculation from such experiments are that upon transformation, the SV40 DNA becomes integrated into the host DNA and the mRNA coded for by the viral genome is part of a polycistronic message which may contain a host recognition signal for translation on ribosomes after interferon treatment. In this manner, the viral mRNA could pass as host mRNA, be bound and translated (226). Todaro & Green (228) reached somewhat similar conclusions from their experiments with the SV40-3T3 system by measuring the effect of interferon on the frequency of transformation at different times after infection. By using synchronous cultures of 3T3 for infection they found that resistance to inhibition of transformation by interferon occurred when the cells began the period of cellular DNA synthesis. This observation fits in with the findings of Oxman et al. (226, 227) in that the period of cellular DNA synthesis may be associated with the integration of the viral genome into that of the host.

Oxman, Rowe & Black (229) have approached this problem from another somewhat different tack. While synthesis of SV40 T antigen is highly sensitive to inhibition by pretreatment with interferon, synthesis of a T antigen specified by adenovirus 7 is not. The availability of the hybrid virus, adenovirus-SV40, in which the respective DNA molecules are covalently linked, led these investigators to test the effect of interferon on SV40 T antigen synthesis by the hybrid virus. Induction of SV40 T antigen by the hybrid virus was resistant to interferon inhibition, as was induction of adenovirus 7 T antigen. Thus, apparent rejection by ribosomes in interferon-treated cells of mRNA coded by SV40 DNA was lost if the mRNA was, as is currently believed, part of a polycistronic mRNA with adenovirus 7 mRNA. Two possible views of how the recognition is accomplished are that either a SV40 recognition site is missing in the hybrid by which SV40 mRNA is recognized or rejected, or that the adenovirus 7 mRNA contains a recognition site which allows it and the proposed attached SV40 mRNA to be recognized and translated. The former seems less likely since in one of the hybrid strains tested, it was possible to recover fully infectious SV40 virus, indicating that the entire SV40 genome had been associated with the adenovirus genome.

POTENTIAL SITES OF ATTACK

As our knowledge of the biochemistry of viral replication and the mecha-

nisms of action of antiviral compounds increases, it is logical to consider the design of compounds for sites that may be highly specific and lethal for the virus.

Translation Inhibitory Protein

The hypothesis of Marcus & Salb (*221*) suggests that interferon exerts its antiviral activity by causing the formation of a protein that inhibits the translation of viral mRNA but not that of host cell mRNA. The isolation of this protein and elucidation of its structure might enable the protein chemist to synthesize polypeptides that could mimic such activity.

Transfer RNA

Recent studies by Subak-Sharpe and co-workers (*230*) indicate that herpes simplex virus may specify tRNA. Can this molecule be affected so that it is not recognized by the appropriate aminoacyl synthetase, thereby interrupting that protein synthesis uniquely vital for the replication of the virus and of no concern to the host cell?

Virus Specified Enzymes

Viral infection may cause an increase in the activity of a number of enzymes, the specific ones varying with the infecting virus (13, *231*). The induced enzymes appear in many instances to be qualitatively distinct in that they may have different chemical and physical properties. Can advantage be taken of these differences between host and virus-induced enzymes with respect to susceptibility to inhibition?

Viral Protein Synthesis

Grollman (*232*) has investigated the possibility of inhibiting the binding of viral mRNA to ribosomes with minimal or no effect on the attachment of host cell mRNA to ribsomes. Studies *in vitro* showed that this approach may be feasible since aurintricarboxylic acid, a triphenylmethane dye, did indeed interfere with the interaction of mRNA to ribosomes without affecting mRNA already bound. A problem of cell transport may exist with these dyes and also, unless preferential inhibition occurs, marked toxicity would result.

Understanding of the composition and synthesis of proteins by viruses is being pursued actively in several laboratories and from such information opportunities for attack may develop (*233–238*).

DNA-Bound Proteins

Histones bound to DNA have been postulated by some to regulate the transmission of genetic information from DNA to mRNA. It is conceivable that in time understanding of the specificity of interaction of specific amino acid sequences with specified base sequences may afford the knowledge required to synthesize polypeptides that selectively bind to purine and pyrimidine sequences that are unique to the virus nucleic acid.

Initiation of RNA Synthesis

Travers & Burgess (239) have discovered the existence of a protein, identified as sigma, whose function is stimulation of the initiation of RNA synthesis. The question posed is whether sigma can be modified so that after combining with minimal enzyme it either preferentially binds to host cell DNA, or forms an irreversible but inactive complex with vital nucleic acid. Whether a synthetic polypeptide with sigma-inhibitory properties can be formed either enzymically or synthetically is also a question of interest.

Amino Acid Activation

By inhibition of amino acid activation, protein synthesis is inhibited. If one could block specifically amino acid transfer to viral tRNA, then a selective inhibition of the synthesis of viral protein would result. Of interest, therefore, is the demonstration by Deutscher (240) of inhibition of glutamyl tRNA-synthetase by natural polynucleotides. Whether smaller molecules capable of being transported into a cell can be isolated or synthesized remains to be seen.

Other Potential Antiviral Agents

Each year a large number of substances are reported that have antiviral activity in cell culture and of these very few generally have antiviral activity *in vivo*. The limitations imposed on the reviewers preclude the possibility of critical evaluation of the numerous compounds with antiviral activity. Recently an excellent service has been provided which enables the listing of titles, journal reference, authors and author addresses for each viral chemotherapy article published. This valuable listing is being published in the journal *Chemotherapy*.

CONCLUSIONS

It is clear that every biosynthetic step concerned with the formation of an infectious viral particle is subject to attack. It is desirable, however, to find, if possible, those steps in this process that are unique and essential for the virus and not the host cell, and then to find substances that preferentially inhibit these reactions.

The imagination of the organic chemist in synthesizing compounds has no restraints; however, for it to be applied relevantly for the production of antiviral agents it should be interdigitated with the knowledge and sympathetic understanding of the biologist (virologist, biochemist, pharmacologist, etc.). It is clear that our understanding of molecular events in viral replication is increasing rapidly and it would be advantageous for such knowledge to be used as a guideline by the organic chemist. One can anticipate continued success in the development of vaccines against human viral infections and this is an ideal approach, since vaccination confers protection for long periods of time up to an individual's lifetime. Although the spectrum of an-

tiviral activity of the vaccines is very limited, this is not a factor except for those diseases where the virus undergoes rapid serological transformation. The possibility of interferon formation through the administration of nontoxic inducers is receiving considerable effort and has the advantage over the vaccine of being effective against a broad range of viruses even though for a limited period of time. The success of the few antiviral compounds that are available encourages one in continued synthetic efforts, hopefully within the constraints imposed above. The disadvantage of synthetic compounds is claimed to be the limited range of antiviral effectiveness. Although this is true for amantadine, it surely is not true for IUdR, which is effective against a broad spectrum of DNA viruses. The limitation in the usefulness of IUdR is a pharmacological problem in that one must overcome the problems of getting the drug into the infected cell, because once there, the desired antiviral activity will be attained. Perhaps it is, therefore, incumbent upon the pharmacologist to exercise his imagination to overcome the obstacles that nature has imposed.

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