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REVIEW ARTICLE

Antiviral Chemotherapy

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Summary. The chemotherapy of virus diseases is one of the greatest challenges of modern medicine. Fruitful research in the field is retarded by the very intimate association of viruses and the host cells, since viruses absolutely depend on the host metabolic processes for their replication. Thus, at best, so far only subtle differences have been revealed between host biochemical events and those that are virus-specific. To meet the challenge several antiviral substances have been tested. These may either be naturally occurring biological products or synthesized compounds. Few of these substances are of current clinical application. However, all the inhibitors studied have helped in certain ways in the elucidation of host–virus relationships, which is an essential first step in the ultimate solution of the problem. The antiviral compounds of clinical use are 5-iodo-2′-deoxyuridine, isatin thiosemicarbazone and amantadine hydrochloride which are active respectively against herpes simplex, pox and influenza viruses.

Résumé. Le traitement des affection virales est un des plus grands problèmes de la médecine moderne. Les recherches dans ce cas ont été retardées par les relations intimes qui existent entre les virus et les celluleshotes. Plusieurs traitements ont été essayes: les uns sont des drogues naturelles, les autres des médicaments synthétiques. Quelques uns de ces produits sont utilisés en clinique.

Les antivurus étudies, sont importants à considérer pour le compréhension, des relations hôte-virus; et ceci est essentiel pour la solution de ce probleme. Les substances a visée thérapeutique antivirale '5-iole-2-deoxyurisine' 'isatin thiosemi corbosone' et 'amantadine hydrochlorine' qui sont respectivement actives contre l'herpes 'simplex' la variole, et la grippe.

INTRODUCTION

One of the greatest challenges to modern medicine is the search for effective chemotherapeutic agents against viral infections. Thus the study of antiviral substances, apart from

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providing tools for basic research in host–virus relationships which would help elucidate the patterns of pathogenesis of virus diseases, also looks forward with optimism into the future when virus diseases, like most bacterial diseases, will be brought under medical control. Excellent reviews emphasizing various aspects of antiviral chemotherapy have been written by many authors (Matthews & Smith, 1955; Hurst & Hull, 1956; Tamm, 1956; Cutting & Furst, 1958; Staehelin, 1959; Sadler, 1963; Wagner, 1963; Thompson, 1964; Pienta & Groupé, 1964; Kaufman, 1965; Eggers & Tamm, 1966; Prusoff, 1967). However, in view of the large catalogue of antiviral substances already compiled, and new ones being reported, it is difficult to cover the field adequately in a single review. Furthermore, the modes of action of some of the substances already reported have been revised, while those of others are being extended, in the light of recent knowledge.

In this review emphasis will be directed to chemically synthesized compounds, and naturally occurring biological products which when introduced into a given host–virus system have the ability to block or suppress virus multiplication, or to cure or ameliorate virus infection. Substances which exert a viricidal effect on the inert virus particle will be excluded from the discussion. The agents of the Psittacosis–Lymphogranuloma Venerum–Trachoma group will also be excluded, since the majority of workers no longer consider these as 'true' viruses (Weiss, 1955; Moulder, 1964). The emergence of resistant virus strains to some of the antiviral agents will be spot-lighted.

Since detailed and exhaustive reviews of interferon have been written (Baron & Levy, 1966; Finter, 1966), this antiviral substance will be mentioned in this review only in connection with certain of the substances discussed, which manifest their antiviral activity through the induction of interferon.

Finally, some of the author's unpublished data will be reported under 'Miscellaneous antiviral substances'.

2. ANTIVIRAL AGENTS AND THE PHASES OF VIRUS MULTIPLICATION

(a) *Adsorption and penetration*

The first step in virus infection of a susceptible host cell is adsorption and penetration. Adsorption is mediated through electrostatic forces, Van der Waals' forces, and hydrogen-bonding between virus particles and receptor sites. For stable adsorption to take place there should be both electrostatic and geometric complementarity between the adsorbing surfaces. The lack of the latter has been advanced as a possible explanation for the insusceptibility of certain lines of cells to virus infection (McLaren, Holland & Syverton, 1959) since naked nucleic acid extracted from such virus particles infects susceptible as well as insusceptible cells (Holland, McLaren & Syverton, 1959; Mountain & Alexander, 1959) by circumventing the requirements for receptor sites. The progeny virus particles resulting from such infections are serologically indistinguishable from the parent virus providing the nucleic acid.

Little is known about the details of the mechanism of penetration of animal viruses into host cells. However, for influenza virus a process of 'viropexis' has been suggested (Fazekas de St. Groth, 1948a). This process is analogous to 'pinocytosis' (Lewis, 1931) by which cells ingest colloidal particles. On the other hand, Hirst (1943) suggested that penetration was

mediated through the hydrolytic enzyme neuraminidase or sialidase on the surface of the virus.

Several antiviral substances acting at these early stages of the infective cycle have been reported:

Receptor destroying enzyme (RDE)

This is an exo-enzyme isolated from a number of bacterial cultures, such as *Clostridium welchii* (McCrea, 1947), *Vibrio cholerae* (Burnet, 1948) and *Diplococcus pneumoniae* (Lee & Howe, 1966). Its action is similar to neuraminidase—the splitting of N-acetylneuraminic acid from myxovirus receptor sites on host cells, thus rendering these sites impaired for virus adsorption.

Treatment of susceptible host cells with RDE completely or partially inhibits infection of such cells by myxoviruses (Stone, 1948a; Cairns, 1951). However, such cells are not permanently impaired since the receptors are regenerated with time (Stone, 1948b; Fazekas de St. Groth, 1948b; Finter *et al.*, 1954).

Carbohydrate substances

Certain carbohydrate substances, acting as analogues of the receptor substance of host cells, inhibit virus infection by binding firmly to the virus and thereby precluding adsorption (Woolley, 1953). Such substances are usually complex molecules like muco-protein, muco-polysaccharide, glyco-protein, or lipo-protein-polysaccharide (Ginsberg, 1960). For the myxoviruses such substances usually, but not always, inhibit both haemagglutination and infection. The active component is sometimes associated with N-acetylneuraminic acid. However, the possession of the acid does not, *ipso facto*, make the molecule an inhibitor. Orosomucoid (Mayron *et al.*, 1961) is attacked by neuraminidase, but it is not an inhibitor. On the other hand, the ganglioside of brain (Bogoch, 1957) is an inhibitor, but not a substrate for the enzyme. An inhibitor present in the normal extra-embryonic fluids of chicken eggs inhibits haemagglutination, but does not neutralize infectivity (David-West, 1963). The latter may be due to the ease with which the virus enzyme hydrolyses the receptor analogue (Fig. 1).

Cohen (1960) showed that a horse serum inhibitor protected mice from the lethal action of influenza virus, especially when the inhibitor was given by the intranasal route. By this route the substance was active even when given 48 hr prior to virus instillation. The inhibitor acted by blocking virus receptor sites.

Synthetic substances

Ackermann & Maassab (1954a, b) demonstrated that α-amino-p-methoxyphenyl-methane sulphonic acid (AMPS) prevented the adsorption and penetration of influenza virus to chick embryo cells when added within 30 min of virus inoculation.

One of the compounds of great current interest in antiviral chemotherapy is 1-adamantanamine hydrochloride (Amantadine). A synthetic organic compound of molecular weight 187, it is a stable, colourless, crystalline primary amine. The parent compound—adamantane (from the Greek word for diamond) is a hydrocarbon in which the carbon atoms have the same configuration as in the diamond molecule (Fig. 2). Because of the highly symmetrical structure of the molecule, the drug also goes by the name 'Symmetrel'. It was synthesized by two Du Pont chemists, Marvin Paulshock and John C. Watts.

Amantadine possesses significant antiviral activity in a variety of culture conditions. Its action *in vivo* is specific for influenza A viruses (Davies *et al.*, 1964) although a strain of influenza C, parainfluenza 1/Sendai, pseudorabies and rubella virus have also been shown to be sensitive either in tissue culture or *in ovo* (Neumayer, Haff & Hoffmann, 1965; Cochran & Maassab, 1964). In mice inoculated intranasally with sensitive strains of influenza viruses amantadine produced both a delaying effect in the time of death and a sparing effect on the number of survivors (Grunert, McGahen & Davies, 1965). The drug is equally effective when given by either oral or intraperitoneal routes, simultaneously with the virus. It is also active even when given as late as 72 hr post-infection.

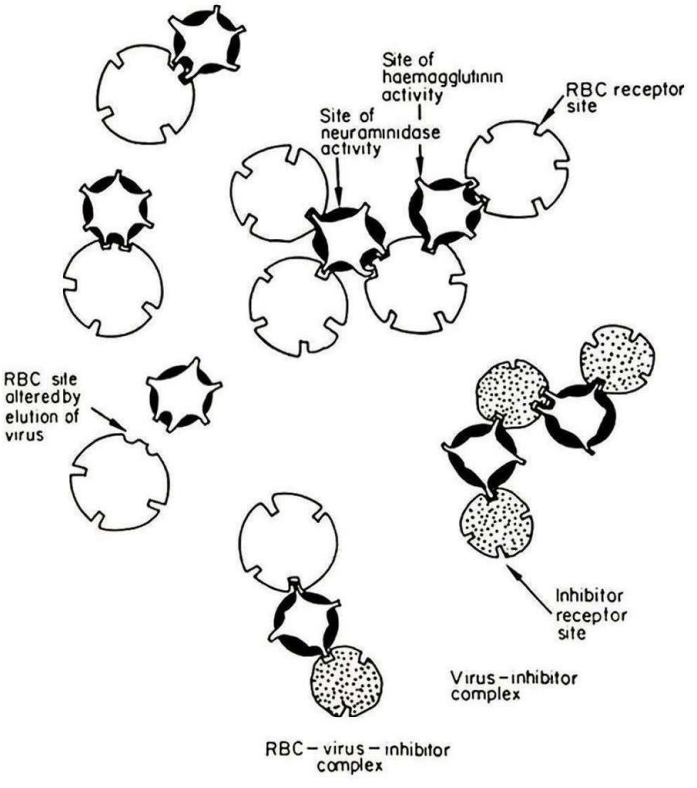

FIG. 1. Interaction of myxovirus, red blood cells and muco-polysaccharide inhibitor (diagrammatic representation).

Treatment does not cause a complete inhibition of virus multiplication. Mice surviving infection as a result of amantadine treatment were immune to subsequent challenge with the same virus, as a result of antibody induction (Davies *et al.*, 1964). Davies and colleagues also showed that the activity of amantadine was enhanced by specific antiserum. However, specific antibody may not be a necessary complement to the antiviral activity (Cochran *et al.*, 1965), since with a small dose of virus inoculum, some of the treated mice fail to show antibody response (Davies, Grunert & Hoffmann, 1966).

Amantadine acts by forming a molecular barrier to virus penetration into host cells (Davies *et al.*, 1964; Hoffmann *et al.*, 1965). It exerts no effect on the action of influenza

virus neuraminidase on red blood cells (Hoffmann *et al.*, 1965), neither does it modify the red blood cell receptors for virus attachment (Schild & Sutton, 1965). Schild & Sutton also showed that recently isolated influenza A virus strains are generally more sensitive than viruses passaged under laboratory conditions.

The drug is one of the few antiviral compounds of successful clinical application (Wendel, 1964). It is rapidly absorbed, and possesses a wide margin of safety (Bleidner & Herrmann, 1964).

Influenza virus readily developed resistance to amantadine *in vitro* (Cochran *et al.*, 1965), although such variants of the virus have not been demonstrated in *in vivo* experiments in mice (Grunert *et al.*, 1965; Cochran *et al.*, 1965). Influenza virus variants resistant to amantadine, also resist inhibition by AMPS. This suggests a common locus of activity for both drugs (Cochran *et al.*, 1965).

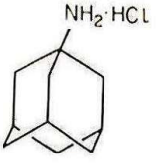

FIG. 2. Crystal structure of 1-adamantanamine hydrochloride: specific inhibitor of influenza virus.

Fletcher, Hirschfield & Forbes (1966) reported that 2-diethyl aminoethyl 4-methyl piperazine-1-carboxylate is similar to amantadine in both mode of action and spectrum of antiviral activity.

Caprochlorone is highly effective against influenza virus multiplication in de-embryonated eggs (Liu *et al.*, 1957a) and in mice (Liu *et al.*, 1957b). An intracellular site of action was suggested by Liu and colleagues. However, more recent work has shown conclusively that caprochlorone inhibits virus penetration (Stanfield, Haff & Stewart, 1966). In this it is comparable to both amantadine and ammonium ions (Eaton & Scala, 1967).

Another synthetic compound, 'Cephaloridine', interferes with the adsorption of vaccinia virus in tissue culture (Nishmi, 1966).

(b) *Eclipse and maturation*

Viruses do not contain the necessary enzymes for the biochemical events of their replication. Their reproduction is therefore very closely tied up with the metabolism of the infected host cell. In such an association the virus either utilizes enzymes already present in the host cell, or induces new virus-specific enzymes. The observation that the induced enzymes may be qualitatively different from those of the host cell (McAuslan, 1963) has obvious implications for selective chemotherapy of viral infections.

The viral nucleic acid contains all the coded genetic information for the replication of the particle. In the case of deoxyribonucleic acid (DNA)-containing viruses, the viral nucleic acid serves as the template for its own replication as well as that of specific messenger ribonucleic acid (RNA) molecules which direct the synthesis of virus-specific proteins. However, RNA viruses are unique in that their RNA serves as the template for its own replication, with the aid of virus-induced RNA-polymerase, and also acts as messenger

RNA. The different virus specific macromolecules are assembled into the intact virus particles, at the phase of maturation.

The 'rational' approach to antiviral chemotherapy is thus directed towards finding ways and means of selectively interfering with the viral nucleic acid and protein syntheses.

(i) *Antagonists of nucleic acid synthesis*

Benzimidazoles. The antiviral action of benzimidazole was first reported by Thompson (1947). Modifications introduced in the purine bases were shown (Tamm, Folkers & Horsfall, 1952) to exert some selective inhibitory activity on virus multiplication. Several benzimidazoles were synthesized and screened for antiviral activity; 5,6-dichloro-1-β-D-ribo-

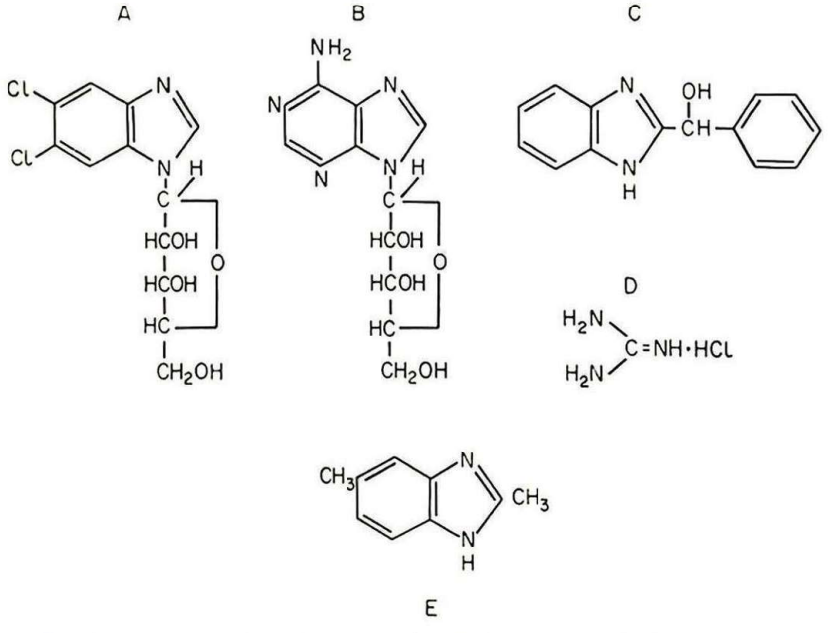

FIG. 3. Chemical structures of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (A); adenosine (B); 2-(α-hydroxybenzyl)-benzimidazole (C); guanidine hydrochloride (D); 2,5-dimethylbenzimidazole (E).

furanosyl-benzimidazole (DRB), 2-(α-hydroxybenzyl)-benzimidazole (HBB) and 2,5-dimethyl-benzimidazole (Fig. 3) were more extensively studied (Tamm, Folkers & Horsfall, 1953a, b). There seems to be a relationship between the structure of the benzimidazoles and their inhibitory activity (Tamm & Overman, 1957; Tamm *et al.*, 1961).

The alkylated and halogenated benzimidazoles were originally considered to be antagonists of vitamin B_{12}, which contains the 5,6-dimethyl-1-α-ribofuranosyl-benzimidazole moiety. However, none of the data on the antiviral activity of these compounds associate the inhibitory activity with the vitamin (Tamm & Eggers, 1963b).

DRB prolonged the latent period of the multiplication of influenza virus and also reduced the final virus yield. It was found to be 35 times more active than 2,5-dimethylbenzimidazole. In contrast with 2,5-dimethyl-benzimidazole, however, the inhibitory action of

DRB persisted after removal of the compound following exposure of the tissue (Tamm *et al.*, 1954).

Structurally, DRB may be considered an analogue of adenosine, and it has been shown to inhibit the incorporation of adenosine into RNA (Tamm & Eggers, 1963a). DRB inhibits the replication of influenza virus, an RNA-containing virus, and the DNA-containing adenovirus and vaccinia virus. Herpes simplex virus is also inhibited (Diwan *et al.*, 1968). This lack of selectivity of the action of DRB may either be due to the participation of RNA in the replication of DNA-viruses (Ikegami, Kato & Kamahara, 1960) or the observed antiviral effects may be secondary to an impairment of some essential host metabolic processes needed for the multiplication of both types of viruses (Diwan *et al.*, 1968).

HBB is a selective inhibitor of picornaviruses (Eggers & Tamm, 1962). It inhibits both virus multiplication and cytopathic effects (CPE) in tissue culture. It is not a metabolic antagonist and its inhibitory action is reversed by removing it from the medium (Tamm *et al.*, 1961).

The action of HBB is similar, but not identical, to that of another selective inhibitor of picornaviruses, guanidine (Fig. 3) (Tamm & Eggers, 1962); these viruses may be grouped, based on their sensitivity to both compounds. Antiviral action is mediated through the inhibition of the synthesis of RNA polymerase (Tamm & Eggers, 1963b; Baltimore *et al.*, 1963). However, the loci of action of HBB may be different from that of guanidine (Eggers & Tamm, 1963a).

In vivo studies with HBB in mice and monkeys have had very limited success, and in some there was enhancement instead of inhibition of virus multiplication (Brown, Craig & Kandel, 1953).

Frequent emergence of both drug-resistant and drug-dependent variants has been reported following the exposure of sensitive viruses to HBB or guanidine (Eggers & Tamm, 1961, 1963b; Ledinko, 1963; Nakano, Iwami & Tagaya, 1963). Infectious RNA extracted from the drug-dependent strains, also showed a dependence on the drug for replication. The progeny virus was also drug-dependent. Back mutations among the drug-dependent variants were either to drug-resistance or drug-sensitivity.

The emergence of drug-dependence is correlated with the loss of neuro-virulence of poliovirus (Loddo *et al.*, 1964). A guanidine-dependent strain failed to produce the characteristic paralysis in monkeys following either intramuscular or intracerebral inoculation; although there was evidence of virus multiplication.

It is of clinical interest that there is very little cross resistance among the picornaviruses with respect to HBB and guanidine (Tamm & Eggers, 1962). Moreover, synergism has been demonstrated between the two drugs (Eggers & Tamm, 1963a).

Thiosemicarbazones. The antiviral activity of thiosemicarbazone was first discovered by Hamre, Bernstern & Donovick (1950), who demonstrated that benzaldehyde thiosemicarbazone, administered orally or intraperitoneally, protected mice infected intranasally with vaccinia virus. These observations were later confirmed by Thompson, Price & Minton (1951), who also showed that benzaldehyde thiosemicarbazone, incorporated in the feed of mice, protected the animals from vaccinia virus given intracerebrally. The drug also prevented the multiplication of the virus in chick embryo tissues. The effects of the compound on neurovaccinia were further studied by Thompson *et al.* (1953a, b), who for the first time demonstrated a marked antivaccinial activity with heterocyclic derivatives of the parent compound. They showed that isatin thiosemicarbazone (ITSC; Fig. 4) possessed a potent

antivaccinial activity, and that the $=N\text{-}NH\text{-}CS\text{-}NH_2$ group appeared essential for the activity of the compound. Although active in mice, ITSC is ineffective in preventing vaccinial skin lesions in the rabbit.

Despite its selective inhibitory activity against the pox group of viruses, ITSC, showed only a moderate antiviral activity against ectromelia virus, which is antigenically closely related to vaccinia virus (Sheffield, Bauer & Stephenson, 1960). However, a modification of the parent molecule, with increased anti-ectromelia activity has been reported (Bauer & Sadler, 1961).

ITSC acts intracellularly. Electron micrographs of infected and treated cells showed an accumulation of immature and abnormal virus particles (Easterbrook, 1962; Sadler, 1965). Such cells produce viral DNA, as demonstrated by acridine orange staining, and also form soluble viral antigens, demonstrable by immunodiffusion (Appleyard, Hume & Westwood, 1965). Appleyard and colleagues also showed that the inhibitory action of ITSC was prevented by DL-p-fluorophenylalanine and actinomycin. It was therefore hypothesized that

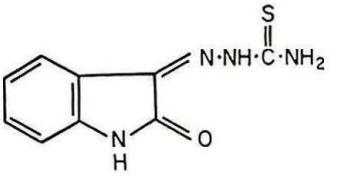

FIG. 4. Isatin thiosemicarbazone: specific inhibitor of pox viruses.

ITSC induced the formation of a new RNA, presumably a messenger RNA, which directs the synthesis of a new protein with antiviral properties. However, Pollikoff *et al.* (1965) failed to demonstrate any interferon or antibody in the brains of ITSC treated mice prior to 10 days after infection.

The drug may also act by an intercalation into the DNA helix (Sadler, 1965), or by the chelation of copper ions by virtue of the sulphur in the side chain (Bauer & Sadler, 1960). There have been successful clinical applications of the drug against smallpox infection (Turner, Bauer & Nimmo-Smith, 1962; Bauer *et al.*, 1963).

Resistant variants of rabbitpox virus have been reported (Appleyard & Way, 1966).

Halogenated nucleosides. In general these compounds act either as competitive inhibitors of the enzymes involved in the synthesis and polymerization of virus precursor macromolecules, or by being incorporated into DNA or RNA, resulting in the formation of 'fraudulent' non-functional nucleic acid. The van der Waals' radii of the halogen substituent in the 5-position of the benzene ring imparts specificity to the compounds (Prusoff, Bakhle & Sekely, 1965; Prusoff, 1967).

An analogue of thymidine, 5-iodo-2-deoxyuridine (IUDR; Fig. 5) was first synthesized by Prusoff (1959). IUDR inhibits DNA viruses, with the exception of Columbia-SK virus and Rous sarcoma virus which are RNA viruses (Force & Stewart, 1964a, b). IUDR-5'-phosphate may be incorporated into DNA in the place of the normal thymidine-5'-phosphate; the further conversion of this compound into the corresponding tri-phosphate and subsequent polymerization into DNA is blocked through the inhibition of the kinases of thymidine and thymidylic acid, as well as DNA polymerase (Delamore & Prusoff, 1962).

The antiviral activity of IUDR has attracted a lot of interest since its chemotherapeutic potentialities against herpes simplex and vaccinia infections were reported by Kaufmann (1963). The drug was found effective in curing herpetic keratitis and ocular vaccinial lesions. Both the 5-bromo-and the 5-chloro-2′-deoxyuridine were also active in experimental animals. But these have limited clinical use partly because of their irritant nature.

Subcutaneous tumours induced by adenovirus type 12 in hamsters (Huebner *et al.*, 1963) and neoplastic conversion of Rous sarcoma virus infected cells (Force & Steward, 1964b) were both inhibited by IUDR.

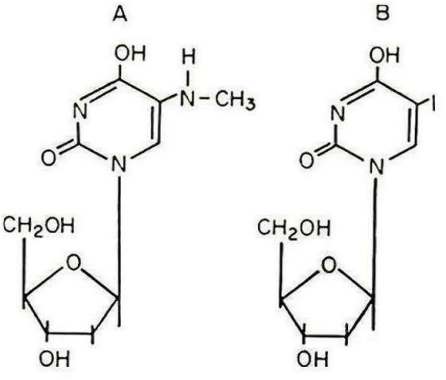

FIG. 5. Chemical structures of 5-methylamino-2′-deoxyuridine (MADU) (A) and 5-iodo-2′-deoxyuridine (IUDR) (B).

From the studies of Smith & Dukes (1964) and Siminoff (1964) the picture emerged that the principal antiviral activity of IUDR is due to an interruption in the assembly of intact virus particles, and not in the production of viral components. In tissue culture, although the production of infectious virus was arrested, the CPE caused by the virus was not prevented.

Two other halogenated nucleosides that are extensively studied, are the 5-bromo-2′-deoxyuridine (BUDR) and the 5-fluoro-2′-deoxyuridine (FUDR). The sequence of conversion and incorporation into DNA of the former is similar to IUDR; with FUDR, however, the corresponding monophosphate, like the normal deoxyuridylic acid has great affinity for the enzyme thymidylic synthetase (Delamore & Prusoff, 1962). But upon conversion to the free fluorouracil, and subsequently to the triphosphate, FUDR can also be incorporated into RNA (Prusoff *et al.*, 1965).

Although both IUDR and BUDR are incorporated into the DNA of pseudorabies virus, a member of the herpesvirus group (Kaplan, Ben Porat & Kamiya, 1965), the synthesis of viral DNA progressed at the same rate in both treated and untreated cell cultures; viral antigens were also synthesized in the presence of the drugs. However, infective virus particles were not produced.

FUDR prevented the formation of infective vaccinia virus as well as the virus haemagglutinin (Loh & Payne, 1965a). The nucleo-protein and the heat-labile-heat-stable (LS) antigens were not inhibited.

Nemes & Hilleman (1965) reported the antiviral action of 5-methylamino-2'-deoxyuridine (MADU) against herpes simplex virus, in both *in vitro* and *in vivo* experiments. It is reported to have a low toxicity, and it is minimally incorporated into host cell DNA, presumably due to the absence of the halogen atom (Fig. 5).

The activity of cytosine arabinoside (1-β-D-arabinofuranosylcytosine.HCL) is similar to that of the halogenated nucleosides (Buthala, 1964; Rapp, 1964; Levitt & Becker, 1967). Like IUDR it is effective against herpes simplex keratitis (Underwood, 1962).

A single passage of herpes simplex virus in the presence of 500 μg/ml of IUDR resulted in the selection of a resistant variant to the drug (Buthala, 1964). However, these IUDR-resistant variants were sensitive to a much lower dose of cytosine arabinoside. A synergistic effect of both drugs was reported (Kaufman, 1963; Buthala, 1964); no resistant variants emerged under these conditions. These findings are of great clinical significance in the use of IUDR for vaccinial and herpetic keratitis. Genetically stable IUDR-resistant herpes simplex virus variants have been reported in human patients treated with the analogue (Kaufman, 1965; Jawetz *et al.*, 1965). IUDR-therapy is further complicated by the observation that some of the resistant strains from human infections were sensitive to the drug *in vitro* and *in vivo* studies in the rabbit cornea (Jawetz *et al.*, 1965). Thus it is difficult to safely extrapolate the results of these various human, tissue culture and animal studies.

Actinomycin D. This is a naturally occurring polypeptide antibiotic. By binding to DNA, actinomycin D interferes with the template function of DNA as a primer in RNA synthesis (Reich *et al.*, 1962; Cavalieri & Nemchin, 1964). In addition, actinomycin D has also been reported to inhibit the 'migration' of radioactive uridine in acid insoluble form from the nucleus to the cytoplasm (Levy, 1963).

Theoretically, the antibiotic should inhibit viruses which replicate in the host cell nucleus. This has been supported in the case of influenza virus whose nucleic acid is synthesized in the cell nucleus; and Newcastle disease virus, which replicates in the cytoplasm. The former is inhibited by actinomycin D, while the latter is not (Barry, Ives & Cruickshank, 1962; Granoff & Kingsbury, 1964). However, the drug is also effective against certain viruses that multiply in the cytoplasm, such as vaccinia virus (Shatkin, 1963), Rous sarcoma virus (Vigier & Golde, 1964), and foot-and-mouth disease virus (Black & Brown, 1968). Vesicular stomatitis virus has been shown by both electron microscopic (David-West & Labzoffsky, 1968a) and immunofluorescent (David-West & Labzoffsky, 1968b) studies to multiply in the cytoplasm. In inhibition studies with actinomycin the virus was found to be resistant under certain experimental conditions (David-West & Labzoffsky, 1968b; Black & Brown, 1968). In some cases there was stimulation of virus growth (David-West & Labzoffsky, 1968b).

(ii) *Antagonists of protein synthesis*

A number of amino acid analogues, and even some naturally occurring amino acids, have been reported to inhibit the multiplication of various animal viruses. These analogues usually exert their antiviral action by competitive inhibition of a reaction involving the natural amino acids, or they may be incorporated into the newly formed virus protein, and thereby giving rise to an aberrant non-functional protein; i.e. a mode of action similar to that of the analogues of purine and pyrimidine bases.

The antiviral action of the amino acid analogues is usually reversed by the appropriate normal amino acids.

One of the most extensively studied amino acid analogues is p-fluorophenylalanine (FPA), which has been shown to inhibit the replication of influenza virus (White *et al.*, 1965), fowl plague virus (Zimmermann & Schäfer, 1960), poliovirus (Scharff, Summers & Levintow, 1965), vaccinia virus (Loh & Payne, 1965b), rabbitpox virus (Appleyard & Zwartouw, 1965), adenovirus (Wilcox & Ginsberg, 1963), mengovirus (Baltimore & Franklin, 1963) and Western equine encephalitis virus (Wecker, Hummeler & Goetz, 1962). It is obvious from the spectrum of viruses which are susceptible to FPA that the analogue is not a restricted selective antiviral agent.

The addition of phenylalanine reversed the inhibitory action of FPA.

DL-Methoxinine inhibited the replication of influenza A (PR8) virus (Ackermann & Maassab, 1954b). The multiplication of vaccinia virus is also arrested (Thompson, 1947). The effect of methoxinine was nullified by L-methionine, but not by DL-methionine (Ackermann & Maassab, 1954b).

A detailed study of the effects of L-canavanine, a naturally occurring amino acid in jack beans, was conducted by Pitcher *et al.* (1955). The multiplication of the Lee strain of influenza B virus was inhibited both *in ovo* and in tissue culture. The acid was not active in mice infected with the virus. L-Canavanine seems to show some selectivity among myxoviruses, since it was not effective against influenza A virus (PR8) or mumps virus.

Structurally L-canavanine may be considered as an analogue of L-arginine, and its antiviral action was completely reversed by L-arginine.

Puromycin is an antibiotic of great interest in the study of protein synthesis. It is an aminonucleoside linked to the amino acid p-methoxyphenylalanine, and blocks protein synthesis by inhibiting the transfer of amino acids from sRNA into ribosomal protein, and thereby interfering with the formation of the polypeptide chains at the ribosomal site (Darken, 1964).

Added early in the latent phase, puromycin prevented the maturation of poliovirus (Levintow *et al.*, 1962). It also inhibited the synthesis of viral RNA. However, when the antibiotic was added towards the end of the latent phase a limited synthesis of RNA was possible. The removal of the antibiotic from the medium, reversed the inhibitory action. It may be concluded from these studies that the synthesis of some 'early' protein was necessary for the replication of RNA (Levintow *et al.*, 1962; Steevalsan & Lockart, 1964).

(iii) *Inhibitors of energy-yielding mechanisms*

Although viruses differ in the extent to which they utilize the energy-yielding metabolic processes of the host cell for their replication, in general, owing to the absolute dependence of viruses on the metabolic activities of the infected cell, any significant alterations of such host activities have a direct influence on the final outcome of virus infection.

Ackermann (1951) showed that the Kreb's (citric acid) cycle played an important role in the replication of influenza virus. Sublethal doses of sodium fluoroacetate administered to mice within the first 12 hr following infection blocked the citric acid cycle; citrate concentration thereby increased in the lungs, inhibiting the replication of the virus. The salt exerted no viricidal action.

Two, 4-dinitrophenol (DNP) uncouples oxidative phosphorylation, and thereby prevents the formation of high energy phosphate bonds essential for cell metabolism. Under these conditions the multiplication of influenza virus was inhibited (Ackermann & Johnson,

1953). The action of butyl 3,5-diiodo-4-hydroxy-benzoate, an antagonist of thyroxin, is similar to that of DNP (Eaton, Adler & Perry, 1953).

(c) *Phase of release of newly formed virus*

There is a significant paucity of information concerning antiviral substances that block the release of the synthesized virus particles from the host cell. The two compounds so far reported are α-amino-p-methoxy-phenylmethane sulphonic acid (Ackermann & Maassab, 1954a, b) and caprochlorone (Stanfield, Haff & Stewart, 1967). Both interfered with the release of influenza virus.

3. MISCELLANEOUS ANTIVIRAL SUBSTANCES

Several extracts of microbial culture brews have been shown to possess antiviral activity. But except in very few cases the exact modes of action of these antiviral brews remain obscure. Furthermore, most of these substances have not yet been produced in chemically pure forms. Thus definitive studies on their chemical structures and details of action are hampered.

Helenine. From a mould found growing on the back of his wife Helen's photograph in Guam, Shope (1953a) extracted a substance with an antiviral activity. The substance was dedicated to his wife, and so was called 'Helenine'. The mould was later identified as *Penicillium funiculosum.* Helenine was active against Columbia SK encephalomyelitis virus (Shope, 1953a), and Semliki Forest virus in mice (Shope, 1953b). In mice infected with poliovirus type 2 helenine treatment delayed the onset of paralysis (Cochran & Francis, 1956). Monkeys are also protected against paralysis by type 1 poliovirus.

Purification and chemical characterization of helenine were carried out by Lewis *et al.* (1959), and it was found to be a ribonucleoprotein; unstable to lyophilization and repeated freezing and thawing.

Helenine acts by the induction of interferon (Rytel, Shope & Kilbourne, 1966).

Statolon, M-8450 *or Agent* 1758. These antiviral substances produced by strains of *Penicillium stoloniferum*, were originally considered to be different. But they are now known to be identical; and the name Statolon has been retained for all of them.

Statolon protected mice against MM encephalomyocarditis virus and Semliki Forest virus infections (Powell *et al.*, 1952). It was also effective against MEF 1 poliovirus in mice (Powell & Culbertson, 1953). In tissue culture it inhibited the cytopathic effect of all three immunologic types of poliovirus (Hull & Lavelle, 1953). Salisbury virus H.G.P. replication was inhibited (Powell, Walcher & Mast, 1961). It also protected against Friend virus leukaemia in mice (Wheelock, 1967).

It is significant to note that in all instances statolon exhibited the greatest antiviral activity when the tissues were pretreated with it prior to virus inoculation.

Chemically statolon has been identified as an anionic polysaccharide (Kleinschmidt & Probst, 1962). Its antiviral action is mediated through the induction of interferon (Kleinschmidt, Cline & Murphy, 1964). It has recently been shown (Ellis & Kleinschmidt, 1967) that virus-like particles in statolon preparations are responsible for the induction of interferon.

Helenine and statolon, although synthesized by different types of mould, may be identical

in chemical composition. The correct picture, however, will be revealed after both substances have been obtained in chemically pure forms.

Ehrlichin. Groupé *et al.* (1951a) reported the antiviral activity of culture filtrates of *Streptomyces lavendulae.* In their 'Contact Test', in which virus and the antiviral extract were mixed *in vitro* and allowed to stand for 2–3 hr at room temperature prior to inoculation into chick eggs, they demonstrated activity against influenza A and influenza B viruses. Pulmonary consolidation in mice infected with influenza virus was also suppressed by Ehrlichin. It was shown not to be viricidal.

Viscosin. A product of culture filtrates of *Pseudomonas viscosa* was found to exert a marked protective effect in embryonated eggs infected with infectious bronchitis virus (Groupé *et al.*, 1951b). There was slight inhibition of the multiplication of Newcastle disease virus and influenza virus in eggs, and of influenza A virus in mouse lungs.

Netropsin. This substance is elaborated by *Streptomyces netropsis* (Schabel *et al.*, 1953). Given intraperitoneally, netropsin protected against neurovaccinia in mice. However, it is ineffective against vaccinial skin lesions in the rabbit. Netropsin thus shares some biological properties with isatin thiosemicarbazone. Mice protected by netropsin develop antibodies against vaccinia; which suggests that there was no complete suppression of virus multiplication.

Propionin. Cutting *et al.* (1960) reported an antiviral principle in cultures of *Propionibacterium freudenreichii.* Propionin reduced the mortality rate of mice inoculated intraperitoneally with Columbia SK virus, when it was given either subcutaneously or orally. Upon further purification a crystalline polypeptide, designated Propionin A, was isolated (Ramanathan *et al.*, 1966). Propionin A was active against vaccinia virus *in vitro* but not against Columbia SK virus in mice. Since the crude extract inhibits both viruses the possibility of more than one antiviral principle in the preparation has been suggested. Propionin did not interfere with the development of specific immunity in treated mice.

Cyclopin. This substance was extracted from cultures of *Penicillium cyclopium* (Nacify & Carver, 1963; Nacify, 1965). Cyclopin inhibited the multiplication of representative viruses of arbovirus groups A and B. The exact mode of action is not yet known, but it was established to act at an intracellular site. Preliminary chemical characterization suggested that the active component is a protein derivative (Nacify, 1965).

Xerosin. Cultures of *Achromobacter xerosis* produced an acid precipitable material, xerosin, which suppressed the development of pneumonia in mice infected with influenza A and influenza B viruses (Goupé *et al.*, 1954). The most interesting biological activity of xerosin is the suppression of nontransmissible pneumonia in mice intranasally infected with Newcastle disease virus (Groupé, Pugh & Levine, 1953). In pneumonia induced by Newcastle disease virus in mice there is no virus multiplication. Thus xerosin does not act directly against virus replication, but elicits its antiviral action through a modification of tissue reaction to virus infection. Histological evidence by Ginsberg (1955) showed that xerosin acts as an anti-inflammatory agent. This property of the preparation was increased by autoclaving (Groupé & Herrmann, 1955). Xerosin did not inhibit the multiplication of influenza virus in the mice lungs or in the allantoic cavity of chick eggs (Groupé, Pugh & Levine, 1952).

Cyclohexamide. This broad spectrum antiviral agent was isolated from cultures of *Streptomyces griseus.* It is active against picorna-, myxo, arbo-, vaccinia, Rous sarcoma, mouse hepatitis, and pseudorabies viruses (Haff, 1964). It acted late in the latent phase.

From the wide range of viruses inhibited, it would appear that cyclohexamide interfered with a host process essential for viral replication in general.

Capsular polysaccharide of Friedländer bacilli. This polysaccharide has been shown to inhibit the multiplication of pneumonia virus of mice in mouse lungs when the virus and polysaccharide were given by the intranasal route (Ginsberg & Horsfall, 1951). Only limited activity was demonstrated when the inhibitor was given intraperitoneally. The replication of mumps virus in embryonated chick eggs was also inhibited (Ginsberg, Goebel & Horsfall, 1948) but other myxoviruses, such as influenza A, influenza B and Newcastle disease viruses, were not sensitive to the polysaccharide. With the sensitive viruses antiviral activity was demonstrated even when the inhibitor was given 4 days after virus inoculation.

Serial passage of mumps virus in the presence of the polysaccharide, selected a resistant variant of the virus (Ginsberg & Horsfall, 1949). A reversion of the resistant variant back to sensitivity was produced upon passage in the absence of the polysaccharide.

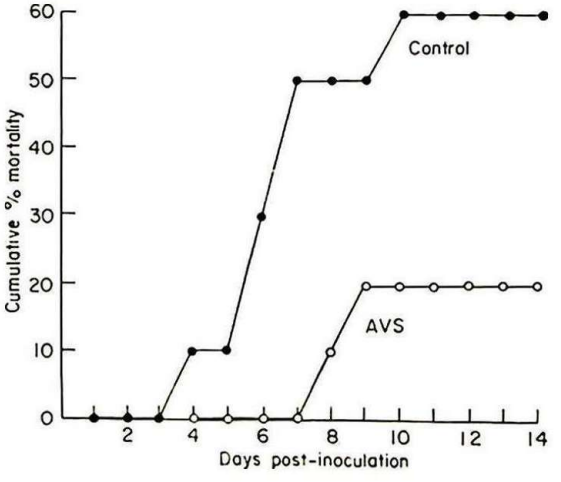

FIG. 6. Effect of *Penicillium cyaneo-fulvum* antiviral substance (AVS) on mice infected with influenza virus (PR8): virus and AVS were given intranasally simultaneously. A single dose of AVS (2·8 mg) was given.

Penicillium cyaneo-fulvum product. A strain of *Penicillium cyaneo-fulvum* isolated in McGill University, Montreal, Canada (Murray *et al.*, 1958) was shown to elaborate an antiviral substance in Czapek-Dox medium—a simple synthetic growth medium (David-West, Cooke & Stevenson, 1968a). The substance inhibited influenza A and B, and Newcastle disease viruses in Maitland-type cultures prepared from the chorioallantoic membrane of chick eggs. The multiplication of these viruses as well as that of mumps virus was not inhibited in *in ovo* studies.

In mice intranasally infected with influenza A virus (PR8) the substance produced both a delaying effect on the onset of death and a sparing effect on the final mortality rate. A better result was achieved when both virus and inhibitor were instilled intranasally (Fig. 6), than when the antiviral substance was given intraperitoneally (Fig. 7). This difference in the response of the mice may be due to an earlier presence of the inhibitor in the target organ (lungs), when the substance is given intranasally.

TABLE 1. Comparison of antiviral substances elaborated by penicillium moulds

Penicillium species	Antiviral substance (name)	Activity		Chemical nature	Dialysability	Heat stability	Viruses inhibited	Need for pretreatment	Mode of action	Media
		Filtrate	Mycelia							
P. funiculosum	Helenine	+	++	Ribo-nucleoprotein	−	−	Picorna Arbo	+	Interferon	Complex
P. stoloniferum	Statolon	++	No report	Anionic Polysaccharide	−	−	Picorna Arbo	+	Interferon	Complex
P. cyclopium	Cyclopin	+	++	Proteinaceous	+	−	Arbo	−	Intracellular	Complex
P. cyaneofulvum	None as yet	++	++	Nucleic acid components and peptides	−	+	Mxyo	−	Intracellular	Simple Synthetic

In mode of action studies it was shown that the substance acted at an intracellular site. It neither blocks virus adsorption nor interferes with the elution of influenza virus or Newcastle disease virus from red blood cells (David-West *et al.*, 1968b). It is also not viricidal.

A comparison of the substance with other reported mould antiviral inhibitors shows that the *Penicillium cyaneo-fulvum* product appears to be different (Table 1).

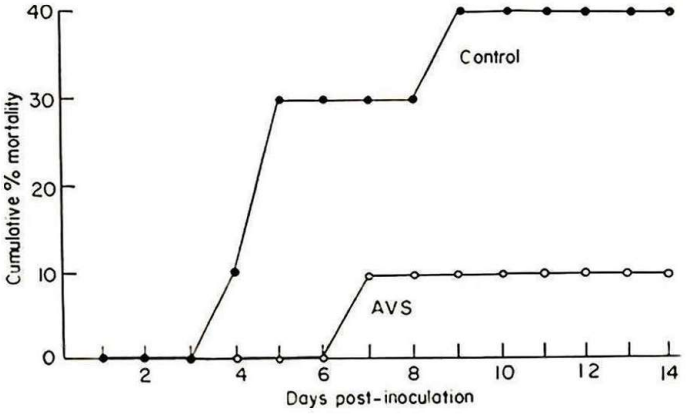

FIG. 7. Effect of *Penicillium cyaneo-fulvum* antiviral substance (AVS) on mice infected with influenza virus (PR8): virus inoculated intranasally, followed immediately with a single intraperitoneal injection of AVS (2·8 mg).

4. GENERAL COMMENTS

The quest for effective antiviral substances is distressingly beset with many setbacks. The first of which is the very intimate dependence of viral replication upon the host's physiological mechanisms. Such an association between virus and host cell greatly hampers selective inhibition of virus multiplication. However, with an increasing knowledge of the molecular details of virus growth, made possible by refined methodology, it is to be hoped that greater differences between the biochemical details of virus multiplication and the biochemistry of the host cell would be unravelled. A signal success in this regard is the selective actions of 2-(α-hydroxybenzyl)-benzimidazole and guanidine, both of which specifically inhibit the synthesis of picornavirus RNA polymerase (Baltimore *et al.*, 1963).

Another problem in the search for meaningful antiviral agents is the laborious and extensive screening procedures required. Moreover, a demonstration of antiviral activity in *in vitro* studies does not necessarily imply a corresponding activity *in vivo*. Furthermore, the same compound may behave differently against the same virus in different cell systems depending on the metabolic pathways of the compound in the different cells.

The development of drug-resistance poses a serious problem in the search for effective antiviral agents, a problem similar to that of drug-resistant bacteria in antibiotic therapy. The study of antiviral chemotherapy has thus revealed that viruses, like bacteria, can also

exist in three genetically stable forms: drug-sensitive, drug-resistant and drug-dependent.

Radiosensitization (Djordjevic & Szybalski, 1960) following the incorporation of the halogenated nucleosides into DNA may also pose a serious problem with the use of such drugs.

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