Approaches towards rational antiviral chemotherapy

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Summary

Present epidemic influenza is uncontrolled by immuno- or chemoprophylaxis. Mutants of varying antigenic composition arise with relatively high frequency in nature and are able to circumvent herd, or induced, immunity. Also, drug-resistant viruses can be selected *in vitro* and this resistance can be exchanged to other viruses by gene reassortment. Combined immuno- and chemoprophylaxis may provide a more effective approach to the ultimate control of the disease. Most antiviral compounds have been selected by random screening in the laboratory. Application of more specific enzyme assays such as the virion-associated RNA transcriptase assays may produce other compounds with a defined mode of action - semi-rational chemotherapy. RNA and polypeptide sequence studies are in progress elsewhere to define transcription and translation initiation sites or virus adsorption sites. Such knowledge could lead to a new generation of antiviral compounds. Specific delivery of virus inhibitory compounds is an interesting problem. Liposomes are lipid spheres, and these have been used for the delivery of antiviral compounds.

MANY substances inhibit virus replication *in vitro* or in animal tests but very few potential antivirals have reached the clinical testing stage (Stuart-Harris and Dickinson 1964; Bauer, 1973). For influenza virus, only amantadine and its derivatives have been shown unequivocally to be useful in preventing influenza in man. The parent compound, a cyclic primary amine, 1-aminoadamantane hydrochloride (amantadine or Symmetrel) was discovered in the early 1960s (Davies *et al.*, 1964). Amantadine (Fig. 1) is used at present on a rather limited scale in England, Europe, and the U.S.A. Its efficacy has been reviewed by Galbraith and Watson, (1977) and Sabin (1978). A derivative, α -methyl-1-adamantane-methylamine, or rimantadine, is being used more widely in the U.S.S.R. as a therapeutic agent (Blyuger *et al.*, 1970; Smorodintsev *et al.*, 1970) against influenza. Since amantadine is the first, clinically useful inhibitor of influenza A virus much can be learnt from a study of the mode of action of the compound. A nucleoside analogue, Ribavirin (Sidwell *et al.*, 1972; reviewed by Oxford, 1975) has

been shown to have mild prophylactic activity for influenza in man but may be unacceptably toxic (Fig. 1) for the prevention of respiratory virus infections although it may prove useful for other infections, with arenaviruses, for example.

FIG. 1. Molecular structures of antiviral compounds used clinically.

Influenza epidemics produce little or no excess mortality in healthy adults but produce appreciable mortality in patients with chronic pulmonary or cardiac conditions (Stuart-Harris and Schild, 1976). Therefore, any antiviral should be without significant toxic side reactions. An optimum situation is to inhibit influenza virus replication sufficiently to abort or prevent clinical signs of the disease but at the same time to allow some synthesis of virus glycoproteins so that the infected person can develop protective antibodies to prevent subsequent re-infection when drug therapy ceases. One problem with chemoprophylaxis is that persons must take

the drug continuously, and immediately become susceptible to infection when drug treatment stops. This is a reason why combined use of drugs and vaccines should be given more careful consideration than it has in the past (Oxford, 1977; Jackson, 1977).

A number of questions arise regarding the development of antiviral chemotherapy. Firstly, are we using suitable and specific screening tests to select new antiviral compounds? Secondly, which virus infections would be most suitable for control, and thirdly, even given a potent antiviral substance, how are we going to use it in the field? It is perhaps surprising that although amantadine is generally accepted by some as a prophylactic compound with an efficacy approaching that of inactivated vaccines, it is little used.

Influenza is the last remaining pandemic infection of man. More is known about the epidemiology, virus structure, antigenic composition and replication at the molecular level of influenza than of any other human virus yet, so far, little progress in the control of the disease seems to have been made. This may reflect some of the unique biological properties of the virus. Thus, the genetic code of the virus is contained in 8 unique polynucleotide sequences, each with genetic information for the synthesis of a virus structural or non-structural protein (McGeoch, Fellner and Newton, 1976). Early studies established a high recombination rate between different influenza A viruses (Simpson and Hirst, 1968). If 2 influenza A viruses co-infect a cell, genetic exchange or reassortment of genes can occur, resulting theoretically in 2^8 possible recombinants. The genetic variability can therefore be exceptionally high, and the results of this are experienced as control of the virus is attempted by immunoprophylaxis or chemoprophylaxis. In the former case, antigenic change or 'drift' rapidly occurs in the face of immunological pressure. This means that vaccine-induced or natural immunity is rapidly circumvented by mutational changes and selection. Furthermore, it is now apparent that amantadine resistance can be induced in the laboratory (Oxford, Logan and Potter, 1970), and this resistance can be transferred by exchange of gene 7 coding for the virus matrix protein (J. Schulman, personal communication).

In spite of the great increase in knowledge about biochemical events of virus replication or about the virus itself, only a few attempts have been made to apply techniques such as polypeptide and RNA sequence analysis to the problem of selection of new antiviral compounds. In short, a more rational approach to the primary selection of inhibitory compounds is desirable. The biological methods which have yielded the successful anti-bacterials and anti-protozoals of the past may not be specific enough to select good antiviral compounds. There-

fore, in the next section of this review, possible specific approaches to the selection of new antivirals using enzyme inhibition are discussed.

Delivery of antivirals to a specific site of action should not be forgotten in any discussion of rational approaches to chemotherapy, and a possible approach is the use of liposomes as drug carriers (Bangham, Standish and Watkins, 1965; Gregoriadis 1964; Ryman, 1974).

Inhibitors of influenza RNA polymerase

Influenza A and B viruses have RNA-dependent RNA polymerase activity associated with their cores (Chow and Simpson, 1971; Skehel, 1971; Oxford, 1973). In addition, RNA-polymerase activity has been detected in the microsomes (Scholtissek, 1970) and nuclei (Mahy, 1970) of influenza-infected cells. The virus-associated enzyme can transcribe, *in vitro*, a large portion of the influenza virus genome, suggesting that the enzyme has an important early function in cell infection (Fig. 2). Inhibitors of the RNA polymerase enzyme might have potential application as chemoprophylactic agents against RNA-containing viruses. Ho and Walters (1971) have described the inhibition of cell-associated RNA-dependent RNA polymerase of influenza A/PR8 (H_2N_1) virus by selenocystine, and the related compound selenocystamine dihydrochloride inhibits the virus-associated RNA polymerase enzyme of a number of influenza A and B viruses (Oxford, 1973). The author and his colleagues have described the *in vitro* inhibition of influenza virus-associated RNA-dependent RNA polymerase by selenocystamine dihydrochloride, bathophenanthroline disodium disulphonate and certain heterocyclic thiosemicarbazones (Fig. 2b). A property common to these compounds is the ability to chelate soft, heavy, metal ions such as zinc and copper. Conversely, similar types of compounds in which the possibility of chelation was diminished showed significantly less inhibitory activity against influenza virus RNA-dependent RNA polymerase. Mass spectrometry and atomic absorption techniques have detected the association of zinc with purified influenza B virus and the hypothesis is advanced that the RNA-dependent RNA polymerase enzyme of influenza virus is a zinc-activated enzyme or a zinc metallo-enzyme (Oxford and Perrin, 1974; Perrin, 1977).

The RNA-dependent RNA polymerase of influenza A and B viruses was inhibited by bathophenanthroline (disodium disulphonate), and bathocuproine (Oxford and Perrin, 1977). Bathocuproine also inhibited the DNA-dependent RNA polymerase of *Escherichia coli*, although at concentrations higher than those required for the inhibition of influenza A/RI-5+ virus-associated enzyme

FIG. 2. (a) Summary of replicative events of influenza virus and points of inhibition by existing compounds. Influenza virus enters cells by viropexis although the possibility of some fusion events is not completely ruled out. Primary transcription commences within minutes in the nucleus and later in the cytoplasm. Virus m-RNA transcripts are polyadenylated and are shorter than virus RNA fragments. Secondary transcription commences before 1 hr post-infection. The RNA replicase enzyme may be a modified form of the RNA transcriptase enzyme. Control is exerted probably at both transcription and translation levels. After virus protein transport and insertion of glycoproteins in the plasma membrane of the cell, budding occurs. Virus neuraminidase enzyme activity ensures rapid release of newly formed virus particles.

(b) Molecular structure of a series of inhibitors of influenza virion-associated RNA transcriptase.

*Indicates atoms involved in chelation.

(Table 1). Certain heterocyclic thiosemicarbazones which are effective chelators of heavy metal ions (Gingras, Suprunchuk and Bayley, 1962) were also demonstrated as being inhibitors.

In practical terms for a drug screening experiment, the influenza virion-associated RNA-dependent RNA transcriptase enzyme has to be activated by incubation of the virus with mild detergent. With a supply of nucleoside triphosphates, one of them labelled with ^3H or ^{14}C and the correct cations (Mg^{++}), the enzyme can transcribe the influenza virion RNA into an RNA copy, precipitable by trichloroacetic acid. For a screening test, therefore, enzyme activity is proportional to the radioactive counts incorporated into TCA precipitable material. It is easily possible to screen 20 compounds per day at varying compound concentrations (Oxford and Perrin, 1977). The results from the scintillation counter can be printed directly on tape and fed into a computer to enable detailed analysis of the results. The test is technically simple, the most care being required to remove the possibility of reagents being contaminated with RNAase, an extremely heat-stable enzyme. The presence of very low quantities of RNAase would result, of course, in the digestion of single-stranded RNA; therefore, the virus sample would appear to have little or no RNA polymerase

activity. It is particularly important to ensure that any inhibition of RNA polymerase enzyme activity by a particular chemical 'inhibitor' is not, in fact, simply due to contamination of the compound with RNAase.

Influenza virus purified from infected egg allantoic fluids has proved satisfactory for this test. Approximately 50 mg of virus protein would be purified from 1000 eggs and can be used for 2000 enzyme reaction tubes (Oxford, 1977). Several different virus preparations are screened in the RNA polymerase test to select a preparation with high enzyme activity and low RNAase contamination.

Table 1 shows the *in vitro* inhibition of RNA-dependent RNA polymerase of influenza A and B viruses by selenocystamine dihydrochloride and other molecules. This compound also inhibited the DNA-dependent RNA polymerase of *E. coli* at a concentration of 0.04 mmol, but had little effect on the DNA-dependent DNA polymerase of *Micrococcus lysodeikticus*. Thus a certain degree of selectivity was detected, particularly for influenza B/LEE RNA polymerase and *E. coli* RNA polymerase although the differences between inhibitory concentrations for the latter enzyme and the RNA polymerase of A/RI-5⁺ were not significant.

Examination of atomic models of selenocystamine

TABLE 1. Effect of chelating agents on virus and bacterial RNA and DNA polymerases

	Concentration of compound (mmol) to inhibit incorporation of ^3H -UMP or ^3H -TMP by 50%			
	A/RI-5 ⁺ RNA polymerase	B/LEE RNA polymerase*	<i>Escherichia coli</i> RNA polymerase†	<i>Micrococcus lysodeikticus</i> DNA polymerase‡
Bathocuproine disodium disulphonate	0.02	0.08	0.08	0.30
2-acetylpyridine thiosemicarbazone	0.003	0.002	0.20	0.20
3-acetylpyridine thiosemicarbazone	1.0	1.0	NT	NT
Isatin 3-thiosemicarbazone	0.06	0.02	0.20	0.20
Isatin 3-semicarbazone	NT	0.50	NT	NT
Isatin 3-amidinohydrazone	NT	0.50	NT	NT
Selenocystamine dihydrochloride	0.009	0.003	0.04	0.3

* Approximately 140 000 d.p.m. incorporated per mg virus protein/30 min at 37°C.

† Standard reaction mixture used with addition of 2 µg of *E. coli* k-12 polymerase protein and 2.5 µg of calf thymus DNA. Approximately 20 × 10⁴ d.p.m. incorporated per mg protein/30 min at 37°C.

‡ Reaction mixture (100 µl) contained: 50 mmol tris-HCl buffer pH 8.0, 5 mmol MgCl₂, 0.5 mmol dGTP, dATP, dCTP and 0.005 mmol of methyl-(^3H)-TTP, 6 µg of *M. lysodeikticus* polymerase protein and 2.5 µg of calf thymus DNA. Approximately 82 × 10⁴ d.p.m. incorporated per mg protein/30 min at 37°C.

Note that 3-acetylpyridine thiosemicarbazone, isatin 3-semicarbazone and isatin 3-amidinohydrazone have little or no chelating activity and also have poor inhibitory effects on virus RNA polymerase enzyme. UMP = uridine monophosphate; TMP = thymidine monophosphate; NT = no test; d.p.m. = disintegrations per min. dATP = deoxyATP; dCTP = deoxyCTP; dGTP = deoxyGTP.

suggested that the compound could act as a chelating agent for metal ions, such as zinc, through the two amino nitrogen atoms and a selenium atom. To investigate this possible mode of action compounds were selected which would chelate metal ions, such as zinc and copper, which are commonly found in metallo-enzymes (Williams, 1971).

Although some of the ligands tested would chelate Mn(II) and Mg(II) under the test conditions of influenza RNA polymerase activity, a molar excess of both Mn(II) and Mg(II) was present compared to the concentrations of chelating agents. Thus, inhibition of enzyme activity could not be attributed to removal of the latter 2 cations, which have been shown to be necessary for the demonstration of influenza RNA polymerase activity *in vitro* (Chow and Simpson, 1971). 2-Acetylpyridine thiosemicarbazone (Table 1) exerts its maximum binding effect when it forms a terdentate chelate with the metal ion by loss of a proton from the thioenolic form, with co-ordination of the metal through the sulphur and 2 of the nitrogen atoms. In 3- and 4-acetylpyridine thiosemicarbazone, only bidentate chelation (by the thiosemicarbazone moiety) is possible, leading to appreciably less stable complex formation. Similarly, isatin 3-semicarbazone and isatin 3-amidinohydrazine, in which the sulphur atom of the thiosemicarbazone is replaced by oxygen and nitrogen respectively, should also have markedly reduced chelating ability for Cu(I) and Zn(II) (Perrin, 1977). These analogues were synthesized (Oxford and Perrin, 1977) and found to be significantly less inhibitory towards influenza B/LEE/40 virus-associated RNA polymerase (Table 1). A correlation was thus established between chelating ability and inhibitory effect on influenza virus RNA polymerase.

Thus, compounds with very different molecular structures, but possessing the common property of chelating metal ions, inhibited *in vitro* the RNA-dependent RNA polymerase of certain influenza A and B viruses. Further, atomic mass spectrography and atomic absorption studies demonstrated the association of zinc with purified preparations of influenza B/LEE/40 virus, although such studies have not excluded the possibility that the zinc is present as a strongly bound contaminant. Prolonged dialysis of purified B/LEE/40 virus against 0.1 mmol of the chelating agent bathophenanthroline in 50 mmol tris-HCl buffer, pH 8.0, failed to remove the zinc associated with the virus (J. S. Oxford and D. D. Perrin, unpublished). It was proposed, therefore, that the compounds described above inhibited the virus-associated RNA polymerase enzyme by formation of a ternary enzyme-metal-ligand complex (Oxford and Perrin, 1974).

If the RNA polymerase of influenza virus is a

metallo-enzyme, more active and more selective chelating agents might be designed and tested. The compounds tested at present also inhibit *E. coli* RNA polymerase, which is a zinc metallo-enzyme (Scrutton, Wu and Goldthwait, 1971) but any inhibitory effect on mammalian cell polymerases would depend on configuration of RNA polymerase enzyme polypeptides near the zinc binding site and also on the relative stability constants of zinc for the polypeptide ligand and any competing ligand. A more objective method for assessing the metal-binding ability of chelating agent in a biological situation is provided by the use of the programme COMICS (Perrin and Sayce, 1967) which computes equilibrium concentrations of metal ions and complex species in mixtures containing several kinds of metal ions and many kinds of chelating agents. Using this approach and stability constants for copper and zinc complexes of 2-acetylpyridine thiosemicarbazone (Agarwal, personal communication) the author computed that the concentrations of this reagent would need to lie in the region 0.05–0.1 mmol if the level of intracellular free zinc ion was to be significantly depressed. Alternatively, much lower concentrations of ligand would be sufficient if the added ligand showed a relative preference for ternary complex formation through any metal associated with the influenza RNA polymerase enzyme rather than its removal from the enzyme polypeptide.

Thus, it is possible to combine the disciplines of computer technology and pharmacology and virology to select virus inhibitory compounds. An additional problem is to target the inhibitors into virus-infected cells. Attempts to do this were made using liposomes, since some of the above compounds are either relatively insoluble or too highly charged to penetrate the plasma membrane of cells.

The use of liposome-encapsulated chelating agents for the selective delivery of chelating agents to the interiors of cells of the respiratory tract is an attractive possibility (Perrin, 1977). Liposomes are finely dispersed phospholipid spherules, or vesicles around 1–10 μm in diameter, made up of concentric multiple bilayers that incorporate water and low-molecular-weight solutes in compartments between bimolecular lamellae. Liposomes can be taken up into a cell by pinocytosis or can be engulfed by phagocytes. Once inside a cell, the liposome is broken down by lysosomal lipases and the chelating agent or other drug is liberated. Liposomes may protect drugs from metabolic modification and immunological reaction. The lipid composition may be varied considerably, giving a range of membrane structure, and charged liposomes can be formed by incorporating bases such as stearylamine or anionic

FIG. 3a. Liposomes (PC : DCP lipid).

FIG. 3. *Liposomes and virosomes for selective drug delivery.* A mixture of 22.5 mg of phosphatidylcholine (PC) and 7.5 mg of cholesterol (a ratio of 3 : 1) in 10–15 ml of chloroform was thoroughly dried in a 250-ml round-bottomed flask in a rotary evaporator. The flask with the thin film of dry lipid was then transferred to a 37°C water bath. Five millilitres of drug or aqueous phase were then slowly added. The flask was quickly rotated manually to ensure a thorough wetting of the lipid film by the aqueous solution. Constant stirring was then begun immediately for 10 min. The resultant milky suspension of liposomes was then centrifuged at 2000 r.p.m. for

FIG. 3b. Virosomes (PI lipid).

5 min. The liposome pellet was resuspended in about 10 ml of normal saline solution. The same centrifugation and resuspension procedure was performed five times to ensure the complete removal of drug not encapsulated within liposomes. Before use, the liposomes were filtered through a 1.2- μ m Millipore filter. Virosomes were prepared by sonication of a mixture of purified virus haemagglutinin and liposomes for 5 min. Electron micrographs taken by D. Hockley, NIBSC, and virosomes were prepared in collaboration with T. Heath, Chester Beatty Research Institute, London.

species such as diacetyl phosphate or phosphatidic acid (Fig. 3).

By using encapsulation in liposomes, the deposition and tissue-retention of highly charged inhibitors may be significantly increased. In these model experiments Ca-EDTA or Ca-DPTA were used which are effective chelators of zinc but have a low and defined toxicity in experimental animals and man. The rationale of the experiments was to deplete the zinc levels in cells of the upper respiratory tract and hence to prevent the formation of, or inhibit the function of, the zinc containing influenza RNA transcriptase enzyme (Oxford and Perrin, 1977). However, in preliminary experiments in influenza virus-infected ferrets and mice, no antiviral activity was detected with the latter compounds, although only very low concentrations have been tested to date.

In summary, therefore, it was possible to use a semi-rationale approach to selecting and then delivering to a site of action inhibitors of influenza virus. In the final event the model compounds failed to show *in vivo* activity against influenza viruses. However, the study was a relatively small one and similar approaches in other laboratories have since led to the selection of inhibitors of influenza RNA transcriptase with biological activity (E. Helgstrand, personal communication).

Analysis of the synthesis of influenza virus-induced polypeptides and RNA

As well as the application of newer enzyme and biochemical tests to screening for virus inhibitory substances these methods can be applied to establish the mode of action of existing antivirals. An understanding of the mechanism of action of present inhibitors at the molecular level would be expected to lead to synthesis of more specific and effective compounds. Few of the existing antiviral compounds including amantadine have an established mode of action.

In collaboration with S. Patterson and R. Dourmashkin, Clinical Research Centre, Northwick Park Hospital, the effect of amantadine on the early events following influenza A virus infection has been investigated. The results indicated that amantadine at even high concentrations of 50 and 100 µg/ml had no effect on the absorption and penetration of the sensitive virus A/Hong Kong/1/68 (H₃N₂). Electron microscopy demonstrated intracellular localization of virus particles as early as 5 min after incubation at 37°C in the presence or absence of amantadine. Uncoating can be observed only with difficulty by electron microscopy since it appears to be correlated with gradual disintegration of virus structures. However, it was possible to detect late uncoating stages occurring in the presence of aman-

tadine. Therefore, the compound has no effect on virus adsorption, penetration or late uncoating. Separate experiments established that the compound has no inhibiting effect on the RNA transcriptase enzyme, at least *in vitro* (Table 2). The precise point of action of amantadine appears to be after late uncoating or an unknown event accompanying this, before transcription.

TABLE 2. Absence of inhibition of influenza A virion associated RNA dependent RNA polymerase activity* by amantadine

Concentration of compound (mmol)		H ³ -UMP incorporated d.p.m./mg virus protein/hr × 10 ⁻³
Amantadine	100	96.1
	10	84.2
	1	80.0
Selenocystamine	0.1	0
Control (no drug)		97.1

* Enzyme activity estimated as described previously (Oxford and Perrin, 1977).

UMP = uridine monophosphate; d.p.m. = disintegrations per min.

These experiments were also controlled by examining cells for evidence of virus infection. The electron microscopy data could be correlated with intracellular virus replication events. Four hours after virus adsorption, virus-infected cultures were pulsed with ³⁵S-methionine and the labelled polypeptides subsequently analysed by SDS polyacrylamide gel electrophoresis. Examination of the autoradiograph (Fig. 4) indicates the presence of A/HK/1/68 (H₃N₂) virus-induced NP, HA, M and NS₁ polypeptides. Note that synthesis of these polypeptides is inhibited by incubation of the virus-infected cells with 60, 30 or 15 µg/ml amantadine. In contrast, influenza B virus replication is only inhibited by very high concentrations of amantadine (250 µg/ml) which would probably be exerting some toxic effect on cell metabolism even after only 4 hr's incubation. Recent gene analysis (J. Schulman, personal communication) of recombinants between influenza A parents which were resistant or susceptible to inhibition by amantadine indicated that gene 7, coding for the internally situated matrix protein, carries the property of amantadine resistance. Matrix protein may have an important function in late uncoating, perhaps regulating transcription of influenza A virus RNA and hence infection of the cell.

More recently, polypeptide analysis of influenza virus-infected cells has been applied to quantitate the inhibitory effect of amantadine. Thus, Fig. 5 illustrates NP, M and NS₁ polypeptides synthesized by A/USSR/77 (H₁N₁) and A/Victoria/76 (H₃N₂) viruses. Polypeptide synthesis of both viruses was

FIG. 4. Amantadine inhibits polypeptide synthesis of influenza A/HK/68 virus but has no inhibitory effect against influenza B/HK/73 virus. HA, haemagglutinin. NP, nucleoprotein. M, matrix protein. 250, 125, 60, 30, 15 $\mu\text{g/ml}$ amantadine.

Vero cells were incubated with 50 or 25 $\mu\text{g/ml}$ amantadine and infected with 10 p.f.u./cell of influenza virus. After 4 hr incubation at 37°C the cells were pulsed for 30 min with 20 μCi per 60 mm petri dish in 0.2 ml Geys medium of methionine (specific activity 1040 Ci mmol^{-1} , Radiochemical Centre, Amersham). The cells were then washed and lysed with 0.2 ml of a mixture of 2% w/v SDS and 0.6% v/v β -mercaptoethanol, heated at 100°C for 2 min and 20 μl volumes layered on slab gels of 20% polyacrylamide using high resolution discontinuous buffers and electrophoresed for 18 hr at 40 mA. Gel slabs were dried and autoradiographed by standard procedures (Oxford, 1977).

p.f.u. = plaque-forming units; SDS = sodium dodecyl sulphate.

FIG. 5. Inhibition of influenza H_1N_1 virus polypeptide synthesis by amantadine. V77, A/USSR/90/77 (H_1N_1) virus. Vic, A/Victoria/76 (H_3N_2) virus. C, control. 50, 25, $\mu\text{g/ml}$ amantadine. M, matrix protein. NS₁, non-structural protein number 1. NP, nucleoprotein. Experimental details as for Fig. 4.

markedly inhibited by 50 or 25 $\mu\text{g/ml}$ amantadine. Thus, the degree of inhibition of the recent H_1N_1 viruses was comparable to the inhibition of current H_3N_2 viruses. The polypeptide labelling technique is rapid and gives useful semi-quantitative data. Densitometry of the autoradiograph allows more precise quantitation of the degree of antiviral activity.

Conclusions

The 'first decade' of antiviral chemotherapy (Bauer, 1973) has been an exciting one and some

compounds of proved clinical value such as methisazone, idoxuridine, vidarabine and amantadine have been discovered. However, these compounds were either discovered in random screens or were discovered firstly as anti-tuberculous or anti-tumour compounds. The precise mode of action of these molecules has not been established to date. More recently some virus inhibitors including phosphonoacetic acid (reviewed by Hay *et al.*, 1977) have been selected with a rather more precise point of action. Thus phosphonoacetic acid has a selective effect on the herpes virus DNA polymerase enzyme. An

acyclic guanosine molecule (Eilon *et al.*, 1977; Schaeffer *et al.*, 1978) has even more specificity since the compound has to be phosphorylated in the cell to the active form. Acyclic guanosine is more efficiently phosphorylated in herpes virus-infected cells than in control uninfected cells. Moreover, once phosphorylated, acycloguanosine triphosphate has a specific inhibitory effect on the herpes DNA polymerase and is 10–30 times less inhibitory on cellular HeLa cell DNA polymerase. Using present techniques, important virus specific enzymes such as herpes virus DNA polymerase, influenza RNA transcriptase and picornavirus RNA polymerase can be isolated and used in primary screens to select inhibitors with a specific function.

Application of the techniques of molecular biology could now lead to a specific approach. Sequence studies of influenza virus HA protein are in progress in several laboratories (Skehel and Waterfield, 1975). Knowledge of the amino acid sequence of the HA at the site of attachment to cells may enable synthesis of specific inhibitors of virus adsorption to be carried out. Small synthetic tripeptides inhibit the early infection events of measles virus and may be competitive inhibitors of the adsorption site on the virus. Similarly, the analysis of nucleotide sequences of influenza RNA now in progress delineate RNA transcriptase and replicase binding sites and also ribosome binding sites. A. Porter and P. Fellner of Searle Laboratories have partially sequenced several picornavirus RNAs – approximately 250 nucleotides have been determined to date. Such basic information should lead to opportunities for the logical synthesis of specific inhibitors of vital virus functions.

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