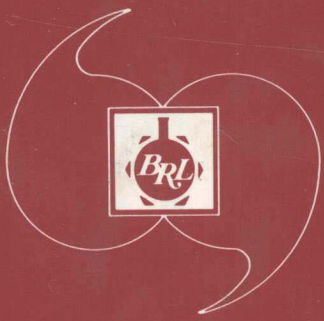
The Beecham Colloquia

A series of occasional meetings on aspects of infection



Problems of Antiviral Therapy

edited by
Sir Charles H. Stuart-Harris
and John Oxford

Academic Press

Problems of Antiviral Therapy

Editors

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Preface

Progress towards effective antiviral chemotherapy has proved both tardy and frustrating. In contrast to the giant therapeutic successes achieved against microorganisms ranging from bacteria to *Chlamydiae*, progress against viruses has been minimal. Today, however, antiviral chemotherapy has been achieved and since the first important successes have occurred against differing virus groups, there is a tendency to be over-optimistic. In fact this area of human endeavour has suffered in the past both from too much pessimism and too much optimism.

The early optimism arose from failure to appreciate the unique biological behaviour of viruses when proof that intracellular parasites were not immune from attack was first shown by active drugs against the *Chlamydiae* and the *Rickettsiae*. Again, viruses are not degenerate forms of bacteria, an idea popularized by microbiologists in the 1930s, and the realization of the peculiarly intimate relationship between virus and host cell has arrived relatively recently. But it is particularly important for positive achievement, however limited, to be made in antiviral therapy if only to sustain the optimism needed for further research in this peculiarly difficult field.

This colloquium was not brought about to recount successes or to give details of clinical trials. Instead the organizers have sought to encourage discussion on the problems which are now being appreciated and which hinder effective therapy. At a fundamental level one may ask whether further progress is likely to result from repeating the mass screening of existing compounds or whether we should look to rational developments in methods of attacking the virus, its enzymes, nucleic acid or proteins. Unfortunately, the quirk of unexpected discovery still exists, as in the case of amantadine. Probably we should not rule out any way by which new compounds with antiviral properties may be found. This introduction is intended in part to counter criticism that progress in antivirals has been too slow, but justification for the delay exists and no apology need be made.

April 1983
Sheffield

Sir Charles Stuart-Harris

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Antiviral Chemotherapy: an Introduction and Apology for the Slow Progress

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Over the past 40 years the fortunes of antiviral chemotherapy have swung to and fro with alternating moods of optimism and pessimism. As this has also been the time when chemotherapy of bacterial and parasitic infections has shown the greatest advance in history, antiviral therapy has suffered by comparison. Very large numbers of substances have now been screened in viral culture systems. Relatively few have shown activity and, of these, fewer still have proved to be active in experimentally infected animals. Yet the remarkable conquest of rickettsial infections by chloramphenicol and successful use of sulphonamides and tetracycline against *Chlamydiae* had shown that intracellular parasites are not protected by their position against attack. This raised hopes of ultimate success against the viruses but, alas, in those early days of the 1940s, their unique biological nature was not fully appreciated. Nor had the peculiarly intimate relationship of virus and host cell been outlined, so that viruses appeared to be designed by Nature to resist human efforts to accomplish their destruction. A glance at early developments in antiviral therapy may be useful in providing some perspective on the apparently slow progress achieved so far.

EARLY DEVELOPMENTS IN ANTIVIRAL THERAPY

Bauer (1972) gives the credit for the first successful achievement of antiviral

therapy to Hamre and her colleagues (1950), who found that *para*-aminobenzaldehyde thiosemicarbazone possessed therapeutic activity in both eggs and mice against vaccinia virus. Ten years later, Bauer and Sadler (1960) reported the activity of isatin thiosemicarbazone in mice inoculated intracerebrally with a strain of alastrim (variola minor) virus. Within three more years, a trial of human prophylaxis among variola contacts in Madras by Bauer *et al.* (1963) using 1-methyl isatin β-thiosemicarbazone proved successful.

These encouraging developments with pox viruses illuminated a field increasingly darkened in other directions. Efforts to show that inhibitors, which were effective *in vitro* in uniting with influenza virus haemagglutinin, came to grief in *in vivo* systems. Indeed, though Green and Woolley (1947) found polysaccharides such as apple pectin could inhibit influenza virus in the fertile hen's egg, they were totally inactive against influenza in mice. When the first information came that amantadine, a compound "taken off the shelf", was actively inhibitory to influenza virus infection in both eggs and mice (Davies *et al.*, 1964), no previous clues to its activity existed. Excitement increased when first the prophylaxis of induced influenza in volunteers proved successful (Jackson *et al.*, 1963) and later trials in family contacts were similarly beneficial (Galbraith *et al.*, 1969). Therapeutic activity under field conditions (Wendel *et al.*, 1966; Togo *et al.*, 1970) was demonstrated later but the benefit of a reduction of illness by a few hours only seemed to be offset by potential side-effects of a psychological nature. The drug had no action of any sort against influenza B virus and clinicians were faced with the impossible task of making a clinical distinction between influenza A and B in order to harvest a doubtful order of benefit. Small wonder that Sabin (1967) was able to criticize the hard-won efforts of clinicians who nevertheless had established that amantadine was indeed an antiviral. Its arrival on the scene had already produced one benefit in that the controversial drug virugon (ABOB-X), which was apparently active in mice infected with influenza virus (Melander, 1960) but inactive in man (Jackson *et al.*, 1961), underwent a speedy demise. The contribution of many clinicians dedicated to determining the true value of any drug is often denigrated but those early trials of amantadine have fully stood the test of time and the Consensus Development Conference held at the National Institutes of Health in 1979 (*Science Research News*, 1979; Symposium, 1980) gave the drug unquestioning support. More may be recounted during this Colloquium since the mechanism of action of amantadine is still largely unknown.

Meanwhile, the history of interferon must be symbolic of the problems of establishing the scope of usefulness of antiviral substances. It was in 1942 that Andrewes (1942) established virus interference between the lung strain of WS (H1N1) influenza A virus inoculated into tissue cultures and a later challenge infection of the same tissue cultures with the neurotropic NWS virus developed from the WS virus in 1939 (Stuart-Harris, 1939). Viral interference

was well known in other fields at that time and later in the 1940s Isaacs, then working in Sheffield, challenged the orthodox view that interference occurred extracellularly when the first infecting virus "shut the door" on the entry of a second strain. Isaacs and Lindenmann (1957) proved the intracellular formation of interferon by extracting it from infected cells and Isaacs *et al.* (1963) showed that it could be secreted in response to non-infective nucleic acids. Five years after the first recovery of interferon, successful prophylaxis in human infections with vaccinia virus was demonstrated (Scientific Committee on Interferon, 1962; Jones *et al.*, 1962) and its wide range of antiviral activities for a time made it appear a unique antibiotic for virus infections.

Can it now be said that interferon has been established and shown to be of practical value after 20 years' more work? Everyone knows that interferon has proved a most difficult substance to prepare, has limitations in terms of species-activity and gives conflicting results in actual practice. But the present use of interferon (Cartwright, 1980), particularly in cancer patients, and its preparation by recombinant DNA techniques has renewed interest in the drug. The obscurity of its mechanism of action (Williams *et al.*, 1980) and heterogeneity according to its cellular or clonal origin (Goeddel *et al.*, 1981) both add to the problem of defining its use.

THE PROBLEM OF VIRUS REPLICATION

Overshadowing all other problems in developing antivirals is the major puzzle of virus replication. Each small piece of information concerning nucleic acid, either in its chemistry or in the peculiar manner by which viruses persuade cells to distort their metabolism, is eagerly awaited in the hope of solving the jigsaw puzzle. Rational chemotherapy certainly has many difficulties and unexpected results are still the mode. When the first step of changing the structure of one of the bases used in building up nucleic acid is made on rational grounds in the laboratory, the end-result in terms of antiviral activity may differ from that which was foreseen. Thus, the development of acyclic compounds from nucleosides seemed to promise competitive inhibition of virus replication. When guanosine was chosen as a compound from which to produce an acyclic derivative, it had become known that it was incorporated into the cRNA of influenza virus during replication by transcriptase action (McGeoch and Kitron, 1975). Yet when acycloguanosine was tested in the laboratory it was not inhibitory to influenza virus though highly active against herpesviruses (Schaeffer *et al.*, 1978). The inhibitory effect of acycloguanosine through the action of the virus' own thymidine kinase is, of course, of great theoretical interest. Meanwhile the hunt is now on for even more active compounds against herpesviruses as described by De Clercq and others (1980) and the exploration of structure–activity relationships may well be successful.

The need for more and more knowledge about biochemical relations between virus and host cells is now well appreciated. Unfortunately, the basis of the dependence of the virus on specific chemical elements within the host cell is not fully known. An instance of this extraordinary relationship has come from the work of Krug and his co-authors (1979) on the priming of transcription of the negative strand of influenza virus RNA. For some time it had been suggested that transcription to cRNA messenger required the host cell's help and it was also known that the amount of cellular RNA increases in the early hours of replication of viral RNA. Plotch *et al.* (1981) have now shown that viral transcriptase requires a primer derived from cell nucleic acid and consisting of a short length of 10 to 15 nucleotides from capped RNA. This is split off by a viral endonuclease and is responsible for priming the transcriptase to perform its function in the formation of viral messenger RNA. Such an intimate interplay of host and virus chemistry suggests that the ability of a chemical compound selectively to inhibit the one and not the other should be regarded as a remarkable achievement. There is little need to apologize for the slow progress of antiviral research when so much has yet to be learnt.

The Hindrance to Antiviral Action by the Pathology of Virus Infections

Much has been said and written about the need for early diagnosis in virus infections and this is certainly true in short-lived infections of the respiratory or alimentary tracts (Gardner and McQuillin, 1974; World Health Organization, 1981). Yet it must be recognized that very considerable replication of viruses occurs during the incubation period. It is, therefore, a fact that by the time a patient presents in the clinic, it may already be too late to intervene with a specific therapeutic substance before either recovery commences spontaneously or cells are destroyed. For instance, the peak in virus titre in the spinal cord during poliovirus infection precedes paralysis and occurs during the pre-paralytic stage of vague symptoms, difficult if not impossible to specify as poliomyelitis. Much the same applies to the exanthemata in which virus replication in internal viscera precedes the appearance of a rash. Even early diagnosis may not be as helpful as has been thought and yet unless a therapeutic substance can be given at as early a stage as possible it may prove ineffective.

Viral infections of a chronic character such as herpetic encephalitis are not thus affected for with these virus replication continues for days or weeks. There is, however, a great need to establish the viral nature of herpetic encephalitis, particularly because the clinical picture embraces many other conditions. Controversy now appears to exist concerning the need for a brain biopsy since brain-scanning can give much diagnostic help (Caplan, 1977). In clinical trials, however, it seems to me essential that identification of virus or viral antigen should be obtained (Whitley *et al.*, 1977, 1981).

Finally, chronic conditions though originally initiated by viruses may become complex in pathological character because of autoimmune processes. This is probably true in chronic active hepatitis and it may account for conflicting reports of the effect of interferon in this condition (Greenberg *et al.*, 1976; Weimar *et al.*, 1980). Lack of benefit from specific antiviral therapy may be the result either of inadequate therapeutic effect or of immunopathology or even of the particular stage of infection in the individual patient. Clearly the evaluation of therapy in chronic viral infections is fraught with difficulties.

CONCLUSION

Forty years are not a long time in the battle between man and his environment. It is natural for human patience to be tried by slow progress, yet in the case of antiviral therapy the difficulties of interfering with virus–host relationships are much greater than in infective conditions in which the parasite is extracellular for much or all of its life-cycle. Nevertheless, antiviral therapy exists successfully and this is an exciting moment to discuss the problems which hinder further progress and the lack of knowledge which must be remedied.

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DISCUSSION

Jackson Sir Charles, you have identified the themes that are in the minds of all of us. I appreciate your evaluation of the studies of amantadine; the early studies were easily able to be reproduced. The problems of implementation are manifold. The biology of virology in the intact cell, and intact tissue, is where I think we are just on the starting blocks. Whilst the chemistry and some knowledge of the replicative nature of viruses has progressed quite skilfully, my own feeling in that the era of antiviral chemotherapy, with some successes, is going to lead to a number of surprises and a tremendous amount of information about the biology of viral infections in cells in intact tissue. Your introduction did not seem to be so much of an apology as to have put us at the beginning of the log phase of growth that this Conference might help develop.

Tyrrell I have three things that I would like to say. The first is that there has been an over-emphasis on the importance and significance of finding that something has an antiviral effect *in vitro* or even in an animal. That is a perfectly good starting point, but metabolites of substances that do not work *in vitro* might work in the whole animal. Dr Davey of Imperial Chemical Industries once told me that something like one in a hundred substances in a screening programme which had some desirable biological effect turned out to be worth

thorough investigation, and of these less than one in ten actually turned out to be a useful drug. So something like one in a thousand of the antiviral substances found in the laboratory could be expected to be useful in a clinical situation. It was lucky that amantadine came up better than one would have expected on those sort of odds. Secondly, I have noticed a fault in relation to interferon work, namely that over-optimism has been followed by excessive pessimism. An American scientist once said to me, "You are wasting your time—exogenous interferon can never be any use. You can never make enough of it, and instead you ought to go for interferon inducers to make the body make its own interferon". Such strong opinions and strong statements may have held up steady work on the subject, although probably not disastrously so.

Thirdly, the future. We now have antivirals and can begin to use them in the next few years. Probably the ability to inhibit virus replication may teach us more about the pathogenesis of infections. In the introduction the reverse view was taken, namely that lack of an understanding of pathogenesis made it difficult to decide how to use drugs. I suspect that we may be able to find out answers to the question that was asked in the laboratory by Frank Horsfall in the Rockefeller Institute years ago—how much virus replication is occurring at the time when one begins to be ill with influenza? If all the replication has ceased by then or if there is very little new virus replication on new cells available to become infected, then one cannot achieve very much by inhibiting the virus. If there is still more to occur, however, then one can. As answers have partly come through using amantadine, similar instances may happen in the future.

Choppin It seems to me that the antiviral field is the prime example of the advantage of knowing exactly what the basic mechanisms are before designing reasonable agents to combat them. I remember that 25 years ago when I was first learning virology, one of the things we were taught was that there was only one virus-specific enzyme, the neuraminidase of influenza, and now we have more viral enzymes than we have viral proteins in some cases, which are obviously potential targets for specific antivirals. Along the way of muddling through and learning about the biology of viruses in order to be able to deal with them, however, there has been a tremendous spin-off in what virology has taught us about cell biology. That is another reason why I think that the apology for slow progress in antivirals does not have to be too profuse.

Bauer The responsibility for finding new antiviral compounds lies fair and square with the pharmaceutical industry, but it is very handsome for a distinguished medical academic to apologise on our behalf for our performance. You may have given the impression that it is very difficult to find antiviral compounds. This is not so. Anybody who has run antiviral screens knows that leads are constantly turning up; when we were still screening against vaccinia we quite often found antivaccinial activity in completely unrelated com-

pounds. More topically, it is quite easy to find compounds active against rhinoviruses, which is fine, but the trouble comes when you ask—are these sufficiently active to be worthy of further development?

There is another point. If one could find a lead against, say, measles, is it worth developing this at all? To develop a compound to the stage at which one can test it in human volunteers one has to spend about 10 million dollars. If the market for measles, for example, is extremely small, there is no chance of getting the money back. So any project like that is stifled at birth, and industry goes for the things with a much wider application such as the rhinoviruses. This means that we shall never have compounds active against viruses such as adenoviruses, measles, mumps and so on. The only possibility of these being found is some kind of screening programme funded by some supranational agency. I think WHO at the moment is funding chemotherapy against the less popular parasitic diseases, and unless this is done with antivirals there is a whole range of viruses diseases which we may never be able to treat.

Field Could I take up the pessimism regarding the fact that the period of virus replication is often succeeded by immunopathological processes? Regarding Tyrrell's earlier comment, the experience with very effective antiviral agents such as acyclovir in herpes simplex suggests that even when the disease in experimental animal situations is under way, the moment therapy is instituted there is a rapid ameliorating effect, although we know that by this time the disease is very much mediated by immunopathogenic mechanisms.

This gives grounds for optimism that probably even immunopathological processes require a continued antigenic drive. Therefore anything that effectively inhibits virus replication could very quickly be reflected in amelioration of the disease.

Stuart-Harris My plea was also that clinicians cannot function until a patient actually presents. If the patient is sitting at home saying, "Oh, I've only got a cold, I won't go to the doctor" when really he is in the beginning phase of something far more serious, then the doctor misses out and those valuable few wasted hours may lead to catastrophe.

Philipson I would like to emphasize that history also poses the question whether we have sufficient background knowledge to develop antivirals in a rational way? In general screening, the hit frequency is small, as indicated by Tyrrell and one in a thousand seems to me to be high; it might be even lower than that. When molecular biology was developed, it was natural to look at the viral enzymes and compare them with the corresponding host enzymes in order to develop a rapid screening procedure. But we are again facing a time when we lack sufficient background knowledge in the area of virus replication. During the last 10 years we have mapped most of the genes of the viruses and have a fairly good idea about the architecture of the viral genomes and expressed proteins. In most cases, however, fine details and also control mechanisms at the molecular level concerning virus replication are lacking. The example of