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DIAGNOSIS OF VIRAL DISEASE AND THE ADVENT OF ANTIVIRAL DRUGS

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1. INTRODUCTION

The data are substantial; there are an array of substances that inhibit viral multiplication without causing harm to the host. Antiviral drugs are available in a number of countries; therefore, it requires no great courage to predict that in the near future more antiviral drugs will be available and their proper use could often depend on some knowledge of the virus causing a disease.

At present it may not be obvious that laboratory diagnosis should go hand in hand with the few antiviral drugs now available. Indeed, few viral diagnostic laboratories are readily accessible to most medical practitioners. In contrast, bacteriological diagnostic laboratories were widely available even prior to the advent of antibacterial drugs. Yet, it is commonly held that there are many times more viral induced diseases (albeit, often mild and self-limiting) than bacterial diseases. Routine diagnosis of the viral diseases seen in medical practice seems, therefore, to be overdue; especially since such experience would provide an invaluable foundation for the future rational use of antiviral drugs.

In that antiviral drugs and viral diagnostic laboratories currently touch lightly on the routine practice of medicine, there is little prior experience on which to base predictions. The following discussion will, therefore, require a significant degree of conjecture about how medicine might be practiced when antiviral drugs are commonplace. It would be foolhardy to presume that in the future the use of antiviral drugs will always be based on a sound diagnosis, any more than is true today with the use of antibacterial agents. It must be acknowledged, however, that certain viral diseases can be rather firmly diagnosed on clinical and epidemiological grounds alone. We will not, therefore, take the unrealistic position that all viral disease must be diagnosed in the laboratory; indeed, it is more likely that in the foreseeable future few of the milder and self-limiting viral diseases will result in a laboratory diagnosis. We will, nonetheless, review human viral diseases and the problems of practical laboratory diagnosis. Predictions as to the nature of future antiviral drugs will at times require certain flights of fancy.

2. THE PRESENT

2.1. LABORATORY DIAGNOSIS OF VIRAL DISEASE

Let us assume that chemotherapeutic drugs are available for all virus diseases that can presently be diagnosed in the laboratory. Would present diagnostic procedures be adequate? Let us further assume that viral diseases are handled in a manner similar to bacterial diseases; that is, an infected patient visits a physician, a hospital or a clinic, and ideally a culture is taken and sent to the laboratory. A bacterial pathogen is isolated, a presumptive identification of the type of organism is made, confirming tests and, in some cases, a drug sensitivity test is undertaken. In the meantime, the patient is frequently started on a course of antibacterial chemotherapy based on a number of factors such as the degree of illness, some distinctive clinical signs and symptoms, or simply on whimsy or intuition. Such treatment may be stopped or altered on the basis of laboratory results. This is a one on one

situation, one patient at a time, with only slight regard for the infections that are prevalent in the general population, for such information is not always available to the physician.

In making comparisons of viral diagnostic methods to those used in bacteriology, it is often assumed that the latter are very rapid and results are available within 24 hr. This is frequently not the case. When antibacterial sensitivity tests are required, results cannot be reported sooner than 48 hr after submission of the specimen. Too frequently, all bacterial diagnosis is equated with the rapid isolation of beta hemolytic streptococci, but defining whether the isolate is *Streptococcus pyogenes*, the more pathogenic streptococcus belonging to Lancefield group A, takes additional time. In bacterial diagnosis a presumptive rapid report can sometimes be made based on stained smears of specimens prior to cultivation of the organism, but it is not often appreciated that cultivation and identification of a specific bacterial pathogen can take considerable time. Generally, then, how do present virological methods compare to such bacteriological methods? As will be discussed, in certain cases they compare rather well, but in most cases not so well if viewed in the classical medical situation just described.

2.1.1. *Virus Detection*

More human viruses can be detected more quickly by the use of cell cultures than by any other method and this is based on a viral cytopathogenic effect and on the ability of certain viruses to hemadsorb or hemagglutinate red blood cells. Ninety-seven per cent of virus diagnostic laboratories surveyed in the United States and Canada isolate viruses in cell cultures, and over 25 per cent use this approach exclusively (Herrmann and Herrmann, 1976a). Such a fact is not obvious from the published papers devoted to viral diagnosis that seem overwhelmingly to emphasize serological methods. But are cell culture methods adequate if an antiviral agent is to be used?

Table 1 presents the experience in isolating more than 5000 viruses in cell culture. The data suggest that under optimal conditions, using specimens rich in virus, many viruses are detected after 1–2 days incubation, others in 3–4 days. Regrettably, conditions are very rarely optimal. The quantity of virus in a specimen is often slight, so the time for isolating 50 per cent of the viruses is no less than 3 days and more frequently longer. Clearly this is not completely adequate if a physician wishes to treat a patient with a drug specific for a certain kind of virus infection. In the overwhelming majority of viral infections, the laboratory data will be generated at a time when the patient is either well or recovering. The data in Table 1 are for the detection of a virus, the lengths of time do not involve specific viral identification, which may take weeks. The viral identification problem is not insurmountable for in many cases the virus either need not be identified, or at least need not be identified as to its specific serological type, as will be discussed later. It is clear, however, that cell culture methods for detecting human viruses require considerable improvement. Further, a full 80 per cent of specimens produce no viral isolate, even from patients with viral-like illnesses.

But what about other viral diagnostic methods, how would they fare if subjected to the logistical problem of treatment of viral disease in routine medical practice? In general, they fare less well than do cell culture methods. Any test that measures a rise in specific antibody using paired sera, that is, one blood sample taken at the acute stage of illness and a second taken 2–4 weeks later, could only rarely have any pertinence to drug treatment. Further, the measurement of antibodies requires specific viral antigens and since there are at least 70 such antigens for the enteroviruses and 120 for the rhinoviruses, the cumbersome nature of this system and its limitations become obvious. A similar problem exists in using fluorescent or other labeled antibodies to detect viruses either in specimens or in cell cultures. The fluorescent method is rapid (1–2 hr) for diagnosing herpetic infections but significantly less sensitive than viral isolation in cell cultures (Cho and Feng, 1978). The large number of serologically distinct viruses one might find on a throat swab (Table 2), for example, makes this method impractical for general use and despite the fact that fluorescent methods have been known for well over 20 years, they have been used in a limited and specific way when used at all in the general diagnosis of viral disease.

There has been significant interest for many years in the use of the electron microscope (EM) for rapid diagnosis of viral disease and this interest has been much enhanced by the recent discovery of a variety of viruses in stools of patients with gastrointestinal upsets (Middleton *et al.*, 1977). None of these viruses detected by the EM can be routinely cultured in the laboratory. In addition the EM has played an important role in defining the A and B viruses causing hepatitis (Dane *et al.*, 1970; Feinstone *et al.*, 1973). Certainly the EM is a most

TABLE 1. *Time for Detection and Reporting of Virus Isolates after Receipt of Specimen**

Virus	Earliest detection (days)	⩾50 per cent detection (days)	Isolates (No.)
HSV†	1(67)‡	3	1266
Cox.B	1(26)	3	428
Cox.A, T.C.	2(14)	4	66
ECHO	2(43)	4	400
Polio	2(8)	4	87
Influenza	2(46)	4	675
Adeno	2(61)	5	681
Rhino	2(13)	6	484
V–Z	3(5)	8	43
CMV	3(3)	11	49
Mumps	3(11)	6	254
PI	4(39)	8	702
Cox.A, mice	4(11)	10	196
RSV	5(14)	10	239

* Cell cultures were read thrice weekly.

† Abbreviations: HSV—herpes simplex; Cox.B—coxsackievirus type B; Cox.A—coxsackievirus type A; T.C.—isolated in tissue culture; ECHO—echovirus (enteric, cytopathic, human orphan virus); V–Z—varicella-zoster virus; CMV—cytomegalovirus; PI—parainfluenza virus; RSV—respiratory syncytial virus.

‡ Figures in parenthesis are number of isolates reported at this time.

TABLE 2. *Antigenic Types of Viruses Isolated from the Human Upper Respiratory Tract, 1962–78*

Virus	Antigenic types* (No.)	Isolates (No.)
Adeno	7	681
Cox.A, T.C.	2	66
Cox.A, mice	?†	196
Cox.B	5	428
CMV	1	10
ECHO	16‡	300
HSV	2	666
Influenza	4	675
Mumps	1	254
PI	4	702
Polio	3	52
Rhino	?§	484
RSV	1	240

* Does not count closely related subtypes of a major antigenic group as for example the various subtypes of Hong Kong influenzavirus type A (Victoria, Texas, London, etc.).

† These isolates defined by typical mouse paralysis and not serotyped, 22 human serotypes are known in addition to types 9 and 16 which were isolated in cell cultures.

‡ A total of 33 human serotypes are known.

§ Not serotyped, defined by biochemical tests (sensitivity to a pH of 3 and resistance to chloroform), about 120 human serotypes are known.

valuable research tool in defining the nature of viruses infecting humans but its expense of installation and operation, which can easily be $150 for a single viral diagnosis, is far in excess of what should be paid for diagnosing the average viral infection. Also in most viral disease it is difficult to obtain sufficient virus (10^6 particles) for ready detection. Use of the electron microscope can detect herpes simplex virus in fresh vesicles but it cannot distinguish these from the virus of varicella-zoster, a distinction that can be important in a clinical diagnosis. But even with such problems it would seem worthwhile for the virus laboratory to at least share some limited time with others using the EM since in some cases its potential for rapid diagnosis could lead to effective therapy.

The measurement of early arising specific antibody of the IgM type has attained some popularity in the diagnosis of rubella, cytomegalo and herpes simplex virus infections in the infant. There seems to be some concern, however, whether this method is as definitive as it needs to be for accurate diagnosis (McCracken and Newman, 1975). In many cases the detection of viral specific IgM antibody can be rather suggestive in the somewhat chronic viral disease and even permit the inception of effective therapy. With most viral disease, however, it is unlikely that the IgM antibody level would be detectable early enough for the effective use of antiviral drugs. It would be difficult to envisage a program that would have to measure hundreds of different types of IgM antibodies in the diagnosis of most viral diseases. But again there will likely come a time when some viral infections are most readily diagnosed by this method.

2.1.2. *Virus Identification*

The definitive technique for identification of a virus isolate has long been its interaction with a known, specific antibody using a wide variety of test methods. It is the need for specific antibodies that can make this a time-consuming and expensive procedure, likely the most expensive part of viral diagnosis. Is this entirely necessary? In many cases, it is not. Indeed, attempting to serotype one of the 120 rhinoviruses is an impossible task for a general virus diagnostic laboratory. The emphasis on serotyping viruses goes back to the hope of vaccines for controlling most viral disease, where the antigenic nature of a virus is important. What is its importance in chemotherapy? The vast number of serotypes of pneumococci or salmonella, for example, is of little importance to a chemotherapeutic attack on these bacteria (albeit, not always successful). Some may feel that use of amantadine and rimantadine, both of which are active only against influenza type A viruses and not type B, justifies serological identification. The fact that these two types of influenza viruses differ sharply in their sensitivity to amantadine establishes they are biochemically distinct and it may well be only incidental that they are also serologically different. Biochemical markers would likely be more useful in virus identification. Even the sensitivity to antiviral agents may be useful as a biochemical identification test.

The present serological methods do permit, after some effort, defining that one has at least isolated a certain type of human virus and if it proved to be an echovirus, for example, then a drug specific for echoviruses could be used. It is likely that by the time one has this added information, a drug would be all but useless. Likewise, if one defined a pathogenic bacterial isolate strictly according to *Bergey's Manual*, this information also would be too late for use in applying appropriate therapy, so some compromises are in order. What is required, of course, is identification that is pertinent to therapy. If one knows he is dealing with an echo, coxsackie or even a rhinovirus, all of which are picornaviruses, which have been shown *in vitro* to be sensitive to the same compounds, then a single drug might well be used on infections caused by any one of them. How might one presently recognize such viruses in general?

As explained elsewhere (Herrmann, 1970) the approach of the bacteriologist can be used. Three cell culture systems represent the virologist's differential media since certain viruses produce a cytopathic effect or hemadsorption in certain cells and not others. The distinctive nature of the cytopathic effect, or the presence of hemagglutination or hemadsorption is analogous to the bacteriologist's use of colonial morphology. And, of course, the

bacteriologist's biochemical tests have counterparts in virology; some viruses are sensitive to pH 3 and lipid solvents, others are sensitive to pH 3 only and still others are resistant to both. There are also specific viral inhibitors that can be used. But biochemical tests might well take more time than seems practical in guiding the use of a drug by a single physician on a single patient.

The nature of the cytopathic or hemadsorption effect and the cell type involved when coupled with other information can often give a better indication of which type of virus one has isolated. Knowing the type of specimen helps; fecal specimens will contain only certain types of viruses, dermal specimens will contain very limited types of viruses, etc. Taking into consideration the season of the year can be helpful; certain viruses are prevalent largely during the summer, others are prevalent in the winter and fall. The clinical picture cannot, of course, be ignored; some viruses induce distinctive illnesses or lesions, in some cases distinctive enough so that laboratory diagnosis is unnecessary.

This comprehensive approach to viral diagnosis has most certainly not been fully perfected and some mistakes in naming a viral isolate are easily made. Yet, in many cases, one can readily detect and recognize an echo, herpes simplex, coxsackie, adeno, parainfluenza, influenza, rhino, mumps or respiratory syncytial virus in cell culture. But mistakes will occur. For example, a coxsackie A9 virus could be called an echovirus. Presently, this is of little significance clinically and since they are both picornaviruses maybe it will also have no significance in drug usage in the future; if there is a drug available for use against all picornavirus disease.

Even if one reports a virus isolate on its first detection in cell culture and correctly guesses at its proper grouping, this in most cases will still not be rapid enough so that most patients can obtain useful drug treatment, that is, of course, if we are dealing with a typical situation found in routine medical practice, such as occurs with bacterial infections. All is not hopeless, however, for a combination of clinical observations and an epidemiological approach could go far in guiding the use of antiviral therapy and this will be discussed in detail in the following sections.

In discussing general viral diagnostic methods, it should be pointed out that there are certain well established and commonly found laboratory procedures that can be used to diagnose a limited number of viral diseases. The use of the heterophile antibody test is one such procedure. Certainly this test could be a useful guide to potential treatment of infectious mononucleosis in that detection of the Epstein–Barr virus or its specific antibody is still beyond the average virus laboratory. Also the isolation of hepatitis A and B viruses in cell culture has not been achieved, but detection of the massive amounts of a portion of the hepatitis B virus is routinely done in many places using blood of infected persons. Even this test could aid specific antiviral drug use, especially with the more chronic or carrier cases. Rubella also can be diagnosed within one week of the onset of the disease by a serology test and certain protective drug measures might be possible if a gravid female has been exposed to a rubella case. Mosquito-borne virus infections caused by a variety of viruses, including those of the togo group, are still best diagnosed by measuring antibody in paired sera. Here the diagnosis may be too late for chemotherapy for a single patient, but establishing that such a virus outbreak has occurred can well lead to drug treatment early in the course of other similar cases prior to a definitive diagnosis.

In these latter instances virus isolation methods using cell cultures would be preferred as less expensive, often quicker and certainly, in the case of viral hepatitis and likely infectious mononucleosis, more sensitive. None-the-less even these somewhat indirect diagnostic methods could be helpful if appropriate drugs were available but only if such information is rapidly reported, and even better, widely disseminated using a virus alert program as will be explained later.

2.2. Antiviral Drugs

What antiviral drugs are in clinical use today, which are still being studied in clinical trials, what drugs are on the horizon and how will this all influence the demand for laboratory

diagnosis? It is suspected that when more drugs are available, there will be a somewhat greater demand for specific laboratory diagnosis since so often one hears the complaint, 'Why diagnose viral disease, you cannot treat it anyway?' Too frequently, however, such undiagnosed infections are then treated with antibacterial drugs. One should not be too optimistic, however, in that it is unlikely laboratory diagnosis will grow in exact proportion to the number of available antiviral drugs. So far as can be determined the present availability of antiviral drugs seems to have little impact on demands for viral laboratory diagnosis.

Amantadine is on the market in the United States and has been available since 1966. This drug and its more potent relative—rimantadine, have been available for some time in the USSR (Marks, 1976). With either drug it is essential to establish if there has been an outbreak of influenza caused by the type A viruses since these compounds are inactive against the type B influenzaviruses as well as other known viruses. Amantadine is widely used in continuous therapy of Parkinsonism, suggesting that it is very well tolerated and would certainly be so when used for prophylaxis during an influenza type A outbreak. Amantadine is also approved for management of such viral infections, with some therapeutic benefit if used early in the course of influenza (Togo *et al.*, 1970). Wisdom would dictate that monitoring for the presence of influenza viruses in a community is the minimum requirement for proper use of these anti-influenzal drugs, otherwise amantadine might well be used on patients not infected by influenzaviruses of the A type. Such a use might be considered poor judgment. The narrow antiviral spectrum of amantadine and rimantadine is a significant disadvantage which, hopefully, is not an indication of the nature of future antiviral drugs. If, however, this turns out to be the case, laboratory diagnosis would be significantly more important in the appropriate use of such drugs.

Idoxuridine (5-iodo-2'-deoxyuridine, IUdR) has long been marketed in an aqueous base for treatment of herpes simplex virus induced keratitis. Recently adenine arabinoside (araA) has been marketed for the same purpose. Although both compounds are inhibitory to herpes and poxviruses' DNA synthesis, neither is helpful for infections of the eye caused by adenoviruses which are also DNA viruses and largely resistant to these inhibitors. It is unlikely that an adenovirus induced conjunctivitis would be easily confused with a classical dendritic ulcer produced by herpes simplex virus (HSV). It can be presumed, however, that both IUdR and araA are used often in the absence of any virus laboratory tests. There are certain HSV eye infections that are deeper and more serious and might not be readily recognized as induced by this virus. In such cases there is much justification for laboratory confirmation of such infections. Generally, however, it is best to know early that the infection is indeed associated with HSV since, in our experience, many specimens taken from those with eye diseases produce no virus isolates, suggesting that certain eye infections may not be all that easy to diagnose by clinical observations alone.

Neither IUdR nor araA, in an aqueous base, have any influence on dermal herpetic lesions. There are, however, convincing data that topical application of idoxuridine in dimethyl sulfoxide produces therapeutic effects on HSV and zoster virus induced dermal lesions (Herrmann and Herrmann, 1977a). In many cases 'cold sores' and 'shingles' can be recognized for what they are, but on some occasions the causal agents for these two diseases can be confused. This may not be of great importance since both are herpesviruses and sensitive to idoxuridine. Where viral diagnosis seems required is where there are lesions of the mucous membranes, many of which are not known to be of viral origin. Certain oral lesions are associated with HSV and coxsackieviruses of the A type and indeed, in our experience, sometimes with the B type virus. Further, the type A coxsackieviruses can produce dermal vesicles much like the vesicles produced by herpesviruses, and DNA inhibitors would have no effect on these RNA containing picornaviruses. Coxsackie A viruses tend to be summer visitors in temperate climates and with proper monitoring of the viruses in the community, one could be alerted to their presence, thus perhaps minimizing the confusion.

It is suggested that even when effective treatment for 'cold sores' becomes widely available, and two such treatments are in fact marketed in Europe (IUdR, DMSO, prednisolone, Viruguent, Hermal–Chemiekurt and tromantadine hydrochloride, Viru–Merz, Merz & Co., both W. Germany), that typical labial herpetic lesions will likely still not result in a

laboratory diagnosis. However, both HSV and varicella-zoster virus can cause lesions on various areas of the human skin and, in our experience, the more atypical infections do induce a request for a laboratory diagnosis. It is worth noting, however, that about 66 per cent of dermal specimens produced no virus isolate (Herrmann and Herrmann, 1976b). Certainly a number of factors other than viruses can cause vesicular lesions, as for example, poison ivy or other allergies.

There are many antiherpesvirus compounds reported in the literature. Trifluromethyl-2′-deoxyuridine is one such compound that seemed a useful drug for treatment of the HSV infected eye (Wellings *et al.*, 1972) and now is likely to be marketed for this use in the near future. There are indications that certain compounds related to araA, especially the monophosphate derivative, will be investigated for potential use in a variety of HSV infections. Of some interest is phosphonoacetic acid (PAA), which, although not strikingly potent in cell cultures, has shown remarkable activity when applied topically to HSV infected mice (Klein *et al.*, 1977). Apparently this rather simple organic compound binds specifically with the DNA of herpesviruses. Although PAA is too toxic for human use, perhaps trisodium phosphonoformate will be better tolerated (Helgstrand *et al.*, 1978), and it may turn out that 9-(2-hydroxyethoxymethyl) guanine is the best of all the antiherpes compounds (Schaeffer *et al.*, 1978). There are still more herpesvirus inhibitors, many so far unpursued, as a result of drug firm screening programs.

Much publicity has resulted from trials of araA on cases of herpes simplex virus encephalitis (Whitley *et al.*, 1977). These positive results emphasize the importance of an early laboratory diagnosis. If drugs like araA are to be useful in this disease, which produces up to a 70 per cent mortality rate, then clearly no present HSV diagnostic method is rapid enough. It now appears that the diagnosis and treatment must precede any significant brain damage and in the case of HSV encephalitis, this would be very early indeed.

With the advent of a number of antiherpes drugs, how is one to deal with oral lesions caused by HSV, which is only one of many causes of such lesions? What indeed is to be done when finally it is agreed that HSV can also cause much ulcerative pharyngitis (Evans and Dick, 1964; Herrmann, 1967)? Certainly, here are diseases that will require laboratory diagnosis to define which of the many potential causal agents is involved. It is possible that topical treatment with certain antiherpes drugs will be useful. More likely a systemically acting drug, taken orally, perhaps something like araA, will be required. With herpes simplex virus infections we can be encouraged, for here is a virus rapidly isolated and recognized in the laboratory (Herrmann, 1967), causing widespread disease that is sensitive to a number of antiviral agents, so it seems likely effective treatment of these diseases is close at hand.

But what of interferons? It has been over 20 years since Isaacs and Lindenmann (1957) first suggested the role of these substances in viral interference. If the often made promise of the interferons as broad-spectrum antiviral drugs were achieved, it would have a dramatic impact on the need for laboratory diagnosis. Those viral diseases that are clinically distinct would likely need no laboratory diagnosis (typical mumps, herpetic lesions, chicken pox, etc.) and with the remaining viral diseases, only determining that a virus is involved would be sufficient. Significant questions remain, however, as to whether interferons play a role in recovery from viral disease or whether they can even be used therapeutically in routine medical practice. It has also not been resolved whether much of human viral disease can even be prevented by continuous use of interferons or their inducers. As synthetic, specific antiviral drugs become more prominent, interferons appear to be declining in practical appeal. One would have hoped that in these 20 years some clinical study would have emerged showing an interferon as active as the established antiviral drugs, but so far this does not seem to be the case.

Another approach to broad-spectrum antiviral drugs is the use of immune modulators such as inosiplex (Ginsberg and Glasky, 1977) and levamisole (Hadden *et al.*, 1977). Again, if such agents had the promised effect on viral disease, much laboratory diagnosis would not be required. Despite some suggestive clinical studies (Waldman and Ganguly, 1977; O'Reilly *et al.*, 1977), largely unconfirmed, there are serious questions about the effectiveness of these compounds. That is not to say that other agents with similar effects might not be useful, it is

just that the hypothesis of immune modulation has yet to be proven in some practical way in viral diseases.

Ribavirin too has been studied in detail as a broad-spectrum antiviral with an inhibitory effect on a number of viruses (Sidwell *et al.*, 1972). One hastens to add that in some cases its effects may be *via* normal cellular mechanisms but without the production of interferon. Despite substantial laboratory data, definitive, confirmed clinical data have not been forthcoming. At the moment there are doubts about the future of ribavirin. One can hope that broad spectrum antiviral drugs will be the wave of the future, but it is very doubtful that *all* viruses will be susceptible to even a 'broad-spectrum drug', and defining the specific agent causing certain viral disease may remain a necessity.

A limited number of drug companies in the United States and overseas have active investigations of antiviral drugs and specific antiviral activity is a common result of most of their antiviral screening programs. So far as can be determined, few such agents are antiviral to a large variety of viruses. Some compounds are active against DNA viruses, others are active against picornaviruses, and still others active against only certain influenzaviruses. As things appear now, the various virus groups are biochemically distinct enough that one would not expect specific inhibitors of viral multiplication to inhibit all viruses. Even the interferons are not uniformly protective in all of viral disease. This suggests that if the somewhat limited spectrum of present antiviral drugs is an indication of the future, then laboratory viral diagnosis will seemingly be an important adjunct to the use of such drugs.

It seems important to mention some antiviral agents that are being neglected, at least in the United States. Rimantadine is one of these that could be useful in influenza A virus infections (Wingfield *et al.*, 1969). This drug may also have a significant impact on viral excretion and hence contagion (Schulman, 1968). *Bis*-benzimidazoles also are not being pursued for use in rhinovirus infections (Shipkowitz *et al.*, 1972). Hydroxybenzylbenzimidazole and guanidine, inhibitors of certain picornaviruses, have long been rejected as having no *in vivo* activity, a rejection based on very little data. Recent studies using mice indicate these drugs may well have *in vivo* activity (Eggers, 1976). If one is faced with the need for laboratory diagnosis as a guide in the use of these drugs, which inhibit only some of the viruses causing upper respiratory disease, then the demand for laboratory diagnosis could become extensive, providing, of course, that such diagnosis is rapid enough.

The use of photodynamic inactivation as a treatment for certain viral diseases continues to be controversial, based on claims by some, using only cell cultures, that such a procedure enhances virus induced cancer (Rapp and Kemeny, 1977). Others deny and cannot confirm these data (Melnick and Wallis, 1977). Since there is as yet no proof that any virus induces cancer in humans, one wonders at the wisdom of this controversy. Preliminary clinical investigations have emphasized photodynamic treatment of herpetic mucocutaneous lesions (Melnick and Wallis, 1977). If this treatment were found useful, then laboratory diagnosis would likely not be common with most treatment based on the clinical picture. The use of dyes and ordinary light could not, of course, be used for systemic herpes simplex virus diseases.

3. THE FUTURE

3.1. LABORATORY DIAGNOSIS OF VIRAL DISEASE

What is likely to be the wave of the future? One suspects that if viral diagnosis follows the history of bacterial diagnosis it will be much like the past, that is, isolation of the organism and its identification relevant to patient care. A close look at virus detection by cell culture methods, however, suggests that little has changed since the human diploid fibroblast was introduced over 15 years ago. In some cases the inability to detect certain viruses in cell culture has encouraged the use of a number of additional methods such as those to detect hepatitis B virus antigen by radioimmunoassay. In that electron microscopy and fluorescent antibody methods have found only limited use (such as detection of rotaviruses in infant diarrhea) in the comprehensive virus diagnostic laboratory, these methods would likely

be supplanted by cell culture procedures that are intrinsically more sensitive, if such methods existed. It would seem the future will demand continued improvement of cell culture procedures, to be used in such a manner that specific chemotherapy of viral diseases is possible. It is regrettable that in the last decade very little work has been done to improve cell culture methods while substantial efforts have been made on numerous tests for viral antibody. Such a continued lag will have serious effects on the wise use of antiviral drugs in the future.

Hopefully some day there will be better cell culture media, but most especially there is a great need for better cell types. Micro cell cultures have eliminated expense as a significant concern (Herrmann and Herrmann, 1977c) but there are still hundreds of cell lines, many derived from human and animal cancers, that as yet seem unstudied for virus isolation purposes. Only recently has there been some hope that a number of type A coxsackieviruses, most of which are now detected only in infant mice, may be detected in a rhabdomyosarcoma cell (Schmidt *et al.*, 1975). This cell, with its bizarre morphology, does not seem as yet completely adequate but it does illustrate that perhaps somewhere there is a cell that can detect these viruses that now are only detected expensively and inconveniently in infant mice. There is a substantial need for a substitute for primary rhesus monkey kidney cells which are so important for the detection of myxoviruses. This primary cell type ,which is almost always contaminated with monkey viruses, may not be readily available in the near future due to an Indian embargo on rhesus monkeys. Perhaps the declining availability of these cells has increased interest in a substitute. Recent studies indicate that a canine kidney cell line (MDCK), with trypsin and EDTA in the medium, is quite sensitive to human isolates of influenzaviruses A and B (Meguro *et al.*, 1979). It is still not clear, however, if these cells will be adequate for the isolation of the parainfluenzaviruses.

Better cell types to substitute for those now commonly used would not be enough if they only detected those viruses we already detect. The common experience in a viral diagnostic laboratory is of extensive outbreaks of what certainly appears to be viral disease but with no virus isolations. It is difficult to guess at how many viruses there must be that cannot now be detected by cell culture procedures or, indeed, by any procedures. At present, hepatitis virus A and B, as well as non-A and non-B types, Epstein–Barr virus and a number of viruses associated with gastrointestinal upsets, cannot be cultured in a viral diagnostic laboratory. Add to this what seems an immense number of viral respiratory infections from which there are no virus isolates, and it is clear that a very comprehensive search is needed for better cell types.

There are suggestive data that some substances added to cells in culture can induce a more rapid detection of certain viruses (Herrmann, 1974). This is another neglected area of study where an organized search is needed for agents that encourage visible virus activity in cell cultures. The problem here is that such additives should be able to enhance detection of some viruses without suppressing the detection of others. Using certain halogenated pyrimidines (Staal and Rowe, 1975) to induce the detection of some viruses might be undertaken by using separate cultures. Such an approach could lead to a substantial duplication of cell cultures if it turns out that a number of substances are needed to enhance detection of many viruses. This use of multiple cell culture types, with and without inducers of viral activity, would be like the multiple media used for bacterial isolations. The number of types of cell cultures might be limited, however, by the knowledge of the nature of the specimen to be tested and perhaps even the nature of the illness. A specimen from a dermal lesion, for example, would contain far fewer classes of viruses than a throat specimen.

The methods for identifying a virus isolate using specific typing antisera are relatively expensive and time-consuming. These techniques could be dispensed with in certain cases. HSV, from rather typical skin lesions, can be very often recognized by the type of cell culture it attacks and the nature of the cytopathic effect (Herrmann and Rawls, 1974). This is often also true for varicella-zoster and cytomegaloviruses (Benyesh-Melnick, 1974; Schmidt, 1974). In the case of a rhinovirus isolate, few laboratories even try to define its serological type. This class of virus can be defined by its cytopathic effect in certain cultures, its sensitivity to a low pH and resistance to lipid solvents. The use of differential cell cultures, the

nature of the viral cytopathic effect and the use of biochemical tests would seem to be the wave of the future with only occasional need for specific typing antisera. To properly use an antiviral drug, there is a need to know the biochemical nature of a virus which may or may not be related to its serological nature. Why serotype an echovirus if, in fact, all echovirus disease responds to the same antiviral drug?

Antiviral drugs themselves may become useful in defining the nature of the virus isolate. Indeed, in the future it may become necessary to define the sensitivity of a virus to antiviral drugs, as is now done in much of bacterial diagnosis. Viruses can become resistant to antiviral drugs (Herrmann and Herrmann, 1977b). What is not known is the impact this will have on the clinical use of such drugs, since laboratory observations of drug resistance are by no means directly transferable to the clinic. Antiviral drug sensitivity tests, therefore, would seem to be part of the future both for defining the type of virus isolated and to determine the best drug to use for a specific viral disease.

Much, but certainly not all, virus disease occurs in sharp outbreaks. This fact can be used to alert community physicians to what virus is 'going around' and the nature of the clinical disease associated with any particular virus. This 'virus alert' program has been in existence for some years (Herrmann and Herrmann, 1977c). Publication in the news media alerts the public and the medical profession so there is at least some hope that the ill-advised use of antibacterial drugs can be avoided. Such an approach can be valuable with outbreaks of influenza, paramyxovirus, and enterovirus infections but is of little help with sporadic adeno or herpesvirus infections or other viral infections unassociated with a significant disease outbreak. Presently the approach to insect-borne (togavirus) viral disease is along the lines of a virus alert program. Although detection of such a virus infection is largely limited to a serological diagnosis, it can alert the community to the problem such drugs, were they available, could be used on likely cases, along with improved mosquito control. It is unlikely that rapid laboratory diagnosis in cell culture will be easily achieved for insect-borne viruses because of the general inaccessability of the virus, also a serious problem with HSV encephalitis. If there is no great improvement in the swiftness of viral diagnosis generally, then clinical diagnosis, along with the knowledge of the prevalence of certain types of viruses, may become the primary guide for using antiviral agents. This is not to say this is the most satisfactory approach. The exact definition of the infecting organism in each patient is always more desirable. The use of the 'virus alert' program seems a necessary compromise and can also make serological procedures of greater pertinence where there is an outbreak of influenza, paramyxovirus, rubella and togavirus disease. Serological diagnostic procedures would still not be useful as a guide in the treatment of most sporadic viral disease, nor in outbreaks that involve virus groups with many serotypes, as is the case with the picornaviruses. With all of this, however, it is likely the use of antiviral drugs will be based most often solely on the clinical picture. Broad spectrum antiviral drugs, therefore, seem a necessity if patients are to benefit.

3.2. THE FUTURE OF ANTIVIRAL DRUGS

A critical study of viral disease will convince one that there are more avenues open to attack in these diseases than in bacterial disease, as suggested in Table 3. It is unlikely, for example, that one would attack bacterial disease by using drugs that produce only symptomatic relief, for in certain cases the bacteria would continue to multiply, producing an unhappy outcome. With viral diseases there are only a few that are life-threatening. One could in many cases let the virus multiply, resulting in a normal immune response, and just treat the symptoms and clinical signs of the disease. This is what is so frequently promised by over-the-counter drug advertising. Only recently, however, has aspirin been studied for its worth in the common cold, and found rather unimpressive if not likely to make the patient more infectious (Stanley *et al.*, 1975). If there are little or no data to support the present nostrums' abilities to produce symptomatic relief, this is far from saying it cannot be done. Even though we presently know very little of the pathogenic mechanisms of self-limiting viral diseases, one can still speculate that the part that bothers the patient most is, in fact, a virus

induced toxemia. The virus may multiply in a limited area of the body such as the upper respiratory tract, but the illness is felt throughout the body. Tests for drugs that affect this toxemia would likely best be performed on human subjects, although some studies might be undertaken in mice using influenza virus and its long neglected associated toxin (Henle and Henle, 1946a,b). It would seem worthwhile to study those diverse drugs presently on the market, under double-blind, controlled conditions, to find if any of a variety of pharmacological actions have some effect on the most common viral respiratory diseases, those that are truly self-limiting. Do various antiinflammatory drugs, analgesics or even certain tranquilizers have some useful effect? If so, then this would give an indication of the type of drugs that might be useful. It is not suggested that the drugs that are tested necessarily be the ones of choice but rather those that have similar activity and are very well tolerated. It is most clear, however, that if such drugs were found useful, then they would have to be relatively inexpensive and readily available if they were to have much impact on the vast number of viral ailments. One thinks, of course, mostly of the many types of human viral infections commonly lumped together as 'colds'. It is likely that there would be little viral laboratory diagnosis associated with the use of such drugs. It seems fair to say that there is almost no laboratory diagnosis of such diseases now and the vast majority of cases are not even seen by a physician. The physician tends to see the more severe and troublesome viral infections and these would be the ones requiring laboratory diagnosis, now and well into the future. This would be especially true when the disease was found not to respond to the hypothetical palliative measures suggested above.

Perhaps in considering the advent of antiviral drugs, viral diseases might be divided into two general classes, those that are self-limiting, never cause death or serious illness, and those that do. For the former group, well tolerated easily obtained drugs are a necessity and individual viral diagnosis perhaps not essential. It is the latter group that must be dealt with in the viral diagnostic laboratory and treated more vigorously with potent drugs. It is possible that in the future certain drugs, both specifically antiviral and palliative, will be found effective for viral diseases where it is either not possible to isolate the virus, or the diagnostic procedures are too time-consuming and expensive. It seems reasonable to assume that drugs effective against all known rhino or coronaviruses might be equally useful against their undetected relatives.

One can predict that there will be no one answer to viral disease treatment. Vaccines will continue to play a role in certain viral diseases just as do bacterial vaccines. Other protective measures will perhaps find a use for certain viral infections and this can be passive immunity with gamma globulin containining a high level of antiviral antibody or even, in some cases, the use of interferon. It seems unlikely there will be a panacea for all viral diseases and, as each new approach becomes available to clinical practice, this will in large measure determine what laboratory tests must be undertaken, just as now occurs in bacteriology. The bacteriology laboratory must seek to determine the causal pathogen in relationship to potential treatment. For the future, therefore, one would expect that a virus isolate would be defined in a similar manner; hence, a major portion of the virus laboratory effort would be in classifying viruses according to their drug sensitivity. It must be expected that resistance to antiviral drugs will occur, inducing the search for new drugs or the use of drug combinations that do not encourage resistance development. As has been suggested, however, any drug approach to viral disease that is indirect and does not attack the specific viral induced mechanisms would not be likely to induce drug resistance (Herrmann and Herrmann, 1977b).

The present trend is to look for specific drug inhibitors of viral associated metabolic pathways, almost to the exclusion of other approaches, not unlike the emphasis used in the search for antibacterial agents. This approach has been productive but it will be limited since most viral disease cannot be defeated by highly potent metabolic inhibitors that are expensive and must be obtained by prescription. What other approaches might one suggest for the future, new discoveries that also come from screening programs but do not involve specific metabolic inhibition? There are a number, as suggested in Table 3 and there is evidence even today of certain possibilities.

TABLE 3. *Stages in Human Viral Infections Open to Attack by Drugs*

Interrupt transport to susceptible host cell
Block attachment to cell
Inhibit entrance into cell
Prevent uncoating of viral genome
Inhibit transport of viral genetic information
Inhibit synthesis of viral specific enzymes and structural proteins
Inhibit or alter viral nucleic acid
Inhibit cellular metabolic systems essential to viral multiplication but not essential to maintaining cellular integrity
Prevent assembly and maturation of viral particles
Prevent incorporation of necessary cellular substances into viral particle (lipids)
Inhibit release from cell
Prevent viral induced cell pathology
Neutralize or block induced biochemical pathology (toxemia)
Enhance host defense mechanisms
Enhance host recovery mechanisms

Naturally occurring substances that neutralize viruses have long been studied but neglected for practical use. It has long been known that a variety of macromolecules, some with polysaccaride moieties, tannins, glycoproteins and lipoproteins, neutralize viruses as do some small molecular weight compounds and this neutralization is not usually as specific as antiviral antibody. In serious viral diseases, these neutralizing agents might be given intraveneously but this use seems limited. Could such agents be given orally to produce some levels of protection in the membrane secretions? More likely would their daily use as a protective respiratory spray confer some protection to certain viral infections. It should be recalled that many persons are infected with viruses but not all become ill, so it is possible that with just a little help the body might be able to abort viral disease. Perhaps it only requires a slight lowering of the amount of infecting virus and a protective neutralizing spray might do this. So far as is known, none of the many known neutralizing substances for influenza virus type A have been tested for this effect, despite the fact that some are even found in human serum. This approach is chemoprophylactic and it would seem that viral laboratory diagnosis is not needed. But is this really true? It may come to pass that the use of well-tolerated viral neutralizing substances, artificial antibody so to speak, are still specific enough that there has to be a general awareness of what viruses are within the community before their use would be effective. Again the virus alert program could perform this function. One substance might be used for parainfluenza virus infections, for example, another for an influenza outbreak and perhaps still another for an echovirus outbreak. The idea would be to protect the upper respiratory tract from primary viral infection or at least decrease the magnitude of the infection.

It is widely held, without substantial proof, that the production of interferons may be the limiting factor in human viral diseases. If this were true, then inducers of interferons would indeed be chemotherapeutic and encourage recovery from viral disease. So far this has not been shown conclusively, but the concept is most valid. This does not involve specific antiviral substances, which in fact interferons are not, but rather a treatment that encourages the body's recovery mechanisms, and such mechanisms could differ for different virus infections. The future should hold some further information on what permits humans to recover uneventfully from most viral disease and how this can be encouraged by chemotherapeutic measures.

3.3. IMMUNE MODULATORS

It has been suggested that immune modulators can play a role in recovery from viral disease and this remains to be seen (see Section 2.2). If there are possibilities for drugs that perform this function, then what impact would it have on viral laboratory diagnosis? Barring a utopian panacea, there would seem to be some necessity at least to define the prevailing community viral problem. With sporadic viral infections, however, each case might have to be specifically diagnosed and the question remains whether this could be done rapidly

enough to institute specific chemotherapeutic measures directed at hastening recovery of a patient already recovering. The inducing of higher levels of antibody or interferons most readily comes to mind, but it seems likely that other recovery mechanisms must exist to explain intrinsic immunity exhibited by those immune virgins who become infected but do not become ill. Whatever these mechanisms are, they are the ones that might also have to be enhanced. If we are so fortunate as to find that enhancement of such mechanisms decreases all viral illness or enhances recovery in virtually all viral diseases, then perhaps much viral laboratory diagnosis would become unnecessary. One should, however, not be too optimistic about this possibility.

3.4. General Considerations

There are other aspects of viral infection that seem worth attacking, such as those mechanisms by which the virus must get into the cell. Would agents that block cell receptors be useful? Long ago this was suggested by mouse experiments using receptor destroying enzyme (neuraminidase) (Stone, 1948), but again only a protective effect was shown. Would agents that influence virus entrance into the cell be useful? Presently amantadine is suggested as one such agent, although this is not likely to be its only action-mechanism. Amantadine has a chemotherapeutic effect early in the course of viral disease but, like rimantadine, it is more potent when given prophylactically. Use of drugs with such antiviral specificity would certainly seem to demand some viral laboratory diagnosis, as is now the case with the use of amantadine or rimantadine. One can perhaps safely assume that viroplexis is a complex function of the cell–virus interaction, but it should not be neglected as an area open to an antiviral attack.

In addition to agents that inhibit production of nucleic acids, proteins, glycoproteins and other structural components and enzymes specific for viral synthesis, one must include the potential inhibitors of mechanisms for uncoating of virus nucleic acid, within the cell, the virus assembly mechanisms with final production of the infectious virion, and even those mechanisms of escape of virus from the cell by other than simple cell lysis. Indeed, is cell lysis itself a mechanism specific enough for attack by a chemotherapeutic drug? Drugs that inhibit specific mechanisms essential to viral multiplication would be the ones most likely to act on a relatively limited group of viruses.

Would, however, a drug that gives support to the virus-infected cell, so that cellular damage is minimal, be of a more broad spectrum type? In some cases the host cells tolerate substantial viral synthesis so well that no observable cytopathology can be observed. There certainly may be a biochemical defect in such cells that is responsible for the illness, but whatever the cellular problem, there may be drugs to aid the infected cell in its time of need and so limit the illness. This may not be considered a classical antiviral approach and it is difficult to say how specific it might be for certain viral infections. If such a hypothetical drug were beneficial in many types of viral disease, then laboratory viral diagnosis would be less essential. In all of this, one can be optimistic for certainly viral disease offers many possibilities for chemotherapeutic or chemoprophylactic approaches. In truth, of course, no one can predict the eventual role of the viral diagnostic laboratory until each new drug reaches general use. Barring the discovery of a utopian antiviral agent, it seems reasonable to predict that some viral diagnosis will be required when there are a significant number of antiviral drugs in use.

4. VIRAL DISEASES OF THE UPPER RESPIRATORY TRACT AND ORAL CAVITY

4.1. The Problem

It has been estimated that in the United States alone there are about one billion cases (3–6 episodes per person per year) of respiratory tract disease each year (Huebner, 1963). Most of

this disease is viral induced, relatively mild, self-limiting and frequently not seen by a physician. Physicians in the United States see perhaps 50 million cases of viral respiratory disease each year and the majority of these patients are children. In the practice of pediatric medicine as many as 33 per cent of the patients have respiratory disease and most of this is seemingly caused by a virus (Glezen, 1976). Indeed, it is thought that overall there is likely 4–5 times more viral than bacterial respiratory tract disease (Glezen, 1976) and it is likely that ill-advised antibacterial treatment is routinely used for many such viral diseases. For the purposes of the following discussion, however, upper respiratory tract disease will obviously not include those infections of the bronchial tree and the lungs, which will be presented later.

There is a long list of known viruses that can be isolated from throat, oral and nasal specimens, as indicated in Table 2. One might guess that there are at the very least twice as many additional viruses that infect the human upper respiratory tract that cannot as yet be detected.

In discussing the known viruses that cause upper respiratory tract disease, it is common to mention those of the common cold and influenza as well as infections caused by parainfluenza, respiratory syncytial and adenoviruses. There must, however, be additional consideration given to the role of the enteroviruses and herpes simplex virus in such disease. Enteroviruses are commonly associated with childhood cases of pharyngitis and fever (Hable *et al.*, 1970; Herrmann *et al.*, 1972) but this aspect of enteroviral disease is too frequently ignored while the seemingly more serious infections, such as those associated with meningitis, are emphasized. Certainly enteroviruses, especially those of the coxsackie group, as well as herpes simplex virus, cause disease marked by oral lesions, a common problem in children. What is not so widely known is the role of herpes simplex virus in producing both pharyngitis and stomatitis in the adult (Herrmann, 1967; Sheridan and Herrmann, 1971). It was long assumed that most adults were immune to herpes simplex virus primary infections, and this was largely true for adults from a lower socioeconomic group. It is not so true of those from higher socioeconomic groups where at times as many as 50 per cent of young adults are not immune to this virus (Wentworth and Alexander, 1971). More emphasis then should be placed on the role of HSV as a common cause of oral lesions and especially its role in acute ulcerative pharyngitis in those of all ages. It is perhaps unwise always to conclude that HSV isolation from a pharyngitis is due to haphazard shedding of this virus unassociated with disease. If a drug attack on respiratory disease is to be effective then the role of enteroviruses and herpes simplex virus cannot be ignored.

Viral upper respiratory tract disease is a very demanding problem in that hundreds of serologically distinct viruses are involved. Many of these viruses contain RNA, others contain DNA and most produce rather superficial diseases, resulting in solid immunity to reinfection (enteroviruses) in some cases, or partial immunity (parainfluenza and influenzaviruses) in others. In certain cases, as with rhinoviruses, the illness is marked by no objective pathology while other viruses, such as those causing influenza, can denude the epithelium of the respiratory tract. Some infections are 'cold-like' while others are 'flu-like' and certain viruses can take their turn producing both kinds of illness. Of all viral diseases, those involving the upper respiratory tract are the most difficult to diagnose on clinical grounds and yet it will be these very diseases that will most likely be treated with little or no laboratory diagnosis.

4.2. POTENTIAL DRUG SOLUTIONS

With most viral upper respiratory tract disease, the pathology is very limited and sequelae rare, so what seems needed is relief from the symptoms and clinical signs. Aspirin has long been recommended by advertisers to control the fever of 'colds' and 'flu' even though it is well known that adults suffering from rhinovirus infections rarely experience a fever, and as indicated before, the use of aspirin seems to make such persons more infectious. Some question should even be raised about the wisdom of suppressing a relatively mild fever with aspirin since certain viruses do not tolerate temperatures much about 37°C, or at least seem to tolerate them less well than humans. Indeed, except when there are dangers from too high a

fever, this is not an aspect of viral disease that causes great discomfort. What is really needed is a drug to produce effective relief from the cough, obstruction of the respiratory passages, sore throat, rhinorrhea, malaise, myalgias and the general toxemia that is associated with rhinoviral and other 'cold-like' viral respiratory disease. Symptomatic relief alone would not be sufficient, however, if the infection were caused by entero, herpes simplex or influenza viruses, for example, since these and other viruses can cause more serious disease likely requiring a more potent and specific viral inhibitor. With the young infant, symptomatic relief alone for respiratory syncytial or parainfluenza virus infections would also not be totally adequate.

Adenovirus disease represents a somewhat special case. Although these viruses usually produce little disease in civilian adults, they can cause a pharyngitis in children much like a 'strep throat' that is often ill-advisedly treated for lengthy periods with antibiotics. In some cases the adenoviral respiratory disease can be accompanied by a conjunctivitis; in other cases a conjunctivitis alone may occur. The problem is that these cases can be sporadic and, despite the fact adenoviruses contain DNA, they seem rather rèsistant to the prevalent drugs that inhibit other DNA viruses. What seems most needed is a drug that can inhibit adenovirus synthesis with complete safety to the patient. But again these infections can be relatively mild and in many cases effective symptomatic relief might be all that is needed.

The status of the present drug approaches to influenza has already been discussed. Clearly what is now needed are more rapid acting chemotherapeutic drugs that influence all influenza, whether caused by the A or B virus types. Symptomatic relief should also be considered, since this virus is likely to cause a portion of the illness *via* a toxic mechanism. Further, the course of influenza can be of such short duration that a specific viral inhibitor may have little time to be effective prior to the patient's recovery. Even when the active disease is virtually over, however, some sequelae including general weakness, lethargy and even certain mild depression remain, sometimes for weeks, and certainly symptomatic relief seems in order in such cases. Chemoprophylactic drugs may well be life saving to high risk patients in the absence of an effective chemotherapeutic drug for influenza, but such drugs should permit development of maximal immunity even in those who experience inapparent or mild disease. Even then, when the specific influenza virus inhibitor is being sought, it best be one that is very effective chemotherapeutically in that it seems more practical to treat only those who are ill. Further, many with influenza virus infections will either not be ill or mildly so and they could be left untreated and hence acquire maximal immunity, which might not be the result if a chemoprophylactic drug were widely used. So far as is now known, antiviral chemotherapeutic drugs do not seem to prevent an immune response, likely because viral multiplication has progressed sufficiently by the time therapy is started to provide the same immunity as would normally be produced by the untreated disease.

So much progress has now been made in finding compounds active against herpes simplex virus that it is expected more antiherpes drugs will become available. It is even possible that HSV infections of the upper respiratory tract might be treated by topical drug application. Superficial and limited oral lesions might be treated topically but extensive oral lesions suggest a primary HSV infection with systemic disease, requiring something more than topical treatment. Further, infectious mononucleosis is likely also caused by a virus from the herpes family (Epstein–Barr virus) and treatment of this disease would require a systemically active drug. There should be more concern that progress in the early treatment of herpes simplex and Epstein–Barr virus infections be swifter, for there are constant suggestions that these viruses may be inducers of human tumors. Chicken pox and shingles also would be expected to respond to an antiherpes drug, for they too are caused by a herpesvirus.

4.3. Diagnostic Needs

It is unreasonable and unrealistic to suggest that every case of viral respiratory disease be diagnosed in the laboratory. It does seem worthwhile, however, at least to define what viruses are circulating in the community, using a virus alert program. Not all virus disease outbreaks will be defined but specific antiviral drugs can be used in those outbreaks that are defined.

The relatively mild viral diseases seem best treated by effective, well tolerated, symptomatic relief since a significant number of these infections will either not be seen by a physician or, if they are, will still not receive a laboratory diagnosis, even where it is available.

The more serious illnesses should have a laboratory diagnosis even when it fits those diseases known to be associated with a prevalent virus invasion. The more serious viral respiratory disease case could, of course, be a sporadic infection caused by a virus not known to be prevalent. It is obvious, however, that only a portion, likely the smallest portion, of most virus respiratory infections can now be defined by any laboratory method. Today a virus alert program would fail on many occasions, so it is obvious that much still needs to be done just to rapidly define even those viruses circulating in a community at any one time.

The clinical picture of disease can at times be useful. Certainly most cases of mumps or measles, for example, could be defined on a clinical basis. Some may feel that viral diseases of the respiratory tract can be distinguished from bacterial since the latter so often are localized and lack the generalized toxic phase common in viral disease. It would seem wiser, however, to rule out bacterial involvement by performing a simple throat culture. This would not, of course, define which virus is the responsible agent in the event different drugs must be used for different viruses. Perhaps what is needed is more effort in an attempt to discover those clinical signs and symptoms that are classical for certain viral infections. Certainly an outbreak of influenza is not difficult to define; it is determining whether the responsible agent is the A or B virus that poses the somewhat more difficult question. Similarly, outbreaks of parainfluenza viral infection in children, with the associated croup, is not too difficult to define when pediatricians compare experiences. When a number of infants have bronchitis or bronchiolitis, then respiratory syncytial virus must be suspected and a drug used to attack this virus, if such existed. The classical clinical picture of the common cold in adults, usually without fever, would certainly suggest the use of effective and well tolerated drugs for the rhinoviruses, if it were not for the fact these are certainly not the only viruses that can cause a classical cold. The seasonal pattern of disease could be helpful, however, in that rhinoviruses seem most prevalent in the spring, summer and fall. Coronaviruses apparently cause many of the wintertime colds. In temperate climates, as another example of seasonal prevalence, one is very unlikely to see pharyngitis cases caused by enteroviruses during the winter months. Future diagnosis of viral respiratory disease, therefore, in such a manner as to permit adequate use of specific drugs will likely require interrelating knowledge involving isolating the virus when possible, defining it serologically when this can be done along with the clinical, epidemiological and seasonal pattern of disease. Much more would be required in community–physician cooperation and communication than now seems the case, however, if antiviral drugs are to be used in a rational and effective manner.

Infections marked by lesions in the oral cavity give a clue to the possible viruses involved. Presently herpes simplex virus is a common cause of such lesions even in adults. In children, during summer months, herpangina caused by the coxsackie A viruses can be equally common. It has been found on rarer occasions, however, that intraoral lesions can also be associated with coxsackieviruses of the B type and so laboratory diagnosis may well be necessary to unravel which viruses are involved. In the winter months, with an infected child presenting with severe oral lesions and a gingivostomatitis, one would certainly think of using a well tolerated antiherpes drug. But still some confusion can occur with cases of aphthus stomatitis which so far as known is not caused by a virus. It is claimed, however, that herpetic intraoral lesions can be distinguished from those associated with aphthus stomatitis (Weathers and Griffin, 1970), so here again is an example of the use of the clinical picture as a guide to therapy. Of course, oral lesions associated with herpes simplex virus can usually be readily defined by virus isolation in any average virus diagnostic laboratory, generally within a very few days. But again there is the concern that this will not be swift enough to start effective therapy. Perhaps more must be done to utilize labeled specific, antibody systems for detecting HSV in oral mucosal cells, if rapid therapy based on an accurate diagnosis is to be the goal.

Many factors can cause oral lesions such as allergies and drug reactions. What does not seem so clear at present is the extent of viral induced oral lesions in both children and adults

and whether there are other kinds of oral lesions caused by as yet unidentified viruses. There is a substantial need to define the problem of oral lesions and, until this is done, it will be difficult to say what the diagnostic needs will be in the future. Certainly something more convenient than infant mice are needed to define coxsackie A virus induced oral lesions. The promise of the RD cell, referred to previously as a ready aid in this diagnosis, has as yet not come to pass (Schmidt *et al.*, 1975) but there is every hope some cell type will eventually fulfill this promise.

Perhaps infectious mononucleosis (IM) is one viral respiratory disease, widely accepted as caused by the herpesvirus of Epstein and Barr, where virus isolation is not required, since the heterophile antibody test can so frequently establish this diagnosis. Further, the course of the disease can be relatively long term, thus permitting effective therapy after the laboratory diagnosis. In some cases the heterophile antibody test will constantly remain negative and this can suggest the involvement of cytomegalovirus, which is also a herpesvirus. One suspects that an effective antiherpes drug that is well tolerated could be useful against either of these DNA virus infections. The heterophile antibody test will persist as a worthwhile diagnostic tool so long as EB virus cannot be readily and routinely identified in the diagnostic laboratory. CMV, on the other hand, can be isolated in human fibroblast cells but it is not always an easy task nor is it swiftly performed. With a typical clinical and blood picture of IM there seems some hope that chemotherapy of this disease could be practically undertaken no matter which of these two herpesviruses are involved. It should be added that at one time it was believed children did not have typical infectious mononucleosis, but recent data indicate that they do and that EB virus is involved as is a positive heterophile test (Ginsburg *et al.*, 1977).

5. VIRUS DISEASES OF THE LOWER RESPIRATORY TRACT AND CARDIOVASCULAR SYSTEMS

5.1. The Problem

The extent of acute heart disease, pneumonia, bronchitis and bronchiolitis related to viral infections is not well known. One can presume that the stresses caused by viral disease, such as with influenza, likely contribute to some mortality among heart patients. There is no question that influenza virus infections, especially of the A types, produce fatal pneumonia, as well as additional disease of the lower respiratory tract (Lindsay *et al.*, 1970). The coxsackieviruses of the B group can cause myocarditis, that is sometimes fatal in the newborn, and pericarditis and pleurodynia in those of all ages (Kibrick, 1964). What is not clear is what fraction of the total of lung and heart disease is directly caused by viral infection. Present evidence would suggest no significant role for viruses in heart disease other than that caused by the B group of coxsackieviruses, but this evidence is, of course, based on our knowledge of the presently known viruses.

In the case of viral disease of the lower respiratory tract in adults the picture is not clear. It is not uncommon to hear a diagnosis of 'viral pneumonia', frequently with reference to illnesses seemingly refractory to antibiotic treatment. In the past much 'viral pneumonia' was likely a disease caused by *Mycoplasma pneumoniae* (Denny *et al.*, 1971). Most pneumonia is still probably bacterial in origin, perhaps even bacteria not previously implicated, as exemplified by the recent discovery of the role of the bacterium responsible for 'Legionnaires' Disease' (Center for Disease Control, 1978). Our examination of over 800 lungs obtained from adults at autopsy produced only 35 viral isolates, mostly influenza A viruses, some cytomegaloviruses from immune compromised patients, and a few, very few, other viruses (Herrmann and Herrmann, 1976b). This suggests that severe infections of the lungs do not seem to be primarily a problem related to the known viruses. Influenza virus has been identified infrequently with severe bronchitis, and in fact bronchitis in adults, sufficiently severe to seek medical aid, has not been defined as a viral problem. Among recruits in the armed services adenoviruses can cause lower respiratory tract disease but this is a special case, rarely seen in civilian adults. These viruses do, of course, produce substantial upper

respiratory disease in children but it is rarely serious if left untreated.

Childhood infections of the lower respiratory tract are, however, another matter. Influenza virus infections seem of less importance in children than in adults. Respiratory syncytial and parainfluenza viruses on the other hand lead the list of those important causes of childhood lower respiratory tract disease. It would now appear that the coronaviruses, and probably to a smaller extent the rhinoviruses, can also produce lower respiratory tract disease in children less than 18 months of age (McIntosh *et al.*, 1974). Respiratory syncytial virus produces the more serious diseases in pre-school children, including bronchitis, laryngialtracheal bronchitis, bronchiolitis, epiglotitis and pneumonia. On the other hand, fatal viral pneumonia in children seems relatively uncommon.

There seems little question that the cause of substantial amounts of lower respiratory tract infections in both adults and children have yet to be defined. Many cases of such disease suggest a viral infection but these viruses are not yet known. There is still hope, as has been suggested already, that these unknown viruses may well be relatives of known viruses, known viruses that one day will succumb to antiviral drugs. Such drugs could likely be used in a number of typical viral diseases even when no virus can be identified.

5.2. Potential Drug Solutions

Lower respiratory tract disease is serious enough to require specific antiviral drugs that quickly stop viral synthesis and tissue destruction. Symptomatic therapy, although perhaps beneficial, is still not enough. Treating only symptoms is perhaps also not the proper approach to cases of myocarditis and pericarditis; virus multiplication should be inhibited as early in the course of the disease as possible for it is still not known whether coxsackie B viruses can produce some sequelae, perhaps sequelae not obvious until years later. Drug treatment for infections caused by the numerous types of enteroviruses seems the only practical attack against the variety of disease problems they produce. Effective antiviral drugs also seem required for respiratory syncytial virus infections and perhaps this virus is a close enough relative to the parainfluenza viruses so they too will respond to the same therapeutic approach. Although certain drugs may be useful to treat only the symptoms of the common cold when produced by rhinoviruses and coronaviruses, this too may not be enough when these viruses produce more serious lower respiratory tract illnesses in children. Since rhinoviruses are related to the enteroviruses it could well be that drugs inhibitory for one group will work against all picornavirus and there are a number of antiviral compounds that suggest this possibility, including hydroxybenzimadazole (Gwaltney, 1968), the *bis*-benzimidazoles (Schleicher *et al.*, 1972), the triazinoindoles (Gwaltney, 1970) and AR-336 (DeLong *et al.*, 1978).

There are substantial data supporting the concept, so far unproved, however, that pneumonia caused by influenza A viruses can be prevented and even treated using amantadine or rimantadine and these drugs likely can be useful on the entire spectrum of respiratory disease caused by influenza A viruses (Hoffman, 1973). What is needed, of course, are more potent antiviral drugs that act on infections caused by both influenza A and B viruses and this has been claimed for ribavirin (Durr *et al.*, 1975). Ribavirin, however, does not appear to be a very potent treatment for human infections and its effect is almost undetectable if the influenza case is a mild one (Togo and McCracken, 1976; Cohen *et al.*, 1976). Certainly, a broad spectrum antiinfluenzal agent, perhaps something like ribavirin, is needed, in that an outbreak of influenza is not difficult to detect on clinical grounds but it is more difficult to determine if one is dealing with influenza A or B virus, or on some occasions, both.

5.3. Diagnostic Needs

A 'virus alert' program could aid in determining those viruses that are prevalent in the community and thus suggest what might be expected in the way of lower respiratory tract disease. Yet it would seem prudent to attempt a specific viral diagnosis in each case of lower respiratory tract disease. This should also be done at least to exclude a bacterial pathogen.

Much has been said about the importance of obtaining a specimen for a bacterial lower respiratory tract infection in that many believe throat swabs are not completely indicative of the bacterial flora of the lower respiratory tract and sputum frequently is little more than saliva. It is not so clear that this is a problem in viral infections, for influenza virus seems relatively easy to isolate from a throat swab from patients with influenzal pneumonia (Lindsay *et al.*, 1970). Further, lower respiratory tract infections caused by respiratory syncytial and parainfluenza viruses frequently are diagnosed using only a throat swab (Smith *et al.*, 1971; Herrmann and Hable, 1970), but there is some evidence that throat and especially nasal washings can be more effective specimens for isolating certain viruses (Hall and Douglas, 1975) and these should be obtained, especially in the hospitalized cases. It seems valid to presume that in a lower respiratory tract infection caused by respiratory syncytial virus, where the virus is readily isolated from the nasal passages, extreme measures to sample the lower respiratory tract are not needed. It is also worth noting that even with upper respiratory viral disease a nasal swab can be at times more productive of a virus than is the throat swab (Bloom *et al.*, 1963).

Whatever the virus involved, superior virus detection methods are needed, and this is especially true for parainfluenza and respiratory syncytial viruses that frequently take a week or more to detect, largely depending on how much virus was contained in the specimen. In the case of influenza, it has long been suggested that fluorescent antibody methods can be used to detect this virus in respiratory secretions and this indeed can be a rapid and specific method, but cell culture methods still are required additionally in the event that it was not a true case of influenza, which may be the reason so little influenza is diagnosed by fluorescent antibody methods. In time still more viruses associated with lower respiratory tract disease will be discovered and, as the number of virus serotypes increases, then the use of specific antibody, such as in fluorescent methods, becomes increasingly less practical; hence, better cell culture methods are required if rapid application of a chemotherapeutic agent is to be the goal. Measurement of virus antibody in acute and convalescent sera could rarely aid in guiding therapy for the individual patient. Perhaps even detection of the virus would not be enough, for it might still have to be established that the isolated virus was sensitive to one or another drug before adequate therapy could be undertaken. It would be ideal if such viral drug sensitivity were first established, but the ideal is not always achieved and it is likely that antiviral drugs, even those with narrow antiviral spectra, will be used based solely on clinical observations, and if the disease is stressful enough this could at times be helpful to the patient. Yet a specimen should be taken prior to any therapy to confirm the clinical diagnosis so adjustment in therapy can be made when necessary. It should be reemphasized that so far as is known today, the greatest percentage of viral lower respiratory tract illness is self-limiting and not lethal, with influenza in high risk patients the outstanding exception. So even with lower respiratory tract diseases some relief of symptoms and clinical signs by a palliative drug is also a worthwhile goal.

6. VIRAL DISEASES OF THE NERVOUS SYSTEM AND EYE

6.1. The Problem

Virus diseases of the eye, so far as is now known, primarily involve herpes simplex (HSV) and adenoviruses, the latter causing little serious disease. With two effective drugs (IUdR and araA) available for treatment of HSV induced keratitis, this infection (that can lead to blindness) seems well on the way to a therapeutic solution. Conjunctivitis associated with adenoviruses has yet to yield to any treatment despite the fact that these viruses, like the herpesviruses, contain DNA but yet are largely resistant to certain viral DNA inhibitors (Herrmann, 1968). Most cases of adenoviral conjunctivitis are mild and self-limiting and permanent eye damage seems very rare. There have been occasions when varicella-zoster virus and even the poxviruses, such as the vaccine virus for smallpox, have infected the eye and in these cases the antiherpetic ophthalmic preparations seem helpful (Jones and Al-Hussaini, 1963; Jack and Sorenson, 1963; Kaufman *et al.*, 1963). Recently, in certain areas of

the world, eye infections associated with enterovirus 70 have been detected, and likely certain other enteroviruses can cause a similar problem (Arnow *et al.*, 1977). The extent of this problem has yet to be fully established.

Viral infections of the central nervous system, unlike those of the eye, seem far from resolution since so many diverse viruses are involved, some frequently produce relatively mild and self-limiting disease, such as mumps and enteroviruses, while others produce significant fatal encephalitides, as with herpes simplex and togaviruses. At the outset of any viral induced nervous system disease it is, of course, not clear whether one is dealing with a mild self-limiting type of CNS infection or one that will be fatal. There are many serologically distinct viruses that produce CNS disease and these include mumps, HSV, CMV, coxsackie, echo, toga, measles and varicella-zoster viruses. One must also add to this list the relatively rare human cases of rabies and a growing list of so-called 'slow virus' diseases such as subacute sclerosing panencephalitis (SSPE) (Gajdusek, 1977). The virus induced CNS diseases can be mild with almost indetectable meningitis or can be severe meningoencephalitis, encephalitis and more rarely slow fatal degenerative disease or even peripheral nervous disorders and paralysis. There is little question that the viral induced nervous system diseases are a major problem which has only been partially resolved by vaccines for the prevention of poliomyelitis and measles. It is also, of course, likely that some nervous system diseases involve viruses that are as yet undiscovered since there seem to be many cases that go undiagnosed but resemble viral infections.

6.2. Potential Drug Solutions

There appears to be no particular reason why eye diseases related to adenoviral infections should not in time yield to a specific antiviral drug, perhaps one that inhibits the DNA synthesis of this virus. It seems, however, with the exclusion of adenovirus type 8 and perhaps one or two other adenoviruses that cause epidemic keratoconjunctivitis, most adenovirus infections primarily involve the upper respiratory tract producing a pharyngitis that is sometimes exudative, with fever and this can include at times a conjunctivitis. Topical application of an antiadenoviral drug to the eye would, therefore, not usually be a proper drug solution but rather what is needed is a systematically active drug. In most cases adenoviral diseases would be seen in children and much less frequently in civilian adults.

Despite what seems a ready solution to HSV eye disease, it is still not clear if the problem is completely solved. The question remains whether topical application of antiviral drugs can cure the deeper HSV eye infection or if a systemically applied drug could better achieve a cure. AraA, or one of its relatives, would be a primary choice for systemic treatment of these deeper and more refractory eye infections but this might still involve significant side reactions. Idoxuridine, on the other hand, is very toxic if given other than topically. The problem of recurrent HSV eye infection, a situation analogous to recurrent cold sores, remains and a systemically applied antiviral drug seems required to achieve long-term cures.

Central nervous system infections require some immediate therapy because of the concern for permanent brain damage. Yet, most cases of virus related CNS disease will be relatively mild with no sequelae even when untreated. The greatest amount of CNS disease in temperate climates is produced by mumps and enteroviruses, especially the coxsackieviruses of the B type. Few communities will go through an entire year without at least some meningitis caused by one or another of these viruses; in some cases there will be a meningoencephalitis and even a few cases of encephalitis, but very rarely will it be fatal or even leave any permanent brain damage. The purpose of chemotherapy would be to shorten the course of the disease and prevent any possible potential complications. The drugs required would have to be well tolerated and rapidly halt viral synthesis. A search for drugs for mumps and enterovirus infections has not as yet been successful. There is no reason why specific inhibitors cannot be found that are compatible with drug therapy in that inhibitors for these viruses have long been known but so far seem to lack all those other pharmacological properties so necessary to an effective drug. Clearly, in cases of central nerous system disease, palliatives or drugs for symptomatic relief might be helpful, but still

what seems needed is a specific viral inhibitor.

The insect-borne viruses associated with central nervous system diseases are also responsible for other clinical syndromes such as yellow and hemorrhagic fevers. The greatest problem, however, is to deal with the lethal or serious sequelae that can result from infection with a number of togaviruses and certain insect-borne viruses now classified in other virus categories. These viruses contain RNA and also a lipid envelope essential to the survival of the virus. Not enough seems to have been done in the search for specific inhibitors of the RNA of these viruses or even for inhibitors of their envelope formation. Whatever the action-mechanism, specific inhibitors are required so that viral synthesis can be brought to a swift halt from the very first moment. It seems a very serious ethical problem, however, to save a life that must now spend its remaining days in an institution, a problem that still occurs in cases of bacterial meningitis no matter what the antibacterial treatment. Clearly such a chance must be taken, but the need is for a potent viral inhibitor that rapidly reaches the site of the infection. Symptomatic relief would not be adequate but a prophylactic drug might well be considered, especially in the high risk patient groups, such as the aged, in the face of an outbreak of St. Louis encephalitis.

In the last 15 years it has become evident that HSV is a most important pathogen of the central nervous system, producing a mortality rate upwards of 70 per cent and much brain damage among the survivors (Whitley *et al.*, 1977). It is somewhat of a surprise that there is not much more HSV encephalitis in that this virus is likely to be one of the most widespread of all known viruses, due at least partly to the very common occurrence of the cold sore. It is the type 1 HSV that is most often isolated in both cases of the cold sore and encephalitis. The first steps have now been taken to attack HSV encephalitis with the use of araA. This is not the complete answer since more potent drugs are needed to head off the extensive sequelae that frequently occur in treated survivors (Whitley *et al.*, 1977), but the proof is now in, potentially fatal HSV encephalitis can be altered by therapy and araA will likely be used until a more potent, less toxic or metabolically stable drug is available. It should be noted that the study of araA's use in HSV induced encephalitis has been sharply criticized (Tager, 1977; Morris, 1977). Nonetheless, as with most viral CNS disease, the only drug to consider first is one that specifically, and rapidly, halts HSV synthesis prior to significant brain damage. In addition, extensive supportive patient care also seems important, for this was the only approach used in the apparent recovery of two persons from rabiesvirus infection (Hattwick *et al.*, 1972; Porras *et al.*, 1976). The study of the treatment of HSV encephalitis leaves little doubt that there is no fundamental reason why other viral CNS infections cannot be attacked in the same chemotherapeutic manner once there is a more aggressive search for specific viral inhibitors for all viruses causing such disease. So far, for example, there seems to have been little work done in looking for a drug to combat the rabiesvirus. Such a drug might not only be used for treatment of frank rabies cases but also could be used as a prophylactic measure, to replace the present vaccine and hyperimmune serum procedures. One would hope that harmless rabies vaccine strains could be included in the tests for antiviral activity. It is estimated that in Latin America alone some 300,000 persons receive antirabies treatment (Porras *et al.*, 1976). Certainly a relatively well-tolerated antirabies drug seems preferred over the tedious multiple dose vaccine procedures used on those presumed to have been exposed to rabies.

The slow-virus diseases present a considerable problem in arriving at a suggestion of how to apply antiviral chemotherapy since our present knowledge of the role of the virus is so sketchy. First, a distinction should be made between CNS diseases associated with conventional viruses and those associated with so-called unconventional viruses since so little is known about this latter type of biological entity (Gajdusek, 1977). Nonetheless, if conventional or unconventional virus production involves metabolic pathways that do not exist in normal healthy cells, then theoretically their synthesis can be halted. What is not known is whether the synthesis of either unconventional or conventional viruses is the basic cause of these serious and degenerating diseases. Whatever the process, it is clear that some drug is needed to halt whatever is going on at the very first moment when the disease is recognized. The slow pace of these diseases is such that there is some hope a drug could be

applied with substantial success. SSPE is a fatal disease, and the course can extend over many months. If a drug were available to treat human measles cases then certainly it should be tried for the treatment of SSPE. Amantadine has been tried on such cases and the authors felt it was beneficial (Haslam *et al.*, 1969) even though this drug is not known to affect the measles virus. A search specifically seeking drugs to treat what, for the moment, is a rare disease seems unlikely. An antiviral drug might be found, however, that is poorly tolerated for treating the rather benign common cold, for example, but yet the toxicity would be acceptable in a degenerative, slow virus disease, such as SSPE.

There are instances of nervous system diseases associated with shingles, chicken pox, measles (rare because of the vaccine), vaccinia viruses (rare since discontinuation of generalized smallpox vaccination) and at times other viruses such as that of infectious mononucleosis. The therapeutic approach to such complications could be just an extension of that drug treatment, were one available, that is used when the more common disease form is encountered. If the common disease is treated by an effective drug it would be expected that CNS disease caused by the same viruses would also respond, or even be aborted.

6.3. DIAGNOSTIC NEEDS

Central nervous system diseases, especially meningitis and meningoencephalitis associated with the mumps or enteroviruses should not be difficult to diagnose. In about 60 per cent of the cases of mumpsvirus, central nervous system disease is preceded by a classical case of mumps with parotid gland swelling. Once mumps is detected in the community then mumpsvirus associated central nervous system disease should be expected. In temperate climates, during other than the summer and early fall months, the first guess at the causal agent of CNS disease should be mumpsvirus, once a bacterial infection is excluded. In that mumps central nervous system disease frequently occurs well after infection, the virus is not easily isolated from the upper respiratory tract but it can at times be detected in the urine (Utz *et al.*, 1958; Person *et al.*, 1971). Detection of a rise in specific complement fixing antibody frequently occurs too late to be other than a retrospective diagnosis (Person *et al.*, 1971). The use of virus isolation methods, clinical observations and serology could go very far in at least determining if mumpsvirus infection is widespread in the community. The question arises if any of this would permit prompt use of a specific therapeutic antiviral drug with individual cases? With a frank case of mumps, presenting with classical salivary gland involvement, the use of an antiviral drug might well prevent development of not only CNS disease but a variety of other mumpsvirus caused problems including orchitis.

Serological methods are impractical in the diagnosis of enterovirus disease because of the many serotypes that could be involved, more than 60. In more tropical climates these viruses can be seen year around while in temperate climates they are primarily summertime visitors and usually can be isolated rather readily in the average virus diagnostic laboratory. Coxsackie viruses of the B type seem to be a most common cause of CNS disease and are isolated and recognized usually in just a few days. Echoviruses take somewhat longer to isolate but can be recognized rather readily (Herrmann *et al.*, 1972). Coxsackieviruses of the A types are not always easily isolated, requiring in many instances infant mouse inoculation, but these viruses are less commonly a cause of CNS disease. Certainly a much better cell system is required to detect coxsackie A viruses.

Once certain enteroviruses are detected in the community, using either cell cultures or mice, then, of course, some cases of central nervous system disease should be viewed as likely caused by these viruses and treatment begun with a specific antiviral drug until data are produced to indicate otherwise. Enteroviruses can be readily isolated from the respiratory tract early in the course of the infection and such an isolation carries greater weight than an isolation from feces. Enterovirus isolation, however, can be achieved even many weeks after disease onset but in this case viral excretion may not be related to the disease for it is common for children to excrete these viruses unassociated with any disease. Nonetheless, any enterovirus isolation must be seriously considered. A virus alert program for the entire

community would go far in preparing physicians for the use of specific drugs to treat all types of enteroviral disease.

When herpes simplex virus infects the CNS it presents some considerable diagnostic problems since it is felt serological diagnosis can either be misleading or take too long, and virus isolation is achieved largely by using brain tissue (Johnson *et al.*, 1968). The clinical picture of the disease can be helpful but yet there can be some confusion with other CNS problems (Rawls *et al.*, 1966). At present isolation of the virus from a brain biopsy is thought the most accurate diagnostic method. Tissue rich in virus permits detection in 2 or 3 days in cell culture with the study of stained tissue sections also of some use if classical intranuclear inclusions are detected (which are not strictly specific for HSV but this would still be the most common virus involved). Fluorescent antibody methods are not completely reliable in such infections (Cho and Feng, 1978). In that very early treatment of HSV brain infections is best, then new, swifter, diagnostic tests will be necessary if permanent brain damage is to be avoided. Rapid treatment is very much the key to success, so diagnosis based on clinical impressions seems the most humane approach, with some type of laboratory confirmation following. A closer study, however, of typical cases of HSV encephalitis may further reveal clinical guidelines that will permit early use of antiviral drugs.

It was long thought, based on misleading serological data, that HSV was a common cause of 'aseptic meningitis'. It was found, however, that the virus was infrequently isolated from spinal fluid which is the opposite of what occurs with similar diseases caused by mumps or enteroviruses. It is still not clear if meningitis is sometimes caused by the type 2 venereal herpes simplex virus (Wolontis and Jeansson, 1977). This virus type is less prevalent when compared to the type 1 virus that is so frequently found associated with cold sores. The diagnosis of meningitis related to any type of herpes simplex virus, however, presents some problems. A brain biopsy would not seem justified. The virus should be found in the spinal fluid but this seems a rather rare occurrence (Herrmann, 1972). Again the detection of rapidly rising antibody might be a useful diagnostic method but the relative rarity of this type of meningitis could inhibit establishment of such a test on a routine basis. The use of fluorescent antibody methods to detect HSV antigen in infected cells in the spinal fluid and blood could be a rapid diagnostic test but it has not been shown to be as sensitive as isolation of the virus in cell cultures (Cho and Feng, 1978). Of course, the patient's medical history could be helpful if it suggests a possible recent infection with the venereal or type 2 HSV. A much closer study of the clinical picture now seems required to try and discover if there is something unique or suggestive about type 2 HSV CNS disease.

It would seem prudent to effectively treat even mild HSV induced diseases in the hope this would abort CNS disease. It seems worth mentioning that the widespread use of antiherpes drugs on common HSV infections, such as cold sores, stomatitis, pharyngitis and a variety of dermal and mucosal lesions, might have an indirect influence on HSV induced CNS disease by limiting the huge amounts of virus being poured into the population. Some feel, of course, that latent HSV, induced to activity by some type of trauma, may be the cause of the CNS disease (Rawls *et al.*, 1966). Even if this were proven, however, the wise course would be to effectively treat the common infections for this might also inhibit the latent infections.

Presently HSV seems our most easily detected virus, producing a cytopathic effect that is distinctive and can at times be observed within 24 hours after the culture is inoculated (Table 1). This virus isolation procedure is comparable to bacterial isolation methods and should be an example of what is needed for all viral diagnosis. Further, even in the absence of virus isolation, distinctive inclusions present in the lesions help to implicate herpesviruses. It would seem, therefore, that the combination of present diagnostic methods plus the use of known antiviral drugs should permit an effective attack on diseases caused by the herpesviruses with the possible exception of encephalitis where somewhat swifter diagnostic methods seem required.

The insect-borne virus encephalitides also present a considerable diagnostic problem. A sporadic case of something like California or St. Louis virus encephalitis would at best be diagnosed by serological methods, very late in the course of the illness. Again, however, the virus alert concept could come into play and indeed has been to some extent already used

since the news media widely announce outbreaks of mosquito-borne viral disease. A patient's history of exposure to mosquitos most certainly would be suggestive while on the other hand a CNS infection occurring during the cold months with no mosquito activity would likely rule out this type of viral disease (providing of course the patient had not recently been to a more tropical climate). The serological test often requires both acute and convalescent sera to detect a rise in hemagglutination inhibition antibodies. There should be some encouragement still to test the acute serum even if no convalescent serum was forthcoming, for a high antibody titer can often be suggestive of which type of mosquito-borne virus is involved. Mosquito-borne viruses can be isolated by intracerebral injection of specimens into the brains of mice. Unfortunately, the human blood stream harbors the virus very briefly leaving brain tissue the only useful specimen and this is largely an impractical specimen, especially since much of the disease produced is mild and self-limiting. Death is, however, not an uncommon result of mosquito-borne viral disease and proper treatment with antiviral drugs would have significant benefit if such drugs existed. The treatment would probably have to be prompt using clinical observations alone with later confirmation from the laboratory.

There are cases of encephalitis associated with chicken pox (varicella) where the classical skin eruption occurs first thereby permitting anti-herpetic therapy at an early stage. Lymphocytic choriomeningitis (LCM) virus can also cause a relatively rare encephalitis in humans. The patient's history might in this case reveal some clues in that there should be a close association with infestations of mice and in some cases even with laboratory animals that harbor this virus. For diagnosis, specific isolation of the virus is not practical since the virus is most readily found in the brain and the disease is not so severe as to justify obtaining brain tissue. Disease caused by LCM virus has some clinical distinctiveness but the usual diagnostic procedure is to detect a rise in specific antibody in paired sera, a procedure frequently available through public health laboratories. Indeed these laboratories often perform serological tests for LCM, mumps, herpes simplex and the more prevalent mosquito-borne viruses. Unfortunately the evidence shows (Herrmann and Herrmann, 1976a) the results of these tests are much delayed and of little use as a guide to therapy for an individual patient.

Shingles, a troublesome disease caused by the chicken pox virus, can be associated with some peripheral nerve damage and even permanent paralysis. Prompt treatment, even today, with anti-herpetic drugs might well prevent such nerve damage. The virus can be isolated in human fibroblast cultures from fresh vesicle fluid obtained from the vesicular lesions but very often the clinical picture alone is typical enough for an accurate diagnosis. The isolation of this virus could be time consuming so treatment might best not be delayed until a laboratory result is produced. Again a search can be made for intranuclear inclusions which would not, however, distinguish a varicella-zoster from HSV infections. Such a distinction may not be important, however, in that the same drugs seemingly could be used for both infections.

Hopefully much of the central nervous system diseases with polio and measles viruses will be the thing of the past with widespread use of vaccines to prevent these infections. Presently there seems little concern for even looking for drugs to use on these diseases. Regrettably, however, there are in the United States some rare cases of poliomyelitis caused by the vaccine. Antiviral agents, something like those already mentioned, active against the picornaviruses could well be used for even these rare polio cases, and, of course, for diagnosis any poliovirus can be rapidly isolated in the laboratory from both rectal and throat swabs. The vaccine strains can be distinguished from the so-called wild strains because of the failure of the vaccine strains to multiply in cell cultures at 40°C.

In the case of measles there should be some concern that the vaccine virus, as well as the wild type strain, can be associated with those rare cases of subacute sclerosing panencephalitis (SSPE) (Gajdusek, 1977). As with most so-called slow virus disease, the diagnosis is made primarily on clinical and histopathological grounds. Generally the progress of these diseases, as suggested before, is slow, hence, permitting a diagnosis with institution of proper treatment, if one existed. Certainly the earliest possible diagnosis, however, would be more desirable, especially if this could be done prior to serious illness. Presently the isolation of a

measles-like virus from cases of SSPE is far from routine. The question still remains, however, would specific antiviral therapy be helpful in cases of slow virus disease and if so at what stage in the illness?

A drug that quickly halts rabiesvirus multiplication would seem not to require a laboratory diagnosis to justify its use. Even a drug only moderately well tolerated could be used based on the clinical history alone with somewhat more freedom than now suggested for the use of the present immune antiserum and vaccine treatment. With pursuit of the responsible animal and the use of routine rabies diagnostic methods, a laboratory confirmation could follow. These laboratory methods should include fluorescent antibody to detect specific rabiesvirus as well as a search for Negri bodies and this should be further confirmed by injection of the brain tissue intracerebrally into infant mice. Even without the offending animal, however, chemotherapy could well be used when there might be greater hesitation to use vaccine and immune serum procedures which are not only rather troublesome but on occasion can produce unfortunate side reactions.

Cases of central nervous system disease have at times been associated with infectious mononucleosis (IM). Since most now feel IM is caused by the herpesvirus of Epstein and Barr (Henle *et al.*, 1968) this disease could well be treated with one of the antiherpes drugs now under study. The heterophile antibody tests are still rather useful in diagnosing IM and these positive tests along with a classical clinical history and blood picture would be excellent guides to treatment. So far routine isolation of this virus is not possible. Of course, IM with or without CNS disease would require a systemically acting antiherpes drug and even in those cases caused by cytomegalovirus, where the heterophile antibody test is negative, an antiherpes drug could still be effective. CMV virus can be isolated in human fibroblast cell cultures but it is frequently time-consuming and the virus often missed. If the antiherpes drug is well tolerated there should be no reason to withhold it in infections either caused by E–B or CM virus. Certainly in those serious CMV infections occurring in infants and those that are immune deficient there should be every reason to use an antiherpes drug at the earliest moment based on the clinical picture. Later confirmation of the CMV infection can be done by detection of specific IgM antibody, the virus or the typical inclusions often seen in cells found in the urine.

7. VIRUS DISEASES OF THE SKIN

7.1. The Problem

Many viral diseases produce skin eruptions and this would include measles, rubella and infections caused by certain enteroviruses, adenoviruses and parainfluenza viruses. All of these are systemic diseases whereby the skin eruption is just one manifestation of the infection and are not considered specific skin diseases nor would they be treated topically. Rather they require treatment of the entire infected host. Measles, of course, can be adequately controlled by vaccine and this, hopefully, will also be true of rubella, which is largely a mild childhood disease that might be conveniently ignored if it were not for the attack of this virus on the human fetus. Rashes sometimes seen with adeno, entero (especially ECHO-9) and parainfluenza viruses would be dealt with as discussed elsewhere again emphasizing the systemic diseases produced by these viruses.

The common cold sore, whether it be on the lips or elsewhere on the body, has to be the most common of all viral skin infections. Some data are being misread giving rise to the claim that 20–40 per cent of humans have recurrent herpetic skin lesions (Spruance *et al.*, 1977). Although considerably more data are required, the figure for those having one cold sore episode in a 6–9 month period is about 8 per cent of a study population (Young *et al.*, 1976). But even if this lower figure is true of all human groups, in all locales, then it truly is an enormous amount of viral infection. To this must be added the problem of type 2 virus, the venereal strain, that has been suggested as having an association with cervical carcinoma (Melnick *et al.*, 1974), and which commonly infects skin and mucosa of humans. Indeed, some claim HSV venereal disease is most common (Nahmias *et al.*, 1969) and perhaps frequently

overlooked because it is self-limiting and rarely causes any sequelae. On the other hand it can be recurrent on the genitalia just like cold sores.

Chicken pox is, of course, a generalized disease but this is not true of zoster which is a manifestation of infection by the same virus in those who are partially immune. Zoster is localized to areas of the skin that overlay certain, specific nerves that are likewise infected. Shingles occur mostly in those over 50 years of age but it can occur at any age and it can be quite painful, persisting for months. On occasion nerve damage occurs producing persistent pain and even permanent paralysis to facial, arm and hand muscles. There seem to be no accurate data on the morbidity of shingles but it is a disease common enough that almost everyone has encountered someone who has had this troublesome problem. It should be added that pain and chronicity are not always the hallmark of this disease, for some suffer very little and get over it quickly suggesting that special immune conditions may exist in those with the more troublesome aspects of the disease.

Vaccinia necrosum is an uncontrolled spreading of skin lesions from a smallpox vaccination site. It seems limited to the skin, usually occurs in the very young and suggests an immune deficiency. It can, when untreated, lead to death. This localized infection should be distinguished from the vaccinia infections which can be much like mild smallpox, that occurs in those persons with atopic or other dermatosis. Such a generalized infection requires systemic drug treatment, that has been accomplished with marboron (Bauer, 1977). Likewise, it appears that systemic application of marboron cures *Vaccinia necrosum*. What with the very limited prevalence of smallpox and the limited use of the smallpox vaccine, the adverse reactions from the vaccinia virus infection will be even rarer than before. Smallpox, which now seems to have been brought under control, will not be discussed even though marboron, also an antismallpox drug, is available (Bauer, 1977).

7.2. Potential Drug Solutions

There is no longer any need for conjecture regarding the direction that should be taken for the treatment of herpetic skin lesions. There are adequate data showing that skin lesions caused by herpes simplex virus and those associated with shingles can be treated with idoxuridine in dimethylsulfoxide (Herrmann and Herrmann, 1977a). With the rapid advent of other antiherpes drugs it seems clear that various preparations should eventually become available for topical treatment of these localized infections.

7.3. Diagnostic Needs

The data so far indicate that a drug such as idoxuridine in DMSO is effective in the treatment of both zoster and cold sores, hence there seems little need to distinguish these two diseases by virus isolation. In many cases the diseases are so classical that the clinical picture is quite sufficient for the application of topical antiviral drugs. But in certain cases the clinical picture may not be clear, as with infections of the mucous membranes, and then laboratory diagnosis might well be undertaken. HSV is the most readily isolated and identified of all human viruses so the problem of laboratory diagnosis of these infections seems largely solved. Varicella-zoster virus is somewhat more difficult to isolate, but given fresh vesicle fluid, inoculated into cell cultures at the first possible moment, this results in a significant number of isolates even though it may take a week or more before virus activity is seen. But treatment would have largely been begun prior to a laboratory report in any event; at least this is what should, in all practicality, be expected. Vaccinia virus is also readily isolated in cell culture but the patient's history should point to this problem in any event and laboratory diagnosis usually seems unneeded.

There are those cases of vesicular lesions that might not be induced by the herpesviruses and this is the time when some laboratory diagnosis seems worthwhile, to at least exclude the herpesviruses and this can even be done by cytological studies of cells from the border of the lesions. But as indicated before, this method does not distinguish between HSV and zoster virus infections, if in fact such a distinction is needed. The problems related to herpesvirus

associated skin infections seems well on the path to resolution as regards the treatment and diagnostic needs.

8. VIRAL DISEASES OF THE UROGENITAL SYSTEM, THE NEONATE AND THE NEWBORN

8.1. The Problem

Presently the most important virus infection of the urogenital system is that caused by herpes simplex virus, frequently the type 2 venereal strain. Some of what has been said of the diagnosis and treatment of infections by this virus in previous sections applies here. Certainly these seem to be widespread infections and they can be recurrent (Nahmias *et al.*, 1969). A major concern has been suggestive data associating HSV type 2 with carcinoma of the cervix (Melnick *et al.*, 1974). A second concern, whether it be type 1 or 2 HSV, is the infection of the newborn in its travel down the infected birth canal. Although such neonatal infections can at times be limited and superficial and even subclinical, more serious disease does occur such as infections of the eye (which can, of course, be treated with presently available drugs) and a generalized infection that involves a number of organs and can produce a fatal outcome. Serious infections likely occur in about one in 10,000 births but these figures vary depending on the study population (Hanshaw, 1973), nonetheless there are enough cases to demand more rapid progress in the development of effective treatment using antiherpetic agents that are already known.

Mumpsvirus infection of the urogenital tract is common since this virus can be isolated so readily from urine even for weeks. Specific disease involving this system seems rarer, however, and usually takes the form of monolateral orchitis in post puberty males, bilateral orchitis is rarer and hence sterility is not common. Oophoritis also occurs in some cases of mumps but sequelae from such infections is not well documented, as is the case with fetal or newborn infections.

There have been claims that adenoviruses are associated with hemorrhagic cystitis, mostly in children (Manalo *et al.*, 1971). In that this work has not been fully confirmed (and in fact could not be confirmed by these authors) there is some question that this is a valid problem. It is possible that other viruses do at times infect the urogenital system, perhaps some that are still undiscovered but the problems are as yet indistinct. It is worth mentioning, however, that the very common detection of simian viruses in kidney tissue from healthy monkeys suggests that the human kidney might well also be infected with a variety of viruses. This is not to say that such infections are commonly associated with disease; probably they are not.

Cytomegalovirus, which can even be isolated at times from the urine of healthy children, is another herpesvirus that can infect the fetus, producing a devastating disease (Hanshaw, 1971). Even though the maternal urinary tract can be infected this is likely not the source of most fetal infections and it is even less clear how much CMV disease occurs postnatally. The major problem is infection of the fetus and this likely occurs because of a viremia in the mother. Cytomegalovirus is presumed to infect all humans, if antibody surveys are an accurate gauge of infection, but it seems that most cases go unstudied or are mild with the possible exception of certain instances of heterophile antibody negative infectious mononucleosis and it could be such a case in the mother leads to an infected fetus. Despite what seems the more common prevalence of HSV, the more serious and frequent cases of newborn disease involve CMV infection. Like HSV and varicella-zoster, CMV takes a more serious course in the immunodeficient and immunosuppressed patient and the fetus and newborn might in a sense be considered in this category. It is estimated that CMV infections can be detected in 1–2 per cent of all newborns but there is concern that some cases of mental retardation may also be produced by this virus and even perhaps by HSV, cases that went unrecognized in the newborn (Nahmias *et al.*, 1976).

The role of rubella virus in attacks on the fetus is well known and will not be pursued further here in the hope that the rubella vaccine can in time eliminate this as a significant

problem. The enteroviruses, principally those of the coxsackie B group, are of some importance in newborn infections and it is likely that echoviruses also play some role in diseases of the newborn, no doubt as a result of fecal excretion of these viruses by the mother at the time of birth. Some cases can be fatal in newborns because of a serious myocarditis while other newborns may exhibit a meningitis and even an encephalitis, although in these latter cases mortality seems rare. Considering the prevalence of enteroviruses during the summer months, infection of the newborn resulting in a serious problem seems less frequent than might be expected. Of course any viral infection, producing significant illness in the mother, might cause problems for the fetus but such problems seem rare enough that they are as yet not well defined. What has now been well defined is the role of the so-called rotaviruses in infant diarrheal disease, almost always in those under 3 years of age. It has been established that these infections can be rather serious in the newborn (Carlson *et al.*, 1978).

Newborn children clearly can be subjected to those viral infections that afflict all humans but this is not as great a problem as it might seem since they have an array of antibodies from the mother that can persist for various lengths of time even up to a year after birth. One suspects that in addition there is intrinsic resistance to infection in the newborn that, however, may not be enough in the face of massive doses of virus either prior to or after birth. Again, it seems worth pointing out that many fetal and newborn problems go unexplained and a number of these, including some cases of mental retardation, could be related to infections by viruses still undetected or characterized.

8.2. Potential Drug Solutions

Prior sections have discussed potential if not real drug solutions to herpesvirus infections, whether they are superficial or systemic, and this applies also to infections of the urogenital system and the newborn. How one shall prevent viral attacks on the fetus when a mother has a virus infection presents significant problems, nonetheleast of which is the effect of the drug on the fetus. In some cases, such as with CMV or HSV, the maternal infection may not be obvious. When it is, then it should probably be treated but the benefits should outweigh the risks to the fetus and this can only be done when the cause of the viral infection is well established and any possible action of the drug on the fetus is well known. Having said this, however, antiviral drug treatment of the infected gravid female must be considered as a potentially fruitful approach to protecting the fetus and even the newborn.

If mumps virus infections were treated promptly with an appropriate drug then probably many problems associated with the urogenital system could be prevented since they usually occur sometime after the onset of recognizable mumps. Certainly, as with mumps meningoencephalitis, some cases will not be typical but even fully established orchitis might be treated effectively once a useful drug becomes available. It should be pointed out that a live mumps vaccine is being used. So far 30 million doses have been given in the U.S. and immunity lasts for at least ten years. If this approach continues to be fruitful then chemotherapy for mumpsvirus infections would be required far less frequently.

In that there seems little interest in vaccines for the many enteroviruses, other than polioviruses, specific chemotherapy for these infections seems required, especially when they occur in the newborn and this would, of course, require systemic therapy, as discussed in prior sections. One should still expect that even those diseases that can be largely controlled by vaccines, such as rubella, polio or measles, in some cases will still occur and hopefully, in time, there will be adequate drugs available, perhaps those primarily for other viral diseases, that will also prove useful in the treatment of these rare cases.

8.3. Diagnostic Needs

CMV and HSV infections of the urogenital system and the newborn can in many cases be diagnosed by the clinical picture, as well as by virus isolation and by cytological methods. With all these approaches combined, much can be done so that proper drug treatment can be instituted rather promptly. There are instances when urine sediment or urine samples do not produce a positive result when from a typical case of CMV infection so there is some room for

improvement in the detection of this virus in cell cultures. Also there are cases of HSV infection where no herpetic skin lesions occur or where the virus is not detected in any convenient specimen and this produces the same diagnostic problem as found with HSV encephalitis. Again the question arises whether some early rising specific antibody might aid in the diagnosis so that effective treatment might be undertaken. In some cases the clinical picture would be such that therapy could be started without a laboratory diagnosis, and continued until that time when a CMV or HSV infection is ruled out.

About 70 per cent of mumps cases have a typical clinical picture that would permit early drug treatment. Mumpsvirus can also be isolated in cell cultures, especially from urine and most especially from urine that has been ultracentrifuged. Unfortunately many such virus isolations take 5 or more days to detect and in an atypical case of orchitis this may be too long a wait prior to starting therapy. Treatment could be started prior to the final laboratory reports and then altered depending on what the reports indicated. Likely a rapid mumpsvirus test might be established using sedimented urine cells and specific fluorescent antibody, and although perhaps a useful approach, there may not be a significant demand for such a test, at least not significant enough to establish it on a routine basis. Again, a virus alert program would help to indicate the prevalence of mumps in the community and even with those atypical cases that involve the urogenital system such information could be invaluable in making a differential diagnosis. This same epidemiological approach could be used for enterovirus infections. Coxsackieviruses of the B type cause the more serious problem in the newborn and a monitoring system would readily establish the presence of these viruses in the community. The mother might well be tested for excretion of these viruses prior to delivery even though it is not completely clear that all enterovirus infection of the newborn come from this source. Coxsackie B viruses are readily isolated in cell cultures and many times produce a recognizable cytopathic effect in 2 or 3 days. The question arises whether infections of the newborn by such viruses always permit easy access to the virus, especially in cases of myocarditis. It is not clear that all such cases shed virus in the throat or feces. Further, the cases are rare enough that some physicians may not have adequate experience to recognize the problem based on the clinical picture alone. If it is known that such viruses are in the community then treatment with a specific, systemically applied drug that is therapeutic for enterovirus infections could be used to treat a very ill newborn. It would be hard to imagine that much improvement is required in the isolation of coxsackie B viruses, that are routinely recognized and reported to the physician almost as rapidly as are bacterial isolations (Hable *et al.*, 1970). The echoviruses take somewhat longer to isolate and recognize but they seem to be less of a problem in the newborn, indeed serious illness may be rather rare (Herrmann *et al.*, 1972). Even though most enteroviruses are rather readily detected and recognized the problem has been to define the specific serotype of the over 70 that exist. The hope is that this will not be necessary in that anti-enteroviral drugs will be effective against all the various serotypes and identification could be a simple biochemical test even using the drug as the best indicator of the nature of the virus rather than tedious and lengthy serum neutralization tests.

Some thought must be given, of course, to the varying drug sensitivities of closely related viruses. For example, a newborn could well be infected with either type 1 or 2 HSV. There are suggestive data that these two viruses are not equally sensitive to certain antiviral drugs (Person *et al.*, 1970); hence, it would have to be determined which of the two types is infecting the child. On the other hand it would seem prudent to promote a drug for herpes simplex virus that was equally useful on both types of infections thereby eliminating any need for seratyping HSV. One should be prepared, however, for the emergence of drug resistant mutants. There are sufficient data on such mutants (Herrmann and Herrmann, 1977b) to suggest that drug sensitivity tests will become a routine part of viral diagnosis.

9. VIRAL DISEASES OF THE GASTROINTESTINAL SYSTEM

9.1. The Problem

In recent years great strides have been made in understanding the nature of hepatitis

viruses and there is widespread use of methods that can detect the coat of hepatitis B virus (HBV or serum hepatitis virus) in ill patients as well as in blood donors. These methods still lack some sensitivty but are very useful. Certainly some hepatitis virus carriers still are not detected. Further, such carriers present a public health problem since there is no cure for their infection. Efforts are being made to develop a vaccine by isolation of vast amounts of the virus antigen from infected bloods. The problem would seem to be how can a person that has carried this virus in their blood stream, likely for years, be made immune by injections of still more pieces of the same virus?

At least one so-called infectious hepatitis virus has been characterized, this hepatitis A virus (HAV), like the hepatitis B virus cannot be cultivated in cell cultures and it is recognized by concentration from feces and then visualized in electron micrographs. Work with both these viruses has led to the conclusion that they are not the only viruses that cause hepatitis in humans. Indeed, perhaps 90 per cent of post-transfusion hepatitis is neither A or B virus. There is no clear idea of how many hepatitis viruses there may be. So despite great strides the picture is complex. Something was achieved, however, when it was discovered that purchased human blood was much more likely to contain hepatitis B virus than was blood from volunteers and correcting this has lowered the rate of post transfusion hepatitis (Holland and Alter, 1976).

The second major virus associated problem of the gastrointestinal system is just now emerging. Some had thought that the enteroviruses, such as echo and coxsackieviruses, were a common cause of gastrointestinal upsets. It is clear now that they play a minor role in these ailments. The rotaviruses are the primary cause of severe infantile diarrhea. To this can be added a number of other viruses associated with both adult and childhood bouts of vomiting and diarrhea. These include the Newport type agents, astroviruses, 'mini-reoviruses' and likely others (Middleton *et al.*, 1977). None of these viruses, usually causing a rather explosive short term gastrointestinal upset, can as yet be cultivated in the laboratory. How many more there may be, how many types of viruses they represent, is unknown. The solution to the problem, unlike that of hepatitis, does not seem to require something as vigorous as a vaccine in that these diseases, although of substantial importance, are only life-threatening to infants who have not received proper medical care (Carlson *et al.*, 1978). Such infections might be viewed as the common cold equivalent of the G.I. tract and represent what so many for so long have incorrectly called 'intestinal flu'. So far the data suggest the morbidity from these viruses is most substantial, far in excess of what is seen with bacterial disease of the G.I. tract (Birch *et al.*, 1977).

9.2. Potential Drug Solutions

Hepatitis viruses cause liver damage that at times is fatal and at other times induces chronic liver problems. A vigorous application of a drug to halt virus synthesis or limit the degree of liver damage is warranted. Such a drug could also be used to protect those at risk when in contact with hepatitis cases or even to treat carriers of the virus. Further, until that time when every unit of blood for transfusion can be guaranteed free of any hepatitis virus, the same drugs might be used to protect patients receiving transfusions. Viral inhibition seems mandatory if a treatment is to be effective in halting liver damage. Unfortunately it is not known how many hepatitis viruses we are dealing with and in fact how they may be biochemically related to each other. The A and B viruses are unrelated and each might well demand its own form of treatment. Experiments are underway in an effort to find if interferon might be useful. Here is a case where the antiviral agent is not virus specific, as certain drugs might be, and a broad spectrum agent or drug would seem desirable. Virazole is a drug with a somewhat broad antiviral effect, part of which may not be specifically antiviral, but nonetheless it too has been considered for treatment of hepatitis. Of course, until the time all hepatitis viruses can be cultivated in some convenient laboratory system, we are not likely to be able to search for new drugs specifically active against these viruses. In assuming a specific viral inhibitor is required it must be pointed out that a recent study shows a drug that is not known to inhibit viruses (Blum *et al.*, 1977) may have some worthwhile effect on cases of

hepatitis through some unknown mechanism.

Virus gastrointestinal disease is a somewhat different matter since most cases seem to be relatively mild, self-limiting diseases so perhaps a highly potent specific antiviral drug is not required. It seems true that certain of these viruses do produce some lesions in the G.I. tract but whether this is the direct cause of the disease problem or whether the disease is due to a toxemia is not as yet known. Nonetheless, some consideration should be given to drugs that relieve the symptoms and signs of the disease but are not specifically antiviral. Drugs that act on the toxemia or alter cellular destruction or in some manner relieve the patient's discomfort might be just what is required without making any attempt at altering the virus multiplication. The search for such drugs can be two-fold, first some attempt can be made to study various available and approved drugs for some worthwhile pharmacological effect. As mentioned before, is it possible anti-inflammatory, antihistamine or even tranquilizer drugs have any worthwhile effect? Do in fact, any of the over-the-counter drugs have any effect on human viral diseases? Animal studies could be first undertaken since virus induced diarrhea has been established in infant mice and baby pigs and this does not involve the substantial challenge of common cold virus studies where only humans exhibit a rather subjective illness. Controlling the diarrhea would be a useful definitive criterion for the study of drugs that are not specifically antiviral.

9.3. Diagnostic Needs

Despite the routine methods now used to detect serum hepatitis antigens in the blood of infected humans this is a small beginning when the entire area of gastrointestinal system virus infections is examined. None of the remaining hepatitis viruses (other than those few known viruses that only occasionally cause hepatitis) nor any of the viruses causing GI upsets can presently be diagnosed in virus laboratories without easy access to an electron microscope. One might conclude that a person had viral hepatitis on clinical grounds alone but it seems necessary to define the particular virus exactly before effective therapy could be undertaken. The same seems true of the viruses so far associated with G.I. upsets except in this case bacteria could just as well be the cause. It is obvious that much more needs to be done in isolating and defining all these viruses in a routine way before effective drugs could wisely be used. Since none of the viruses discussed in this section can as yet be cultured in cell cultures there is some question how soon drugs would be discovered specifically for such viruses. It would seem rather more likely that antiviral drugs found for other viral infections would be tested for their effect on viral hepatitis even prior to the time the viruses could be conveniently handled in the laboratory and in fact this is what is being done with interferon and with virazole. Since HBV infections can now frequently be definitively diagnosed, at least in these cases drugs could be tested and hopefully routinely used in what can sometimes be a lethal infection.

10. SUMMARY AND CONCLUSIONS

There can no longer be any doubt that the era of antiviral drugs has arrived. It is possible that antiviral drugs will find widespread use even prior to the general availability of laboratory viral diagnosis, although there is no justified reason that this should be the case. Many antiviral drugs will likely be specific inhibitors of viral infection but it is also likely there will be other types of drugs that do not influence the virus but rather have a pharmacological effect on disease symptoms and clinical signs. Some drugs may well enhance the body's own protective systems by immunmodulation or by an interferon effect. There are, in fact, many approaches to treating viral disease, a number presently neglected, but this is not likely to continue much longer as the economic potential of antiviral drugs becomes fully recognized. The advent of antiviral drugs should result in a significant decrease in the ill-advised use of antibacterial drugs for viral diseases.

The overwhelming majority of cases of viral disease involves the human respiratory tract and much of this disease looks the same even when caused by a great variety of viruses; therefore, it would seem that some type of laboratory confirmation would be required for the

rational use of antiviral drugs, barring, of course, a utopian drug that inhibits many types of viruses. Even if a universal antiviral drug came into being, the need for distinguishing viral from bacterial disease would remain. The rational use of antiviral drugs based on laboratory data is not likely to come to pass, however, until it is accepted that laboratory viral diagnosis can be relatively swift, inexpensive and part of everyday, routine, medical practice. Laboratory viral diagnosis will likely follow the path of bacterial diagnosis with emphasis on isolation and identification of the pathogen plus some indication of its drug sensitivity. In that viral diagnostic procedures may not always be useful for drug treatment of a single patient, a 'virus alert' program should be part of medical practice. The wide transmission of information on what viruses are in the community at any one time would be an aid in the proper use of antiviral drugs. But it can still be presumed that much use of antiviral drugs will still be based solely on clinical impressions which in some cases will be correct and in other cases will be in error. Hopefully, the degree of ill-advised use of antiviral drugs will not reach the level of what occurs with antibacterial drugs since this could cause more problems to the patient than the generally mild viral diseases. Sharp outbreaks of viral disease should be swiftly defined in an attempt to head off the wide use of an inappropriate antiviral drug.

The emphasis must be on relieving the patient's discomfort so as to produce an individual that can function in a fully effective manner. This might well be achieved with drugs that are and are not specific viral inhibitors. Of course, it would be much preferred that viral disease was prevented but there is no convincing evidence that there will be a general preventive approach to diseases produced by the hundreds of viruses that now infect humans. The future would still seem to favor specific antiviral drugs since, as indicated by antiviral studies of the past and present, it is the specific inhibitors of virus multiplication that have produced the most convincing data.

There seems little question that viral diagnosis will continue to emphasize virus isolation and identification but such an approach still seems to be lagging behind drug development. Much more needs to be done to improve virus isolation techniques, not only for the known viruses, but for the many still unknown. The economic rewards for the successful pursuit of an antiviral drug seem obvious. The rewards for those who toil to improve viral isolation procedures is not so obvious, nor well supported financially. Until there is a dramatic change in the acceptance of laboratory viral diagnosis as part of routine, everyday, medical practice, antiviral drugs will not achieve their full potential in the relief of the patients' problems.

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