The tragedy of viral diagnosis

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Summary

The shortcomings of the methods commonly recommended for the diagnosis of viral infections are emphasized.

Most of them are laborious and expensive, and are of very little practical value to the clinician.

Nine years' experience has confirmed that the use of a single swab, obtained during the acute stage of the illness, for bacterial culture and viral isolation (looking for cytopathic effects or haemadsorption) provides the diagnosis quickly in the great majority of viral infections.

Introduction

Most infectious disease suffered by humans affects the upper respiratory tract. If, as has been shown in a variety of studies (Dingle *et al.*, 1953; Hope-Simpson & Higgins, 1969), each person averages seven respiratory infections annually, then there are well over one billion such cases in the United States each year. It has been estimated that on the average this huge morbidity can be related to identifiable microorganisms at least 50% of the time, and these organisms are mostly viruses (Hilleman, 1963). The average virology textbook emphasizes the more serious viral illness requiring hospitalization, when in fact over 90% of viral disease affects the upper respiratory tract. This is the true nature of viral disease. Physicians in the United States probably see no more than 5% of this total disease (Hope-Simpson & Higgins, 1969), which could represent upwards of fifty million visitations per year. Such a burden weighs heaviest on the pediatrician and the general practitioner. Faced with this enormous case-load physicians find meaningful laboratory aid in diagnosing viral disease virtually nonexistent. It is the purpose of the following discussion to examine why this is true and whether it need be.

Retrospective diagnosis

Perhaps one can obtain a clue to the problem in what Lennette, a recognized leader in viral diagnosis, says: 'Isolation and identification of an agent are still, in most cases, relatively costly procedures and seldom give information which cannot be more simply, more rapidly, and less expensively (albeit retrospectively) obtained by serologic methods' (Lennette, 1964). Lennette is also the chief editor of the latest version of another manual purporting to show how viral disease should be diagnosed, and he is consistent (Blair, Lennette & Truant, 1970). The serological approach is emphasized in virtually every presentation on the subject. As will be shown, this emphasis is wrong. The key words in Lennette's remarks are, of course, 'albeit retrospectively'. A retrospective diagnosis is largely an academic exercise, not very useful in the practice of medicine.

The emphasis on serologic methods seems unique to virology, for in the Introduction to the *Manual of Clinical Microbiology* the editor states the manual is devoted to 'the isolation and identification of disease-producing organisms' (Blair *et al.*, 1970, p. 3). Those constructing the virology portion seem to have been unaware of these objectives. In 1961 a respected virologist and his colleagues detailed the case against the serologic approach (Henle *et al.*, 1961). Their plea for an emphasis on virus isolation seemingly was ignored.

How did the diagnostic virologists become isolated from the aspirations of microbiology and from the needs of medical practice? Perhaps they feel themselves to be more serologists than virologists; perhaps it is the early pride and comradeship of workers in the Public Health Laboratories that were successful in making the Wassermann test work that has led them to emphasize programs of complement-fixation tests. After performing thousands of such tests, I have become aware that consistent and useful results are an illusion. The

sine qua non for diagnosis of infectious disease is the isolation of the pathogen, which in virology today is by far the most rapid, least expensive, most comprehensive, useful, and accurate method of diagnosis.

The fact that virus isolation is readily done is now recognized by many. Unfortunately, some serologists still feel that isolation of a potential pathogen is not enough; one must further 'prove' the significance of the virus isolation by showing, with acute and convalescent sera, a rise in some type of antibody specific for the isolate. The term 'proof' is in fact used, indicating a substantial faith in antibody rises (Sohier, Chardonnet & Prunieras, 1965). But a rise in antibody titre even over the course of the disease does not prove what caused the disease. All that has been done is to confirm what would already be known by viral isolation, that the individual was infected. 'Infection' cannot be equated with 'disease'. It is relatively easy to prove someone is infected, but there is no scientific method to prove that the disease was in fact caused by the isolated pathogen. Other pathogens, known and unknown, could have also been present and overlooked. The best that virology can do, with or without confirmatory antibody rises, is to offer the physician a clue to the possible causal agent. This is the working philosophy of the bacterial diagnostic laboratory. How often is it found useful to 'prove' the significance of an isolated bacterial pathogen by measuring antibody rises?

Virology or epidemiology?

The first function of a diagnostic laboratory is to aid in diagnosing disease, and therefore it must be related to medical practice. Most who claim to practice diagnostic virology are in fact involved in epidemiology. Epidemiology, on the other hand, is a productive area of research that aims to produce data to clarify the significance of various micro-organisms isolated from disease situations. The epidemiologist must use every tool available to aid in establishing the role of certain organisms in disease; many have emphasized the measurement of antibody levels, even in the absence of isolation of the pathogen. This has led to significant errors. Antibody measurements are inadequate, for at best they can indicate only that the patient was infected, and at worst they indicate he was infected when he was not (Henle *et al.*, 1961). For the epidemiologist the alternative is to fulfill Koch's postulates, a most demanding procedure, but one that was in fact undertaken in establishing the pathogenic nature of Eaton's agent (*Mycoplasma pneumoniae*) (Rifkind *et al.*, 1962).

How did the emphasis on serologic confirmation arise for establishing the significance of a viral

isolate? As every microbiologist should know, there are only a few pathogens that are not at times present in healthy individuals. Perhaps this fact has made virologists somewhat insecure, so they demand additional evidence that an isolate is pertinent. They would like to be sure the infection was unquestionably related to the disease. Unfortunately, serology cannot produce such security. It is still possible that the patient was infected with a virus, producing antibody rises and extensive virus excretion, yet it still had nothing to do with the disease. This is the nature of medicine; rarely does one have absolute proof.

These traditions may well have started with two historic episodes in clinical virology. First, there was the discovery that normal human adenoid tissue could harbour adenoviruses (Rowe *et al.*, 1953). These contaminating viruses, which were at such low levels that they were almost undetectable, may have led many to believe that such viruses are common in the throats of healthy individuals and are readily isolated. Few have considered that there is a significant difference between the quantity of virus found in adenoid tissue in these original observations and the amount that must be transported via a swab to the virus laboratory to be detected in cell culture. The significance of the quantity of virus does not seem to have been much considered as a further guide to the pertinence of a viral isolate. Despite the fact that over 30 serotypes of adenoviruses have been found in humans, types 1, 2, 3 and 5 represent 90% of the isolations, primarily from children with pharyngitis and fever (Herrmann, 1968). This experience should suggest which serotypes are pathogenic. The ludicrousness of the situation is emphasized when the virus laboratory requests 25 grams of faeces as well as throat swabs and blood specimens in order to make a viral diagnosis. That this is an unnecessary practice in respiratory disease has not been sufficiently emphasized. As might be expected, after the extraction and culturing of such a faecal specimen, one or more viruses are found—which can only confound the diagnosis. Numerous adenoviruses, for example, can be found in the faeces, many serotypes in fact only in the faeces, which have no relationship to human disease (Vargosko *et al.*, 1965).

Faecal specimens

The use of faecal specimens in diagnostic virology can be traced to a second historic episode related to poliomyelitis research. Many attitudes and practices today are based on that experience, which is not related to the nature of most viral disease. Finding a host of viruses in the faeces, often unrelated to any disease, has further contributed to the insecurities

of the clinical virologist, so he searches for additional means to make such isolations pertinent. The frequent recommendation that extracts of faeces are better than a rectal swab only increases the opportunity for isolating viruses unrelated to disease (Blair *et al.*, 1970, p. 533). An acutely ill individual is presumably rather infectious, and extreme measures should not be needed to isolate the causal agent. Naturally if the specimen is taken many days after the onset of illness, viruses will then be more difficult to isolate and a more vigorous approach to collecting specimens may well be in order. Generally, however, the physician sees the patient during the acute stage of illness and such patients are rather infectious.

Insistence on blood and faecal specimens certainly has discouraged patient and physician cooperation. Serologic diagnostic methods are useful when the pathogen is difficult to isolate but they should still not be considered an adequate substitute for isolation of the pathogen. Faecal specimens are useful at times, especially long after onset of the illness since a number of viruses are excreted in the faeces for weeks and even months. Such occasional usefulness cannot, however, justify preoccupation with such specimens. The diagnostic virologist would like to avoid reporting a virus isolation that might have no relationship to disease; but this is not his prerogative. His responsibility is to report what he finds. Healthy persons will at times have potential pathogens and ill persons may have pathogens unrelated to their disease. It is questionable whether measuring antibody titres will resolve this problem.

It seems doubtful that the diagnostic virologists have critically examined their usual practices. Faced with a sick child, who usually has fever and pharyngitis and sometimes a cough, caused by any one of a number of virus serotypes, the usual demand from the virus laboratory is for a throat swab (or less conveniently a throat washing), two blood specimens, at least 2 weeks apart, and even at times, faecal samples. Many texts and manuals actually describe how these specimens can be kept frozen while awaiting the second blood sample. Of course, in some cases the viruses will not withstand freezing, so if such sensitive viruses are likely, it is further suggested that such specimens can be inoculated into cell cultures immediately. Generally, however, there seems to be little need to hurry with all these procedures. If everything goes well and the second blood specimen is received, then, according to usual practice, an attempt will be made to isolate a virus. If one is isolated it must be pooled, titrated, and carefully serotyped. It is a matter of great concern to most virologists to determine whether they have isolated, for example, a type 2 or a type 4 coxsackievirus of the B type. This would, of course, be of

great importance in preparing a paper for publication. Serotyping is usually unimportant to the practice of medicine, however. Once the serotype is determined, the measurement of antibody in the patient's sera is undertaken using the virus that has been isolated. In the event that no rise in antibody titre is observed, then the virus isolated is presumed to be of no significance, and the isolation is either not reported to the physician, or at best is reported but described as being of no significance. On the other hand, if everything is in order then, usually after the passage of some months, a report is made. All this for a sore throat and cough, according to the system.

In hospitalized patients, comprehensive specimens should be obtained. But few viral diseases require hospitalization. Hospitalized cases are so rare that they could hardly sustain a virus laboratory. Some studies may mislead, especially where hospitalization was free and a viral clinical study was underway. Usually many patients are studied who in fact did not need hospitalization.

Repeat blood samples

Bleeding infants and children at least twice seems not to bother most virologists, even though such procedures cannot benefit the patient and could be more traumatic than the disease. There is considerable resistance to being bled when the patient is well, so it is not surprising that up to 90% of the acute blood specimens end up with no matching convalescent specimen. There is little concern about the logistics of obtaining this second specimen, especially the time wasted by the physician which he could better spend ministering to the ill. Frequently much time is expended in convincing the patient or the parents that this is all in the name of Science. Once this ploy has been resorted to, then it is clear that *diagnosis* was not the real objective. Rarely, of course, can blood specimens be obtained at just the appropriate time so that a substantial rise in antibody titre can be shown. Rubella is perhaps one exception, but in most cases everyone is willing to settle for a small rise in titre, even a four-fold rise. This is the rule. No one justifies it or even inquires how it became established. Most concede that a two-fold rise could be due to a technical error, but a four-fold rise is considered diagnostic, even in the absence of a virus isolation. This seems a tenuous basis for diagnosis.

The imposition of the serological approach on viral diagnosis has led to a somewhat unpractical presentation in a recent manual of clinical microbiology (Blair *et al.*, 1970) which de-emphasizes tissue culture procedures for viral isolation, but gives detailed descriptions of virtually every type of serological method, frequently without designating the best method. This manual gives little space to

cell culture methodology; little or nothing is mentioned regarding maintenance and handling of the cell cultures, media to use, or even where cell cultures and reagents can be obtained commercially. The viral diagnostic portion is, however, anomalously located in juxtaposition to a lengthy presentation describing media and methods for growing various bacteria and fungi.

Misinformation on viral isolation

In many manuals and texts, the information regarding virus isolation is frequently in error, or inadequate, or both. Perhaps this reflects the separation of some authors from recent experience in isolating viruses. Lists of cell types are given; only on occasion is it indicated which is the best type for a particular virus. Thus one can find statements such as that herpes simplex virus can be isolated equally well in HeLa cells and diploid fibroblasts (Blair *et al.*, 1970, p. 563). Nothing could be further from the truth (Herrmann, 1967b; Herrmann, 1967c). In our laboratory, in isolating herpes simplex virus over 900 times, hundreds of these isolates would have been missed if only HeLa cells had been used. The implication that mumps virus is isolated equally well from all primate kidney cells (Blair *et al.*, 1970, p. 516) is in error; African green monkey kidney cells are rather inferior to rhesus monkey kidney cells for mumps isolation and there is little or no data indicating the sensitivity of most primate kidney cells to this virus. It is further stated that the classic, syncytial-cell cytopathic effects produced by mumps virus were of little help in diagnosis and haemadsorption is preferred (Blair *et al.*, 1970, p. 516). In isolating mumps virus 220 times we found that at least 80% of the time this virus produced typical cytopathic effects allowing earlier haemadsorption tests and an early report to the physician. The necessity for a low pH and an incubation temperature of 33°C in the isolation of rhinoviruses in diploid fibroblasts (Blair *et al.*, 1970, p. 539) is questioned by experienced investigators (Hamparian, personal communication) and has not been our experience in isolating these viruses over 400 times. Far more serious is the advice that serum-free medium must be changed frequently to properly maintain monkey kidney cells for the isolation of influenza viruses (Blair *et al.*, 1970, p. 499). Certainly such cells must be maintained on serum-free medium to eliminate inhibitors for some viruses, but any attempt to change medium frequently, where certain of the cultures are infected, will lead to substantial cross-contamination to uninfected cultures. When authors indicate that the medium is renewed every few days, then the number of isolations claimed should be questioned. Rhesus monkey kidney cells can be

maintained with no change of medium, by using 3 ml of Eagle's basal medium for a period of 2 weeks or more in the total absence of any serum. We have done this for several years while isolating influenza viruses over 400 times. We found, in fact, that monkey kidney cells were rather granular if any serum were present. It is a break with tradition, however, to use more than 1 ml of medium per culture tube.

Usually no advice is given on how cell cultures can be made less expensive. Miniaturized cell cultures can be made in microtitre plates (Rosenbaum *et al.*, 1963; Sullivan & Rosenbaum, 1967), a technique that likely will revolutionize cell culture and make it so inexpensive that there will be little excuse not to isolate viruses from human illness. The usual advice discourages economy, suggesting the use of multiple cell cultures of numerous cell-types and many so-called blind passages of medium from normal-appearing cultures to fresh cultures in the hope that a virus will finally appear. If blind passages with monkey kidney cells are undertaken, then a virus frequently does appear later, and it is invariably a monkey virus.

The consistency found in most diagnostic manuals is impressive. No matter what the disease problem, there are extensive descriptions of how to measure antibody rises in acute and convalescent sera. This includes patients with mild upper respiratory infections such as the common cold (Blair *et al.*, 1970, p. 541), apparently those with a cold sore (p. 564) and patients with measles, chicken pox (pp. 521 and 576), but not necessarily mumps (p. 519). Most interesting is the suggestion that tests be performed for rises in antibody titres to reoviruses, viruses not known to cause human disease (p. 546), and for para-influenza and respiratory syncytial virus infections, despite the unreliability of such tests (p. 507). Perhaps most striking of all is the serious recommendation that acute and convalescent sera be obtained from patients with possible rabies (p. 554): yet, as indicated by the author himself, all evidence indicates that no human has ever convalesced from rabies. In the rabid patient, brain tissue from autopsy will soon be available from which the virus is readily isolated (Gomez, Siekert & Herrmann, 1965).

Quick identification

Few seem to care that the procedures just described have little to do with the patient's needs. What can be done about this situation? As described in detail elsewhere (Hable, O'Connell & Herrmann, 1970; Herrmann, 1967a, 1967b, 1967c, 1968; Herrmann & Hable, 1970; Hoekstra, Herrmann, & O'Connell, 1970; Rawls & Herrmann, 1964), in the overwhelming majority of cases of viral disease only a single throat or lesion swab need be obtained, and that during the acute stage of illness. The swab is

first applied to bacterial culture media, then extracted for viral isolation. Using as few cell cultures as possible, with as little changing of media as possible, one simply looks for viral cytopathic effects or haemadsorption. When virus activity is observed an immediate report is made to the physician. In isolating 4000 viruses, 74% were reported to the physician within 1 week after receipt of the specimen, and almost 50% were reported in 4 days. This is the overall result for a 9-year period. As experience accumulates the reporting is now even faster. This is effected by only examining the cultures thrice weekly. If cultures were examined daily, another 1 or 2 days could perhaps be subtracted from the reporting time. In addition, the physician is told what kind of virus was isolated. This opinion is based on the nature of the cytopathic effect or haemadsorption, and the type of cell culture most sensitive to the virus. In 84.5% of the isolates the 'guess' as to what virus was isolated was correct. In only 4.48% of the cases was the guess wrong. The remainder of the isolates were just reported as 'virus'. Fewer than 1% of reported viruses could not be confirmed. All virus isolates are later identified using specific antisera or certain biochemical tests. In many cases biochemical tests probably can supplant the traditional serotyping of viruses. This more rapid viral diagnosis depends partially on the cell cultures effected, which is analogous to the use of differential media in bacteriology. The nature of the cytopathic effect, haemadsorption, or even mouse pathology is in some ways analogous to bacterial colonial morphology as used in bacterial diagnosis. The use of the sensitivity of viruses to a pH less than 3, and to organic solvents, most especially chloroform, can be considered analogous to the use of the bacteriologist's Gram stain. Much can be done in determining the nature of the virus isolate by certain biochemical tests, in much the same way as the bacteriologist uses the Gram stain or various biochemical tests.

Since diagnosis is the *sine qua non* of medicine it would seem unnecessary to justify accurate diagnosis of viral disease. This has been discussed rather convincingly in a recent textbook (Horstmann & Hsiung, 1965). It may be worth repeating, however, that viruses can be implicated in much that looks like bacterial disease, with considerable impact on the ill-advised use of antibacterial agents. A more accurate prognosis often can be given, and the physician is also aware of what viruses are circulating within the community. With rapid reporting of virus isolates the clinical features of the disease are still fresh in the physician's mind and on many occasions the patient is still ill. Certainly all of this becomes even more important when the advent of antiviral drugs is considered (Herrmann, 1965, 1969; Herrmann & Stinebring, 1970).

Not all viral diagnostic problems are resolved. Superior cell culture systems are needed which are more convenient and which detect viruses even earlier than at present. Something better than infant mice must be found for the isolation of the majority of coxsackieviruses of the A group. Additional biochemical tests will be required to identify viruses so that there will be no need for the hundreds of specific antisera now used in identifying viruses in a never-ending and expensive program of neutralization and complement-fixation tests. Now, with more than 100 common cold viruses, few virologists will any longer serotype, but rather will identify them by their sensitivity to a low pH and resistance to organic solvents. For over 15 years there has been much said about the use of fluorescent antibody methods for early detection of viruses; this technique should be rejected as further enslavement to the need for hundreds of specific antisera.

In the future viral diagnosis will be much like bacterial diagnosis, with virtually no use of serology. In some cases serology will be used because the virus is difficult to isolate, but this will remain a poor substitute for isolation of the pathogen.

As for the significance in disease of any microbe, this should be resolved by the epidemiologist, who could at times do much better in indicating what organisms are pathogens and how often. In some diseases this has been done but in others, as with herpes simplex virus associated with pharyngitis, there is more myth than fact (Herrmann, 1967c). The need for this research should come as no surprise since bacteriologists still are not certain of the significance of *Haemophilus influenzae* in disease (Branson, 1968; Dick & Carr, 1966). There is no way to produce the security everyone wishes when he reports a potential pathogen. The quest for such security by the virologist has almost totally inhibited the advent of useful laboratory diagnosis. Viral diagnosis today is largely an academic exercise. It may be that patients are weary of academic exercises. Patients may also be weary of the physician saying 'it's a virus of some kind going around'. That type of information can be obtained from friends and relatives.

Inevitably the laboratory diagnosis of viral disease must take its place in the routine practice of medicine, beside the laboratory diagnosis of bacterial, mycotic, and parasitic illnesses. This will only happen, however, when viral diagnosis is practiced, like other microbial diagnosis, with some concern for the patient.

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