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Application of immunofluorescence technique to clinical virology

Virologists for many years have striven to achieve the goal of rapid virus diagnosis. This book has described in detail one of the methods. The achievement of success in a method of rapid virus diagnosis cannot, in itself, be a final aim, otherwise the method would degenerate solely into a rapid routine method for identifying virus agents and the virologist would become a biological philatelist. An applied science, to justify itself, must bring benefit to the main clientele, which is in this case the physicians and the patients. In this chapter it is intended to evaluate the applications of rapid immunofluorescence diagnosis in clinical medicine.

MANAGEMENT OF PATIENTS AND RATIONAL USE OF ANTIBIOTICS

There are a number of cardinal features of acute virus infections which need to be emphasized. The presence of viruses at the site of illness usually means that the virus is associated with that illness and is almost certainly causal. There are exceptions to this, such as enteroviruses in stools and adenoviruses both in stools and the respiratory tract, but even here they have caused infection and, because they have the property of being excreted for a long time, may be found at the site after the acute phase has passed. However, in lesions of the skin, central nervous system and respiratory tract, viruses found in the presence of symptoms should be taken as causal. In the respiratory tract, influenza virus, respiratory syncytial virus and the parainfluenza viruses are seldom if ever found without illness. The rapid diagnosis of a virus infection in 2–3 hours after admission to hospital revolutionizes the attitude of physicians. They are no longer dealing with a disease of unknown aetiology for most, if not all, of the patient's stay in hospital but with one which is a precise entity immediately known.

A basic tenet in virology is that a virus is completely insensitive to antibiotics and that virus-infected patients, particularly children, do not as

a rule acquire secondary bacterial infections. The main exception to this rule is influenza infections in a previously damaged lung. This knowledge and confidence has been acquired over a number of years of investigation in joint virological and clinical studies (Elderkin *et al.*, 1965; Holdaway, Romer and Gardner, 1967; Gardner *et al.*, 1967; Aherne *et al.*, 1970).

A physician can now manage his patients with, in most cases, sure knowledge of the aetiology. This should give him confidence that in respiratory viral infections antibiotics will not benefit the patient, and that nursing and general support therapy should form the treatment. In other infections antiviral agents are now becoming available and even in respiratory infections the future holds promise as will be seen presently. The clinician should be well satisfied with this rapid progress which will at worst at least satisfy his curiosity during the acute phase of illness and not retrospectively, and at best indicate a line of specific therapy to pursue.

THE USE OF ANTIVIRAL DRUGS

There is now a vast literature on the use of antiviral drugs; amongst recent books is Gallasso, Merigan and Buchanan (1979). It is not the purpose of this chapter to review this aspect but to draw attention to the role of rapid virus diagnosis in their use. Drugs may be used in two ways: prophylactically or therapeutically. Rapid virus diagnosis may determine quickly the presence of a virus in a community, for instance a school, factory, family, etc. Should a drug be available for prophylactic use then treatment of the uninfected members of the community may prevent the development and spread of an outbreak. One such drug is amantadine hydrochloride which has been used effectively in the prevention of influenza A.

Infections with the herpesvirus group are those which have, at this time, been the most effectively treated with antiviral drugs. Most studies have been undertaken with herpesvirus hominis, which more often than not, causes just minor skin lesions such as 'cold' sores. However, herpesvirus hominis eye infections are not infrequent and those of the brain, though rare, are often fatal. Both these conditions need immediate diagnosis and treatment with one of the antiherpetic drugs now available. Amongst these drugs with antiherpetic action are idoxuridine, cytarabine, vidarabine and acycloguanosine (Collins and Bauer, 1977). The first has been effective when locally applied but is toxic when administered systemically; cytarabine and vidarabine are less toxic but trial evidence of their effectiveness in herpes encephalitis is still equivocal. Acycloguanosine is reputed to be non-toxic and with a very powerful antiherpetic action; its action is directed against viral enzyme only. It could indicate the lines along which certain antiviral drugs may develop. The results of the clinical trials which are proceeding at

the moment must be awaited with reasonable optimism (Schaeffer *et al.*, 1978).

A naturally occurring substance which has wide application as an antiviral agent *in vitro* is interferon. Difficulties in manufacture and standardization have prevented early practical use but successes have now been claimed for clinical use especially in eye infections with herpes zoster (Merigan *et al.*, 1978). Its use in the immunosuppressed patient is discussed on page 292.

By the time a patient manifests the signs and symptoms of illness, virus infection of cells at the site of infection is widespread. Therefore, if antiviral therapy is to stand any chance of success a diagnosis must be made as near to the time of onset of symptoms as possible. It is only at this stage that antiviral drugs can do good by preventing the infection of further cells and thus further replication of virus. This delaying process will allow the normal immunological defences of the body to respond fully and thus terminate what might be an overwhelming virus infection. The most vulnerable groups are therefore the young, perhaps the very old and those who are immunosuppressed. Even when efficient antiviral agents become available their use will still be dependent on accurate rapid virus diagnosis in order to start treatment sufficiently early to contain the infection.

VIRUS INFECTION OUTSIDE ACUTE STAGE OF ILLNESS

Some patients do not come into hospital at the onset of their virus illness but may have been ineffectively treated at home with antibiotics. These patients can still be diagnosed rapidly on admission to hospital by immunofluorescence. It has already been pointed out in Chapter 6 that respiratory syncytial virus may be identified in many patients late in their illness, frequently as late as the seventh day and occasionally as late as the fourteenth day. In the later stages of the illness infected cells have an altered appearance which was illustrated in *Figure 41* (*see* page 115), the fluorescent green colour being dull and the outline of the cell hazy. This is due to a coating of local antibody acquired by infected cells in the respiratory tract during the course of the illness (Gardner and McQuillin, 1978). The number of infected cells in the secretions is also greatly reduced as the patient progresses to convalescence.

At this stage of illness virus is difficult to isolate and in the majority of cases the diagnosis can only be made by immunofluorescence. Table 2 in Chapter 6 (page 115) illustrates clearly the difficulty of isolating virus after the early days of the illness have passed. The coating of cells by antibody, with the subsequent difficulty of virus isolation, does not occur solely with respiratory syncytial virus but with many other respiratory viruses: the parainfluenza viruses and probably influenza A and B viruses. Armed with

a knowledge of this phenomenon and with a developing experience of the appearance of these coated cells, all these respiratory virus infections can be rapidly diagnosed, in many cases late in the illness.

THE USE OF RAPID DIAGNOSTIC TECHNIQUES IN FATAL INFECTIONS

In the UK there are still approximately 2500 deaths per year from respiratory infections, mainly in those under 1 year of age. It would appear from various studies that the majority of these are of viral aetiology (Gardner *et al.*, 1967; Aherne *et al.*, 1970; Scott *et al.*, 1978). The viruses most frequently associated with infant deaths are respiratory syncytial virus, influenza A virus, parainfluenza virus type 3 and various adenoviruses, but recently others such as parainfluenza virus type 1, 4a and 4b have also been incriminated. Measles, too, occasionally causes death in the normal child but is a major cause of death in the immunosuppressed child, who is usually undergoing antitumour therapy (*see* page 292). The majority of respiratory viruses are labile and by necessity most autopsies need to be delayed at least 18 hours, often much longer, in order to obtain the consent of relatives. It has already been pointed out that the ability to demonstrate the presence of virus antigen persists for longer than the ability to detect virus infectivity. Immunofluorescence has therefore become a key tool in the examination of autopsy material for the demonstration of viruses.

Virus deaths in infancy are usually due to bronchiolitis or pneumonia, the latter often being associated with a congenital abnormality. Recently, the presence of viruses has been clearly demonstrated in about 20–25 per cent of 'cot deaths' or the sudden infant death syndrome (SIDS) (Ferris *et al.*, 1973; Scott *et al.*, 1978). In adults influenza A is still the main virus pathogen but, here, especially in those with previously damaged lungs, bacteria may be associated.

The presence of a virus in the lungs at autopsy does not necessarily mean that the virus was the cause of death. It could be present because of contamination from an unrelated infection of the upper respiratory tract. It is therefore essential for all deaths to be investigated by methods other than the isolation of virus in order to substantiate the aetiological role of any virus found. In the studies already quoted pains were taken to use conventional histological investigations of the respiratory tract and supporting evidence was looked for and found in all those autopsies in which virus was identified. Immunofluorescence, too, supplies a second confirmatory tool, for by its use virus antigen can be actually seen in the alveolar and bronchiolar epithelial cells.

Immunofluorescence can therefore be used to investigate all respiratory deaths where the prospect of virus isolation is small. Respiratory deaths in

infancy and occasionally in adults can be sudden and here too immunofluorescence has an important role to play. Many a coroner, faced with unexpected deaths, is grateful for a report within 3–4 hours of autopsy so that other more sinister causes can be quickly eliminated, while the parents of an SIDS may be relieved of the inevitable feeling of guilt that they have experienced.

VIRUS INFECTION IN THE IMMUNOSUPPRESSED PATIENT AND ITS MANAGEMENT

The immunosuppression of children and adults as part of specific therapy is now commonplace and on occasions it may be produced as an unwanted side-effect of many current drug regimens. Included among the drugs frequently used are vincristine, cyclophosphamide, methotrexate, 6-mercaptopurine, prednisone and azothioprine.

In adults these immunosuppressive measures often lead to greater risk of reinfection or reactivation with the herpes group of viruses. They cause a wide variety of bizarre clinical conditions ranging from ocular complications to death (Porter *et al.*, 1972). Cytomegalovirus is the principal culprit but herpesvirus hominis and varicella-zoster are also frequently involved. A rapid diagnosis is urgently required as survival of graft, retina or life itself depends on achieving a fine balance between the treatment and the virus infection. Moreover the increasing efficiency of the antiherpes drugs and the possible availability of interferon for clinical use in the future makes early diagnosis essential.

The major reason for children becoming immunosuppressed is the intensive and prolonged therapy required for the treatment of acute lymphatic leukaemia. Therapy not only includes the above-mentioned drugs but also radiation. Recent months have seen the development of techniques for the grafting of bone marrow transplants after whole-body irradiation. These measures have proved highly successful as regards the primary condition which now can, in many instances, be cured. However, during the treatment the child is left highly vulnerable and susceptible to infection. Infection has therefore become the major cause of death in acute lymphatic leukaemia, the majority of these infections being associated with viruses. The viruses involved are rhinoviruses, measles, mumps, influenza, respiratory syncytial virus and the other everyday viruses which normal children easily combat. However, children with leukaemia and under therapy can die of rhinovirus pneumonia, and most children who are infected with measles during the course of their leukaemia also die. In a defined study of virus infections in childhood leukaemias, their importance in morbidity and mortality has been clearly demonstrated (Craft *et al.*, 1977, 1978, 1979). The numbers of patients both in remission and relapse in whom viruses were associated with death are indicated in Table 15. A distinct feature of infectious illness in the immunosuppressed child is its

Table 15

DEATHS IN ACUTE LYMPHATIC LEUKAEMIA AND THEIR VIRUS ASSOCIATION

Deaths in relapse			*Deaths in induction or final remission*		
Total	*With infections*	*Associated with virus*	*Total*	*With infections*	*Associated with virus*
9	9	5	15	15	7

unusual symptomology. Measles is especially severe, often fatal, but seldom has a rash; respiratory syncytial virus, less often seen in childhood after 1–2 years of age, causes severe and prolonged symptoms in the older immunosuppressed child. On many occasions, onset of a virus infection can be sudden and severe, with death rapidly intervening. Rapid diagnosis in these circumstances has become a vital procedure; it is a guide both to the amount of immunosuppressive therapy which should be given at this time and to specific antiviral treatment needed.

The coating of infected epithelial cells by locally produced antibody in the normal infected child has already been discussed but the position is often very different in the immunosuppressed child. Depending on the stage of the illness and the treatment regimen, many of these children are incapable of producing an antibody response either locally in the respiratory tract or humorally. Infected cells therefore continue to be excreted for long periods of time and their appearance, when stained by immunofluorescence, is indistinguishable from that of a normal child's cells in the first day or two of an acute illness. *Figure 77* illustrates respiratory syncytial virus-infected cells in the secretions of a child aged 4 years with acute lymphatic leukaemia and a severe bronchitis which lasted for 64 days. It shows the number and brightness of respiratory syncytial virus-infected cells at the thirty-fifth day of illness—there is no evidence whatsoever of coating.

Immunofluorescence has also become an essential tool for diagnosing an infection which is not resolving, for controlling chemotherapy and for determining prognosis. Measles is particularly difficult to diagnose in the immunosuppressed child because of its very varied picture. It is possible that early treatment with interferon might alter what has, up till now, been a very gloomy prognosis; the use of rapid diagnosis and interferon therapy in measles is currently being investigated in a multicentred study. However, the key to any successful chemotherapy must lie in early diagnosis, and the screening of secretions for respiratory viruses even in mild infections should include a test for measles. Immunofluorescence enables this to be done. In the normal child with measles, infected cells, including giant cells, are rarely seen after the third day of rash (McQuillin *et al.*, 1976). The

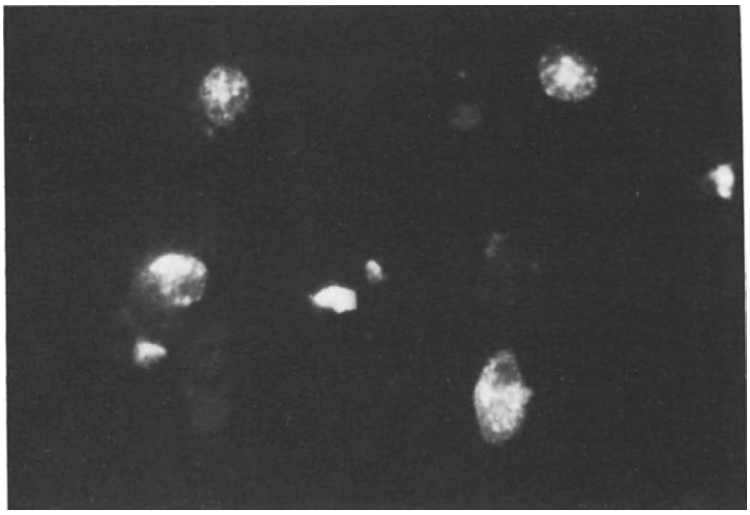

Figure 77. RS virus antigen in cells of a nasopharyngeal secretion, stained by the indirect fluorescent antibody technique. From a child with acute lymphatic leukaemia on the thirty-fifth day of infection with RS virus. Magnification × 600

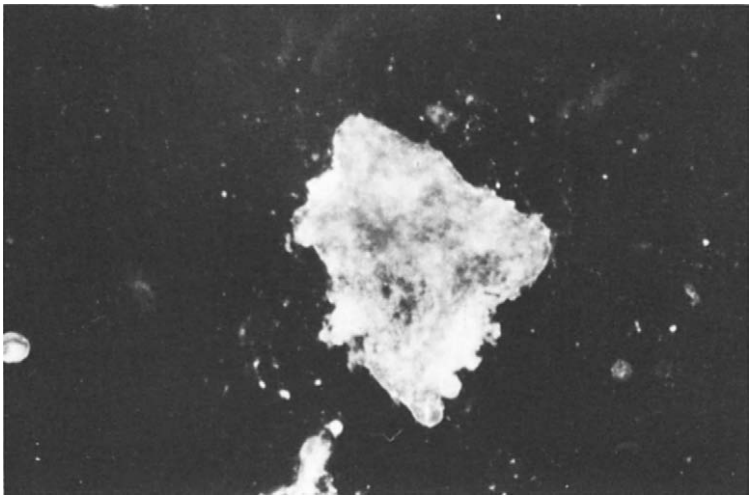

Figure 78. Measles antigen in a giant cell in a nasopharyngeal secretion, stained by the indirect fluorescent antibody technique. From a child with acute lymphatic leukaemia on the twenty-first day of measles infection, complicated by pneumonia. Magnification × 600

picture is totally different in the immunosuppressed child with measles. In these circumstances the secretions are loaded with giant cells which continue to be excreted, usually until the child dies (Pullan *et al.*, 1976). These virus-loaded giant cells are far more numerous and observed for a far longer period than those which occur in the normal child even at the height of his

rash. *Figure 78* illustrates the appearance of such a giant cell on the twenty-first day of a measles illness that had become complicated by the development of an interstitial pneumonia. *Plate 18* illustrates a giant cell in a lung impression smear of a leukaemic child who died with measles pneumonitis. There is every hope that the immunofluorescent techniques will not only simplify the diagnosis of these cases but be a guide to the effect of interferon in the ongoing coded trials.

As long as immunosuppressive therapy remains an integral part of medical treatment, rapid virus diagnosis must be relied upon as the method of controlling the devastating illnesses caused by relatively innocuous viruses which now prevent the full realization of the improving prognoses in childhood malignancy.

FREQUENTLY OCCURRING VIRUSES PRODUCING UNUSUAL SYMPTOMS

In the previous section it was emphasized that ordinary virus infections may, in the immunosuppressed patient, present with unusual clinical features which may make diagnosis difficult. Frequently occurring viruses in normal children may produce unusual symptoms and therefore be difficult to identify; rapid diagnostic methods in these circumstances may be invaluable. Three of these viruses will serve to illustrate this problem.

Measles usually commences with a severe coryza although the rash may not appear for a few days. It is important to exclude these children from the community at this highly infectious stage. This can easily be achieved by immunofluorescent methods (McQuillin *et al.*, 1976). Measles rashes may be modified by prophylactic vaccines and, moreover, be difficult to recognize in the coloured child. The latter point is of great importance in developing countries where measles can be a devastating illness and in the UK where there has been a substantial increase in the coloured child population.

Influenza A is present in the community most winters and frequently reaches epidemic proportions. Normally the diagnosis of influenza A is self-evident with a symptomatology which all have experienced. The child cannot usually complain in a succinct manner though it is now known that he is frequently infected by the virus. The most frequent form of presentation for influenza A in the young child is a febrile convulsion, a symptom still not considered of viral origin by many physicians because respiratory symptoms and signs are often minimal or absent. Immunofluorescence can diagnose influenza A in the cells of these childrens' secretions very readily (Brocklebank *et al.*, 1972).

Similarly, abdominal pain in childhood may be of viral origin. The association of this symptom with influenza B infection was originally noted by Kerr *et al.* (1975). In a similar manner to influenza A in febrile

convulsions, respiratory symptoms may be minimal. When it is known that influenza B is prevalent rapid immunofluorescence diagnostic techniques could prevent unnecessary appendicectomies.

VIRUSES DIFFICULT TO CULTIVATE IN THE LABORATORY

Many viruses are difficult to culture in the laboratory and one example—measles—has been considered already. In measles, rapid immunofluorescent diagnosis is simple and highly sensitive from 2–3 days before the eruption to 2–3 days afterwards; this technique has therefore become the method of choice.

Parainfluenza virus types 4a and 4b are slow growing and the conditions for the haemadsorption technique, necessary for their screening, variable. Perhaps for these reasons their identification in the laboratory falls below predictions based on serological evidence of their presence in the community. Their diagnosis by directly staining virus-infected nasopharyngeal cells by immunofluorescence is easily accomplished, provided suitable reagents are available, and should prove to be the method of choice.

Similarly, coronaviruses probably play an important role in human respiratory infection but their cultivation and identification at this stage can only be considered a research procedure because of the cumbersome and insensitive techniques available. Antisera were prepared against two of these viruses known to be associated with human illness, OC38 and 229E, and used to identify these agents in cells shed from the respiratory tract of human volunteers who had been previously inoculated with them. *Figure 79* shows the appearance of a cell infected with OC38 in an NPS. Virus-infected cells were only found in those volunteers with symptoms (McIntosh *et al.*, 1978). These experiments clearly demonstrate that the now routine method of identifying respiratory viruses by immunofluorescence in cells shed into the respiratory tract would be suitable for coronaviruses and is at this stage the only effective method for their rapid diagnosis.

The new group, rotaviruses, is now known to be responsible for 40 per cent of acute diarrhoea in infants and children (Birch *et al.*, 1977).

The diagnosis of infection by this virus is usually made at present by electron microscopy, enzyme-linked immunosorbent assay (ELISA) or immunofluorescence. All three methods are of similar sensitivity. However, the first method enables the virus to be identified within an hour but an electron microscope is not available to all nor is it particularly suitable for handling large numbers of specimens. Although the virus has not as yet been cultured it is known to penetrate tissue culture cells and give one abortive cycle. Tissue cultures may be so infected and examined the following day by immunofluorescence. This is illustrated in *Figure 80*. The sensitivity of the method may be enhanced by centrifugation of faecal

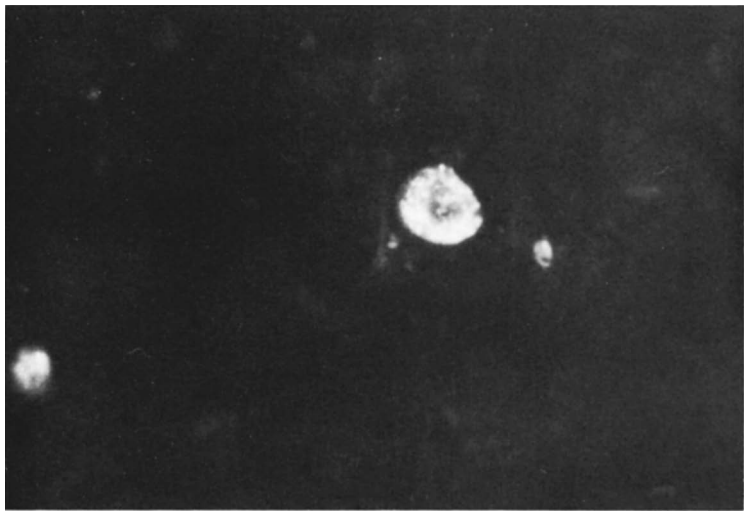

Figure 79. Coronavirus OC38 antigen in a cell of a nasopharyngeal secretion, stained by the indirect fluorescent antibody technique. From an experimentally infected volunteer at the Common Cold Research Unit, Salisbury. Magnification × 930

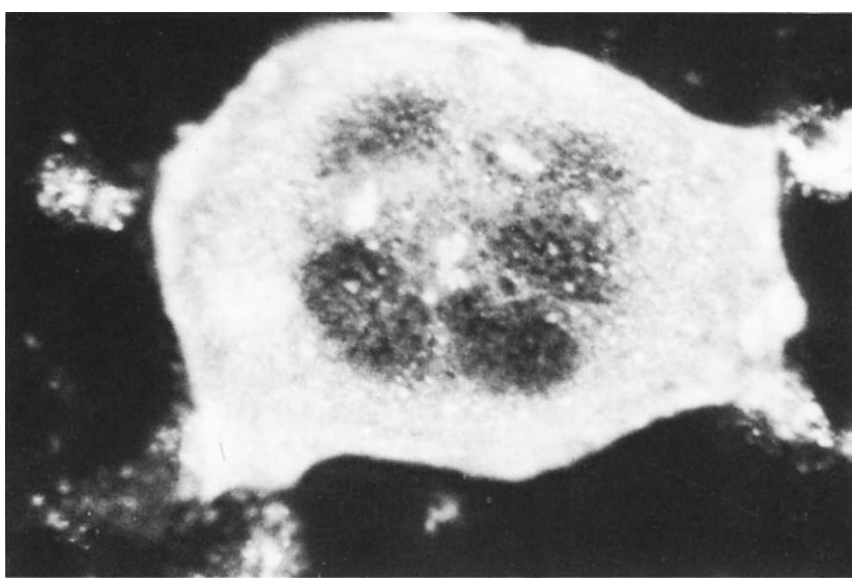

Figure 80. Human rotavirus antigen in a multinucleate giant cell in trypsin-treated LLC-MK tissue culture cells stained by the indirect fluorescent antibody technique. From the faeces of a child with gastroenteritis. Magnification × 990

supernatant on to the tissue culture cells at low speeds (Banatvala *et al.*, 1975); the presence of trypsin has also made the method more sensitive by increasing the number of infective centres in the first cycle (Moosai *et al.*, 1979).

The diagnosis of hepatitis B can now be made rapidly and effectively by many techniques and immunofluorescence has no part to play at this stage. However, though rarely a matter of rapid diagnosis, liver biopsy material can be effectively examined by immunofluorescence (Brzosko *et al.*, 1973). Until the viruses of hepatitis A, non-A and non-B are more clearly defined it is difficult to see a role for immunofluorescence in their diagnosis.

The examples only serve to illustrate how immunofluorescence may be used to identify viruses for which current culture techniques are inefficient and at the same time provide the clinician with a rapid aetiological diagnosis.

PREVENTION OF WARD CROSS-INFECTION WITH VIRUSES

From personal observations even without planned scientific studies it is well known that if a patient with a respiratory infection is placed next to another patient, the latter will often acquire the infection. This is especially true for young children who have not had time to develop immunity to the various frequently occurring virus infections. A defined study was carried out in a number of paediatric wards to obtain information as to which viruses were involved, the severity of these 'cross-infection illnesses' and the chances of acquiring such infections. These studies have been fully reported elsewhere and gave valuable information about a number of viruses, namely respiratory syncytial virus, influenza A and the parainfluenza viruses (Ditchburn *et al.*, 1971; Weightman, Downham and Gardner, 1974; Sims *et al.*, 1975). For the first time immunofluorescent techniques were used in such studies to define these agents and examine staff and well patients for carriage of virus. Table 16 clearly shows the enormity of the problem, over a 4-year period.

Table 16

RECORDED CROSS-INFECTIONS OVER A 4-YEAR PERIOD

	RSV	*Influenza A*	*Parainfluenza*	*Total*
Upper respiratory tract infection	41	38	27	106
Severe lower respiratory tract infection	47	10	15	72
Total	88	48	42	178

RSV = respiratory syncytial virus
(From Gardner (1976). Reproduced by permission of the Editor of *J. gen. Virol.*)

Respiratory syncytial virus infections were the most severe and one notes with dismay that more than half those acquiring infections had severe lower respiratory tract illnesses which required intensive therapy. The position was in fact far worse as all the severe infections occurred under the age of 1 year and in this age range the child who became infected stood a 70 per cent chance of acquiring a very severe lower respiratory tract infection, usually bronchiolitis. Anecdotes may help to emphasize the problem. An infant aged 3 months, immobile because of a fractured femur, was nursed in a two-bedded cubicle and, during the winter respiratory rush, a child with a rapidly diagnosed respiratory syncytial virus croup was admitted to the free bed, which was the only one available. Within 5 days the patient with the fractured femur had acquired a proven respiratory syncytial virus bronchiolitis by cross-infection.

Influenza A

Influenza A also plays an important role in cross-infection. It would appear from Table 16 that it is relatively unimportant with fewer severe lower respiratory tract infections. In fact, the latter illnesses were all pneumonias and a number of these patients died. The patients with influenza A infection admitted to hospital are usually older than those admitted with respiratory syncytial virus infection. The usual reason for admission is a febrile convulsion. Frequently by the time they have been admitted to hospital they have recovered from their convulsion and have lost their fever. Being relatively well at this stage, they run around the ward coughing and secreting virus over patients who can ill afford to acquire infections, such as patients with mucoviscidosis, congenital heart disease or leukaemia or on immunosuppressive therapy. It is the latter group of patients who fare badly when acquiring influenza A. Unfortunately, toddlers are not respecters of cubicles, which to them are no barrier. It is essential that ward staff realize that febrile convulsions in 50 per cent of cases can be of viral origin and when influenza A is prevalent the percentage of febrile convulsions due to this cause and admitted to hospital rises to very much higher than average (Stokes *et al.*, 1976). They must also realize the serious consequences of cross-infection in debilitated children. Rapid diagnosis by immunofluorescence has provided a method for identifying influenza A within 3 hours of admission to hospital. Now that the risks of influenza A cross-infections are known, every child with a febrile convulsion admitted to hospital when influenza A is prevalent must be examined and also confined to a cubicle until the result of the rapid diagnostic test is available. It is only in this way that tragic deaths from influenza in debilitated children can be avoided.

Parainfluenza virus is also spread easily and may cause severe lower respiratory tract infection; once again the severest infections are in

debilitated children. The same principles of diagnosis and segregation are true for this group. The toddler will again cause the problems though he is not usually as well on admission as the child following febrile convulsion; the predominant cause of admission with this group of viruses will be croup.

VIRUS DIAGNOSIS IN AREAS DISTANT FROM A VIRUS LABORATORY

Slides dried and fixed for staining by the fluorescent antibody technique are suitable for transporting over long distances. If preparations of secretions, scrapings, biopsies and post-mortem material, etc. are dried and fixed on slides in the usual way they can be sent to a virus laboratory to be diagnosed by immunofluorescence. Minimal resources are required at the originating centre where staff can readily be trained in the production of satisfactory slide preparations.

A scheme was initiated some years ago with the Medical Research Council Nutritional Unit in Uganda. A small batch of slides arrived by air within 18 hours and 1 child with pneumonia was shown to have a parainfluenza virus type 3 infection. *Figure 81* shows how a slide of a specimen which has been well prepared and well fixed can be stained satisfactorily by immunofluorescence after having travelled a long

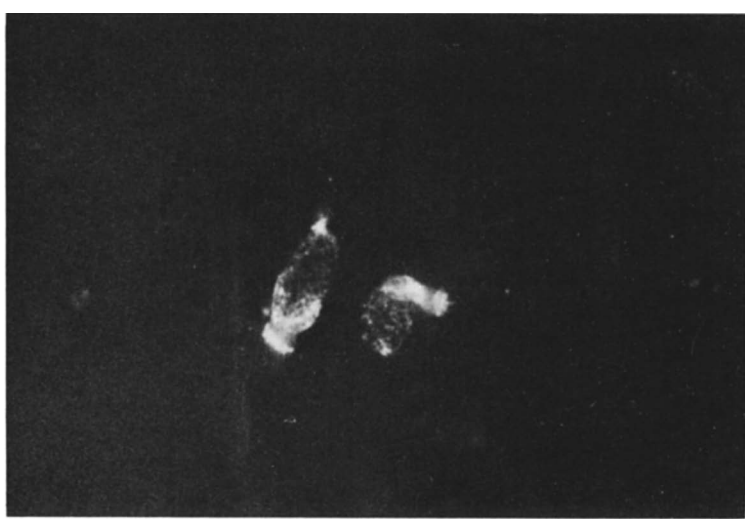

Figure 81. Parainfluenza virus type 3 antigen in cells of a nasopharyngeal secretion, stained by the indirect fluorescent antibody technique after delivery to Royal Victoria Infirmary, Newcastle upon Tyne, from Uganda, by air transport. From an infant with pneumonia in Uganda. Magnification ×*930*

distance. For obvious reasons this planned investigation came to an abrupt end in Uganda but occasional specimens still arrive from tropical countries for diagnosis, for example 5 out of 8 specimens from children with pneumonia in Kenya proved to be influenza A.

These successes inspired us to attempt a similar study in an area closer to home without a virus diagnostic service (Downham *et al.*, 1974). West Cumbria, a rural area, has a single hospital admitting all children from that region of the country. All children admitted with respiratory illness were examined and specimens taken by ward staff and sent to the local pathology laboratory which had no virus facilities. Staff there prepared the slides and fixed them, dispatching them to Newcastle where they were examined. Table 17 shows the results of the study. All the respiratory viruses found in conurbations were also found in West Cumbria causing the same illnesses.

Table 17

RESULTS OF RESPIRATORY VIRUS DIAGNOSTIC SERVICE BY POST WITH WHITEHAVEN HOSPITAL

Dates	*RSV*	*Inf A*	*Inf B*	*Para 3*	*Para 2*	*Para 1*	*Adeno-virus*	*Negative*	*Total*
1.12.72–31. 5.73	33	11	–	–	1	1	–	40	86
1. 6.73–30.11.73	–	–	–	5	–	–	–	22	27
1.12.73–31. 5.74	18	8	3	5	–	–	2	49	85
1. 6.74–30.11.74	–	–	–	2	1	–	–	38	41
1.12.74–31. 5.75	6	7	–	–	3	2	–	52	70
1. 6.75–30.11.75	2	–	–	–	–	–	1	17	20
1.12.76–31. 5.76	18	7	1	2	–	–	–	20	48
Total	77	33	4	14	5	3	3	238	377

(Reproduced from Heath, R. B. (Ed.) (1979), *Virus Diseases*, by kind permission of Pitman Medical Company Ltd.)

However, their incidence was 2–3 times less. Specimens were sent by post and always reached Newcastle within 24 hours of dispatch. They were examined on arrival and the result telephoned the same day. This proved very satisfactory for an area which previously had no virus diagnostic service and indeed under the circumstances could still be considered as rapid virus diagnosis.

RAPID DIAGNOSIS IN EPIDEMIOLOGY

Virus epidemiological studies in the community are difficult and expensive to perform. Collection of specimens, their transportation under suitable

conditions to the laboratory for virus isolation and the sampling of all illnesses from the most trivial to the most severe in all age groups and both sexes have proved to be the chief stumbling blocks. To obtain some idea of the virus epidemiology of an area, the total admission of patients to the hospital covering that area can be investigated. Hospital admissions, however, can only reflect the viruses present in the community as only the most severely ill patients are admitted, but nevertheless much useful information can be obtained. Many such surveys have been carried out both in Newcastle and in various parts of the country (Report by the Medical Research Council, 1978); the most recent report gave valuable information on the occurrence of epidemics of respiratory syncytial virus, influenza A and B and parainfluenza viruses over a 6-year period, the interrelationships of these viruses and the effect of climatic conditions, and admission rates to hospital (Martin, Gardner and McQuillin, 1978). The use of immunofluorescence for rapid virus diagnosis made possible the investigation of a greater number of patients and the diagnosis of a wider range of viruses than could be achieved by routine methods. The study was based on total hospital admissions and included 2311 identifications by immunofluorescence of respiratory syncytial virus, parainfluenza viruses 1, 2 and 3 and influenza A alone.

Further knowledge of community virus epidemiology might well be obtained by using the immunofluorescence technique of collection and transportation as outlined for specimens coming from a distance. The specimens could be prepared in a small side room in the general practitioner's surgery with a nurse or health visitor collecting the specimens. If the general practice is near the hospital the secretion itself, transported on melting ice, could be taken to the laboratory—the time scale here is less critical if isolation of viruses is not attempted and only specific antigen is being identified.

A further epidemiological use of the fluorescent antibody technique can be confidently anticipated for assessment of live respiratory vaccines. Great difficulties have been experienced in making a live respiratory syncytial virus vaccine so urgently needed for infant protection. Control of such a vaccine, when produced both in the investigational stage and the practical stage as regards infectivity, spread and length of time of excretion, will prove to be invaluable.

Other respiratory vaccines such as influenza A are already available and their control will similarly be simplified by these techniques.

The next few years may see the development of a new class of highly specific monoclonal viral antibodies. Minor degrees of antigenic variation might be detected directly for viruses in patients' secretions when changes occur in current antigen types. The method might also be adapted to differentiate between vaccine and wild virus strains. The future is alive with many exciting epidemiological developments employing these newer methods.

IMMUNOFLUORESCENCE AS A LABORATORY AID

It has already been stated that wide-scale epidemiological studies on respiratory virus infections would have been almost impossible if every virus needed to be cultured and identified by conventional means. Virus culture, however, still takes place mainly when the specimen submitted is unsuitable for immunofluorescence, for example when there are too few cells in the secretion or a throat swab has been submitted instead of a secretion. Under these circumstances, an agent causing a suspicious cytopathic effect can be immediately identified by immunofluorescence by scraping the cells off the culture tube, placing them on to a slide and staining in the usual way. This identifies the agent immediately without the necessity for a long and unsatisfactory neutralization test such as for respiratory syncytial virus or for a fastidious haemadsorption inhibition test for the identification of a haemadsorption virus.

Since the initial description of immunofluorescence by Coons, Creech and Jones in 1941 the method has evolved from an academic technique of unreliable clinical value to a routine bedside investigation. The progress from a novelty to routine has been rapid, upholding the aphorism 'nothing ages more quickly than novelty'.

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