Epidemiology

LABORATORY DIAGNOSIS OF VIRUS INFECTION IN THE PUBLIC HEALTH LABORATORY SERVICE

(The following notes are compiled from articles in the April and August numbers of the Monthly Bulletin of the Ministry of Health and the Public Health Service, and passages in the articles are reproduced with the kind permission of the Controller of Her Majesty's Stationery Office.)*

The Public Health Laboratory Service was originally built up as a bacteriological service, but, as a result of the increased interest in virus disease and developments of techniques which make their study possible, a virological service has now been included and most public health laboratories are now undertaking the diagnosis of virus infections and should be consulted when any problem arises. A number of new techniques have been introduced in recent years by means of which some well-known viruses such as poliomyelitis are now much more readily dealt with. In addition several new groups of viruses have been isolated and incriminated as the cause of diseases in man, especially in the respiratory tract and central nervous system. Although this new work has given satisfaction by explaining the aetiology of certain clinically recognizable diseases, it has not completely solved the problem of the aetiology of various syndromes, such as influenza-like illness, febrile catarrh and benign aseptic meningitis. Indeed, some of the problems have become more complex, for example, numerous viruses have been isolated from apparently healthy persons, and knowledge is still being sought about the normal virological flora of the throat and gut. No virus has been isolated in the laboratory from glandular fever, infectious or serum hepatitis, rubella, trachoma, inclusion conjunctivitis, cat-scratch fever, or " winter vomiting disease."

Investigation

For the investigation of those virus or rickettsial diseases which can be dealt with in the laboratory, two types of test may be used: serological—mainly complement-fixation—and virus isolation in fertile hens' eggs, tissue cultures or animals.

Serology. Complement-fixation with known antigens and the patient's serum is likely to remain the mainstay of laboratory diagnosis until more simple and rapid means of detecting viruses or their antigens in the acute stage of the disease are developed. It is much simpler and cheaper to perform than is virus isolation.

*Reprints of the article " A Guide to the use of the Laboratory in the Investigation of Suspected Infections of Man," from the August number of the Monthly Bulletin of the Ministry of Health, may be obtained on application to the Directors of Public Health Laboratories.

The significance of the results for individual patients can only be assessed with confidence when a rise in antibody titre can be shown between a serum taken not more than five days from the onset, and a second, 12—15 or more days from the onset. Single sera are of no value in the acute stage and of doubtful value in convalescence, except in studying an outbreak in a family or community.

There are complement-fixation tests for Q fever, psittacosis, lymphogranuloma venereum, influenza A, B and C, adenoviruses (APC), Sendai virus (a newly recognized virus causing respiratory infection), mumps, and lymphocytic choriomeningitis.

Agglutination tests with Streptococcus M.G. are used to identify a section of the cases of atypical pneumonia for which the cold agglutination test is also suitable.

Virus isolation is an expensive and laborious process compared to that of bacteria. The generally short period of persistence of viruses on tissue surfaces and in excreta, and the lability of the viruses in various excretions, secretions, body tissues and fluids at atmospheric temperature necessitates particular care in the selection of patients suitable for investigation and in the transport of the specimens to the laboratory.

(a) Isolation in fertile eggs. Various tissues of the intact, fertile hen's egg are required for the isolation of smallpox, vaccinia and influenza viruses. This work is only done in a few laboratories because of the extra equipment and space required for holding and maintaining enough eggs of the required age of incubation. The viruses are selective in the age of the embryo as well as the particular tissue in which they grow best. Even under the most favourable conditions it takes at least three days to isolate and identify an influenza virus and the best isolation rate will not be greater than 70 per cent.

(b) Isolation in tissue culture. The obvious requirement is cells which are self-propagating. The cells most readily handled at the present time and about which most is known are derived from the HeLa line (from an epidermoid carcinoma of the cervix) and these are suitable only for the isolation of poliomyelitis and adenoviruses, and one or two others. They are now being used in most laboratories of the P.H.L.S.

Other types of tissues such as monkey kidney and human embryo can be used for the isolation of some additional viruses, for instance Coxsackie and Echo (enteric cytopathogenic agents of human origin).

The present culture techniques are time-consuming and laborious, and usually demand the attention of the most highly trained members of the staff. It may take between seven and fifteen days to obtain a result in a suspected case of poliomyelitis, although a positive result may be obtained in a shorter time, and for other viruses between three days and one month may be required.

In considering which cases to investigate experience has shown that the investigation of a single patient believed to be suffering from an unknown virus infection is almost always a waste of time, whereas the investigation of an outbreak in which there is clear evidence of an infectious agent is often worthwhile.

Collection of Specimens

Because of the varying conditions existing in different laboratories, the local public health laboratory should be consulted whenever there is doubt as to its ability to carry out the necessary tests, and whenever advice is required on the best method of laboratory diagnosis.

All specimens should be collected and transported in clean, and when possible, sterilized containers. Containers and receptacles used in collection (e.g., bed pans) *must not* contain even small amounts of disinfectant. Containers should have screw-on lids—with washers if fluid is enclosed—and should not be more than half full, particularly when packed in ice.

Serological tests. Two samples are required, one in the acute stage and one ten to fourteen or more days from the onset. Because, in many clinical syndromes it is necessary to examine for serum antibodies against an increasing number of viruses, it is best to try and obtain *5 to 10ml. of blood.* Blood should be taken with a clean, dry, sterile syringe and needle, or needle only if necessary. Syringes sterilized by boiling must be completely dry or rinsed in sterile saline before use. Blood-tinged serum is unsatisfactory for complement-fixation tests. Blood should be placed in a clean, dry, sterile bottle, with screw-on lid with washers. Test-tubes with bungs may be used, but the latter must be fixed with tape or wax. Do not use tubes with cotton-wool plugs or corks. Venules are satisfactory.

The blood should be kept in a cool place—*not frozen*—and sent to the laboratory by post or hand within 24 hours of collection.

NOTES ON INDIVIDUAL DISEASES

Adeno-pharyngeal-conjunctival (APC) Infections

Causative viruses. The family of adeno-viruses, of which 17 serological sub-types have been recognized so far.

Illness in man. A variable, though generally small, proportion of patients with influenza-like illness, febrile catarrh or atypical pneumonia have evidence of infection with these viruses. Some sporadic cases of catarrhal or follicular conjunctivitis and some epidemics of keratoconjunctivitis are due to members of this group of viruses. There is also a suggestion that they may be responsible for certain lymphadenopathies—e.g., some cases of mesenteric lymphadenitis. When fever, follicular conjunctivitis, pharyngitis, rhinitis and cervical lymphadenopathy are present together, the syndrome has been termed *pharyngo-conjunctival fever.* APC viruses have also been associated with cases of aseptic meningitis.

Distribution of virus in man and duration of excretion. Viruses of various serotypes have been isolated during the acute stage of illness from the nasopharyngeal secretions and the faeces of patients with respiratory illness, from the conjunctival secretions of patients with conjunctivitis, from the excised adenoids and tonsils or other lymphoid tissue of " normal " children and from the stools and throats of certain cases of aseptic meningitis.

Isolation and identification. From pharyngeal washings, nose and throat swabs, conjunctival secretions or faeces, by inoculation of HeLa cell tissue cultures. The virus is identified at first by the formation of group-specific, complement-fixing (C.F.) antigen and later by serological typing (when required for epidemiological or other purposes).

Serological diagnosis. By an increase in titre of C.F. antibody during the course of illness. As the C.F. antigen is common to the whole group of APC viruses, the use of the C.F. reaction largely avoids difficulties caused by the numerous serological sub-types. C.F. antibody first appears at 10—15 days and rises to a maximum by the 20th—30th day. Acute and convalescent-phase sera are particularly desirable, as residual antibody from past infection is common.

Coxsackie Virus Infections

Causative viruses. Group A—more than 20 immunological types of virus. Group B—five types of virus.

Illness in man. Group A—aseptic meningitis, herpangina. Group B—aseptic meningitis, epidemic pleurodynia (Bornholm disease), acute myocarditis in infants and possibly adults. These viruses may be isolated from healthy persons.

Distribution of virus in man and duration of excretion. In pharynx and cerebrospinal fluid during early stage of illness; faeces for a variable period.

Isolation and identification. From throat washings or throat swabs, cerebrospinal fluid and faeces.

Group A—suspension into new-born mice intraperitoneally or subcutaneously. This requires a minimum of three to five days. A few types may grow in tissue culture but the method is less reliable than mouse inoculation.

Group B—suspension into new-born mice, which requires a minimum of three to eight days, or into tissue culture which takes a minimum of three to six days.

Identification by the use of a specific antiserum in neutralization tests against each type of virus.

Serological diagnosis. By an increase in antibody titre during the course of illness. With most Group A viruses in mice, this is only feasible when the causative virus has been first isolated and typed; it is not a practicable routine procedure. Neutralization tests with Group B viruses are practicable in tissue culture.

Echo Virus Infections

(Enteric cytopathogenic human orphan viruses. Viruses other than poliomyelitis, APC and Coxsackie viruses, isolated from alimentary tract or cerebrospinal fluid in tissue cultures only, not in animals.)

Causative viruses. At least 18 immunological types.

Illness in man. Benign aseptic meningitis (sometimes in epidemic form) and other poorly defined illnesses. Many strains have been isolated from healthy persons, particularly children.

Distribution of virus in man and duration of excretion. In pharynx and cerebrospinal fluid during the early stage of illness; in faeces for a variable period up to several weeks.

Isolation and identification. From throat washings, throat swabs or cerebrospinal fluid by the inoculation of bacteria-free faecal suspensions or throat washings in tissue culture. These viruses have been found to cause cell degeneration of monkey or human kidney and possibly human amnion cells, but not HeLa cells, on primary isolation. Isolation takes a minimum of two to five days. Identification by use of a specific antiserum against each virus type. A neutralization test takes at least two days.

Serological diagnosis. By an increase in antibody titre during the course of illness. Neutralization tests are at present mainly used. Complement-fixation tests are being developed. These tests are feasible in a particular outbreak only when the causative agent has been isolated and identified previously.

Herpes Simplex

Causative virus. One (possibly several closely related strains).

Illness in man. Vesicular eruption on skin and mucous membranes, acute stomatitis in infants. Acute systemic disease in newborn; rash may be absent, jaundice may be present (usually fatal). Conjunctivitis and keratoconjunctivitis. Encephalitis and aseptic meningitis.

Distribution of virus in man and duration of excretion. In skin lesions, eye and mouth, (faeces); occasionally in cerebrospinal fluid and brain; in organs in generalized systemic disease, usually in infants. During acute stage of illness only, except in fatal cases.

Microscopic examination. Smears from vesicles may show multinucleated giant cells, which are also found in chicken-pox.

Isolation and identification. From vesicle fluid and scraping of base of skin lesions, swabs of eye lesions and throat ulcers, cerebro-spinal fluid, (faeces), in the acute stage. From brain, liver and spleen in fatal cases. Inoculation of chorio-allantoic membrane (C.A.M.) of fertile hen eggs; intracerebral or intraperitoneal inoculation of new-born mice; tissue cultures of various cells—virus isolated in two to five days. Appearance of typical lesions on C.A.M.; typical intranuclear inclusions in infected tissues; neutralization test on C.A.M. and in tissue culture with specific antiserum.

Serological diagnosis. By an increasing antibody titre during the course of illness in *primary infections.* Complement-fixation and neutralization tests in eggs, mice or tissue cultures. Sera to be collected at onset and seven to ten days later. Antibody tends to persist after primary infections and serological tests will then not show rising titre, i.e., will not afford a specific diagnosis. Virus isolation is essential for diagnosis of recurrent lesions.

Influenza

Causative viruses. Viruses A, B and C. (Newly recognized Sendai or " New-born pneumonitis " virus also causes mild " influenza.")

Illness in man. Typically a fever with constitutional disturbance which is at first disproportionate to the respiratory illness; but mild cases are common.

Distribution of virus in man and duration of excretion. Viruses are commonly isolated from pharyngeal washings, nose and throat swabs, or sputum collected during the first two to three days of illness or, in rapidly fatal cases, from tracheal washings or epithelium, or lung.

Isolation and identification. From pharyngeal washings or nose and throat swabs, sputum during life, and lung in fatal cases, by inoculation of fertile hen eggs and cultures of certain human and monkey tissues. Result available in five to ten days depending on the number of passages required. Identification by serological means.

Serological diagnosis. By an increase in the titre of complement-fixing (C.F.) or haemagglutinin-inhibiting antibody (H.A.I.) during the course of illness. C.F. antibody begins to appear at 10—15 days and reaches its maximum titre some 15—30 days after onset of illness.

Pneumonia—"Atypical" or "Virus"

Causative organisms. This is a syndrome which may be due to infection with the following viruses or a rickettsia: psittacosis-ornithosis viruses; influenza viruses; adeno-viruses; *Rickettsia burneti*; a virus described by Eaton and his colleagues and associated with the development of agglutinins to human group O red blood cells at 4°C. and to *Streptococcus* M.G.; and, much less commonly, the viruses of lymphocytic choriomeningitis and herpes simplex and the agent responsible for glandular fever.

The disease may be simulated by pulmonary tuberculosis, by certain fungal infections, by toxoplasmosis, and by the pneumonias that sometimes complicate the acute specific fevers—measles, chicken-pox and smallpox. Other viruses may be implicated in the pneumonias of infancy.

Illness in man. A presumptive clinical diagnosis is usually made when the patient has a febrile illness with pneumonia and none of the common bacteria has been isolated.

Serological diagnosis. As this is a clinical syndrome, the diagnosis is made by serological tests for each of the possible causative organisms. Isolation of Eaton's virus is not a practicable procedure at present, but lung from fatal cases should be sent to the laboratory frozen in a special container.

Apart from the specific complement-fixation tests for the various organisms mentioned above, the cold haemagglutinin and *Streptococcus* M.G. agglutinin reactions may be performed. Sera for the cold haemagglutinin reaction must be separated from their blood clots at 37°C. to avoid absorption of agglutinin by the red blood cells. Sera can be stored at 4°C. or better still, frozen at —20°C. but can be sent through the post in the unfrozen state. Sera for the *Streptococcus* M.G. reaction should not be inactivated by heating, as this reduces the agglutinin titre. With both reactions there should be an increase in agglutinin titre during the course of the illness; agglutinins appear during the second week of illness and rise to a maximum by the second to fourth weeks.

Poliomyelitis

Causative viruses. Three immunological types of virus, designated 1, 2 and 3.

Illness in man. Paralytic and non-paralytic poliomyelitis, "aseptic meningitis," polioencephalitis.

Distribution of virus in man and duration of excretion. In pharynx during the late incubation period and the first day or two of illness; faeces, from the onset of illness for a variable number of days or

weeks with maximal amounts within the initial seven to ten days of illness; blood, a viraemic stage may occur some days before the onset of paralysis.

Not found in cerebrospinal fluid. In spinal cord in fatal cases.

Isolation and identification. From throat washings, throat swabs and faeces, by the inoculation of bacteria-free faecal suspensions or throat washings in tissue culture. Isolation takes two to seven or more days. Identification by use of a specific antiserum against each virus type. A neutralization typing test takes at least two days.

Serological diagnosis. By an increase in antibody titre in neutralization or complement-fixation tests during the course of illness. Serum from 5—10ml. of blood should be collected as soon as possible after the onset of illness and again 14—21 days later. Neither test is in routine use but both are helpful in selected cases.

Psittacosis-ornithosis

Causative viruses. A family of viruses, psittacosis—LGV group, of variable pathogenicity for human beings and animals, and possessing a common heat-stable complement-fixing antigen. Commonly found in birds but also present in certain mammals.

Illness in man. Classically an acute febrile illness with toxaemia and pneumonia, but mild cases resembling influenza or atypical common cold may occur.

Distribution of virus. Virus has been isolated from the blood during the early stages of the illness; also from throat washings sputum and pleural fluid and, in fatal cases, from pneumonic lung, pleural and pericardial effusions, spleen, liver and brain.

Isolation and identification. From heparinized blood or blood clot, sputum or pleural fluid during first week of illness, and lung, liver and spleen from fatal cases. *Glycerol must not be used as a preservative.* Inoculation of adult or suckling mice, or fertile hen egg, together with the guinea-pig when there is a probability that the strain is derived from mammals. Identification depends on the demonstration of the organism in the tissues of the laboratory animal by microscopic examination and serological methods. Isolation may be interfered with in patients treated with sulphonamides or antibiotics.

Serological diagnosis. By an increase, during the course of the illness, of the titre of complement-fixing antibody to the heat-stable antigen shared by the whole family of viruses including that of lymphogranuloma venereum. C.F. antibody first appears about the 12th or 15th day of illness and reaches a maximum by the 25th to 35th day. Acute and convalescent-phase sera are necessary, as residual antibody from past infection is not uncommon.

Q Fever

Causative organism. Rickettsia burneti.

Illness in man. Acute fever with severe headache and pneumonia in a variable proportion of patients. Rare complications include encephalitis, hepatitis, epididymitis, oesophagitis and thrombophlebitis.

Distribution of the rickettsia in man and duration of excretion. The rickettsia is regularly found in blood during the fever (three to fifteen days) and is present irregularly in the urine during early and middle convalescence (eight to thirty-five days). Occasionally it has been isolated from sputum, pharyngeal washings, or cerebrospinal fluid in life, and from the liver, spleen and kidneys of the (rare) fatal case.

Isolation and identification of the rickettsia. From heparinized or citrated blood during the fever; urine during convalescence. From inoculation of blood or urine into the guinea-pig or hamster, which is then tested for the development of specific C.F. antibody five to six weeks later.

Serological diagnosis. By an increase of the titre of C.F. antibody during the course of illness. C.F. antibody first appears between the 12th and 18th days, but a few patients may not develop it until a month after onset. Serological diagnosis is the quicker and, in view of the infectivity of the rickettsia for unvaccinated laboratory workers, the safer method.

Modern Pharmaceutical Advertising—Suggestions to counter its disadvantages. J. D. W. WHITNEY, M.B., M.R.C.S. *Brit. med. J.* (1957) **2** Suppl. 7 (July 13th).

Dr. Whitney has raised again the desirability (from a doctor's point of view) of uniformity in the postal advertisements of drug firms, and he offers a form of card index intended to please all interested parties. Those who like regularity and organization may approve of his scheme, but the directors of the advertising firms are likely to continue to strive for novelty as a means of attracting attention.

EPIDEMIC OBSERVATION UNIT

Influenza 1957

The early appearance of influenza and its spread throughout the country is being watched by the Public Health Authorities, and the Unit has made no attempt to collect information about this. Many doctors, however, will have or have had an opportunity of making clinical or epidemiological observations on patients suffering from this disease; a report on any such study would be welcomed by the director of the unit, Dr G. I. Watson, Corran, Peaslake, Guildford, Surrey.

Two special studies are being undertaken by members of the Research Register who are in large partnerships. The first concerns the age and sex distribution of bleeding as a complication (nose bleeding among boys about 9—14 appears to have been quite common). The second is into the incidence of influenza among the doctors themselves, in relation to their contact with the disease, first among their patients and secondly in their own households. If any doctor, whose practice is not yet affected by influenza, cares to have further details about either of these enquiries, he should write to the director of the unit for information.

NOTIFICATION REMINDERS

Bornholm Disease: Dr W. O. Williams, 33 Carmarthen Road, Swansea. Please notify cases or outbreaks. He will send you a questionnaire to be filled up about each case.

Scabies: Dr J. C. Graves, Kitts Croft, Writtle, nr Chelmsford, Essex. Please notify each case suspected or confirmed during 1957 and tell him about any scabies you have seen in the past 12 months. He will ask for further clinical and epidemiological details.

Rubella: Dr G. I. Watson, Corran, Peaslake, Guildford, Surrey. Please notify each case. He will send you a record sheet to be filled up about the case and its family contacts.

Leukaemia or Aplastic Anaemia: Dr D. Crombie, 52 Oakham Road, Birmingham 17. Please inform him of all cases at present under treatment and all new cases encountered. He will send you a notification card and send further details about Dr. Alice Stewart's survey.

Pernicious Anaemia: Dr E. Scott, Suomi, Westwell, Ashford, Kent. See *Research Newsletter* No. 16, for the details which are required.