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RECENT ADVANCES IN VIRUS INFECTIONS: THE NEW ERA IN VIROLOGY*†

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This paper describes some of the advances in knowledge of virus infections of man, with particular reference to those new diseases discovered in the last few years, mainly by the use of improved technical methods.

It may be claimed that virus diseases are now the most prevalent of the microbial infections of man, and by failure to respond to antibiotics they stand out in marked contrast to the bacterial infections. The only effective method of control of most of the virus diseases is prophylactic vaccination, and this method has already proved effective against some infections.

The New Viruses

Twenty years ago only 36 antigenically distinct human viruses were known, and 14 of these could not be propagated regularly in the laboratory—that is, the viruses of molluscum contagiosum, warts, herpes zoster, measles, rubella, varicella, infectious hepatitis, serum hepatitis, trachoma, inclusion conjunctivitis, common cold, Australian X disease, dengue, and sandfly fever (Table I). It is small wonder, therefore, that the study of virus infections was mainly an academic pursuit and of little concern to the physician in his daily practice.

During 1940–59 the situation changed significantly, with the discovery of an additional 116 antigenically distinct human pathogenic viruses, all of which can be easily cultivated in the laboratory (Table II). Over 90 of these were discovered in the last 10 years of the period.

As a further advance, many of the viruses already known in 1939 have more recently been adapted to growth in the laboratory—that is, the viruses of herpes zoster, measles, varicella, trachoma, inclusion conjunctivitis, common cold, Australian X (Murray Valley) encephalitis, dengue, and sandfly fever.

At the present day, only five important human viruses have not been propagated in the laboratory—the viruses of molluscum contagiosum, warts, infectious hepatitis, serum hepatitis, and rubella.

Many of the viruses recently discovered are the cause of acute infections commonly encountered in medical practice, and it may be said that viruses have now been established as affecting almost all the organs of the body.

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It is evident that viruses as a group cause a much wider range of pathological lesions than had been recognized earlier. For example, viruses of the Coxsackie family have been shown to cause acute myocarditis and pericarditis, and some of the insect-transmitted viruses to cause acute gastro-intestinal haemorrhage.

Virus infections are commonly regarded as being acute and self-limiting, with few sequelae. The recent demonstration that acute myocarditis and pericarditis may be caused by viruses, with other evidence of the tissue tropism of viruses, emphasizes the need to investigate the role of these agents in certain forms of subacute and chronic illness—for example, of the heart, vessels, lungs, central nervous system, liver, and gastro-intestinal tract.

Methods for the laboratory diagnosis of virus diseases have been greatly improved and made more generally available. No longer is the study of viruses a purely academic exercise. On the contrary, medical virology is now much concerned with the problems of the practising doctor, particularly in laboratory diagnosis and the development of preventive vaccines.

Advances in Methods of Studying Virus Infections

The Suckling Mouse Technique

A major technical advance, reported in 1948 by Dalldorf and Sickles, was the use of suckling mice. The widespread use of this technique led to the recognition of two major families of Coxsackie virus known as groups A and B. The 19 antigenically distinct types of Coxsackie A virus produce myositis in the limbs and thorax of the baby mouse. The five types of Coxsackie B cause inflammatory changes in the dorsal fat, brain, and pancreas.

Modern Tissue-culture Techniques

The second important development, more accurately described as an improvement of an old technique, was reported in 1949 by Enders, Weller, and Robbins. This team showed that poliovirus would grow in "suspended cell" tissue cultures of minced human embryonic tissue in small conical flasks. The infected tissue fragments slowly degenerated, and in due course stopped metabolizing.

It was soon found that cultures prepared from fragments of tissue planted on the wall of test-tubes were more suitable for the demonstration of the degeneration produced by poliovirus. When such cultures were infected with throat washings or stools of cases of polio

the outgrowth of new cells of the culture degenerated within a few hours. This so-called "cytopathogenic change" could be readily seen under the microscope in wet or stained preparations, and was specifically prevented by the addition of polio antibody.

The essential novelty of the Enders technique was the demonstration that the presence of a virus could be detected by the simple process of examining tissue cultures in test-tubes under a microscope. At one stroke many of the previous technical difficulties of the virologist were removed, and there became available a simple, accurate, inexpensive, and rapid test for the presence of a virus and for specific antibody.

Another major advance was made in 1953, when it was reported that a more sensitive type of culture can be prepared from suspensions of human or monkey kidney extracted with trypsin. Epithelial cells liberated by the enzyme grow in the form of thin sheets of cells in test-tubes. HeLa epithelial cells, originally derived from a carcinoma of the cervix, have also proved sensitive to the presence of poliovirus, as have other cell lines propagated in the laboratory.

Epithelial cells can also be grown in the form of a continuous sheet in Petri dishes. When poliovirus is placed on such sheets, as first shown by Dulbecco, it induces degeneration of the cells with the formation of areas of lysis, or plaques, easily seen with the naked eye. These plaques are analogous to the plaques of bacteriophage growing on cultures of sensitive bacteria. With this new method the virologist has available one of the basic techniques of the bacteriologist, and can obtain

TABLE I.—*Human Viruses Discovered by 1939*

Name of Virus Family	Name of Species of Virus
Dermatropic viruses	Molluscum contagiosum*† Warts*† Herpes simplex Herpes zoster*
Exanthem-producing viruses	Measles* Rubella*† Varicella* Variola
Hepatitis-producing viruses	Infectious hepatitis*† Serum hepatitis*†
Basophilic viruses related to psittacosis virus	Lymphogranuloma Psittacosis-ornithosis Trachoma* Inclusion conjunctivitis*
Myxoviruses (haemagglutinating viruses)	Influenza (A classic) Mumps
Other respiratory viruses	Common cold*
Arbor viruses (arthropod-borne viruses)	North American equine encephalitis (Eastern type) " " " " (Western " ") St. Louis " encephalitis Japanese B " " Australian X disease* Louping ill Russian spring-summer encephalitis Yellow fever Dengue " (single type)* West Nile Bwamba Rift Valley fever Sandfly fever (single type)*
Other neurotropic viruses	Rabies Poliomyelitis type 1 " " 2 " " 3 Virus B Lymphocytic choriomeningitis
Total	36

* In 1939 these viruses could not be propagated regularly in experimental animals, embryonated eggs, or tissue cultures.

† These viruses have still not been adapted to regular propagation in the laboratory.

"colonies" of viruses, which can be subcultured to yield pure cultures. Sabin has used this technique in his experiments on the selection of polioviruses with greatly reduced virulence for the central nervous system.

At this point it may be mentioned that modern tissue-culture techniques have been rendered practical by the use of antibiotics in the culture media, especially penicillin and streptomycin. No longer is it necessary to take elaborate precautions to prevent contamination of tissue cultures by airborne micro-organisms. The preparation of tissue cultures has become almost as routine as the preparation of blood-agar plates.

Application of the Newer Tissue Culture Techniques

Though the original work on the growth of viruses in the newer types of tissue culture was done with polio-

TABLE II.—*Human Viruses Discovered in the Period 1940-59*

Name of Virus Family	Name of Species of Virus	No. of Antigenic Types	Major Clinical Features
Basophilic viruses related to psittacosis virus	Meningopneumonitis Pneumonia virus of mice San Francisco Louisiana Illinois	1 1 1 1 1	Virus pneumonia
Myxoviruses (haemagglutinating viruses)	Influenza viruses Influenza (A prime) " (Asian) " B " C Parainfluenza viruses Sendai Croup-associated Haemadsorption	1 1 1 1 1 2 2 2	Influenza Upper respiratory illness; croup
Other respiratory viruses	Chimpanzee coryza agent JH (2060) Uppsala	1 1 1	Upper respiratory illness
Arbor viruses (group A of Casals)	Chikungunya Mayaro Middelburg Semliki Forest Sindbis Venezuelan equine encephalitis	1 1 1 1 1 1	Dengue-like pains Fever — — — Fever; encephalitis
Arbor viruses (group B of Casals)	Dengue (additional type) Murray Valley Ilheus Bi-undulant fever Omsk haemorrhagic fever Central European encephalitis Kysanur Forest Powassan Uganda S Zika Ntaya Spondweni Wesselsbron	1 1 1 1 1 1 1 1 1 1 1 1 1	Dengue Encephalitis Fever; meningitis " haemorrhages Meningo-encephalitis Fever; haemorrhages Encephalitis — Fever — Fever
Arbor viruses (group C of Casals)	Apu Caraparu Marituba Murutucu Oriboca	1 1 1 1 1	Fever or subclinical only
Arbor viruses (ungrouped)	Sandfly fever (additional type) California encephalitis Bunyamwera Colorado tick fever Crimean haemorrhagic fever	1 1 1 1 1	Sandfly fever Encephalitis — Fever Fever; haemorrhages
Enteroviruses (enteric viruses)	Coxsackie group A Echo " " B	19 5 25	Herpangina; fevers Myalgia; meningitis Meningitis; rashes
Adenoviruses	Adenovirus	18	Myalgia; pharyngitis; conjunctivitis
Miscellaneous viruses	Contagious pustular dermatitis Cytoomegalic inclusion disease Mengo virus	1 1 1	Pustular lesions Generalized infection Encephalitis
Total		116	

virus, it was soon found that many other viruses would grow and produce characteristic cytopathogenic changes. To some extent the nature of an infecting virus can be identified by examination of the microscopic changes induced in tissue cultures.

For example, poliovirus, Coxsackie, and Echo viruses produce an eosinophilic mass in the cytoplasm, and the nucleus becomes pyknotic, shrunken, and pushed to one corner of the cell. Measles virus causes the epithelial cells to become aggregated into giant cells or syncytia, the individual nuclei containing acidophilic inclusions. Herpes simplex, herpes zoster, and varicella viruses also cause the formation of giant cells, but they are smaller, with fewer nuclei. In the early stage of infection the nuclei contain basophilic inclusions; later, the well-known eosinophilic Lipschütz inclusions appear. Adenoviruses also produce intranuclear inclusions, and a characteristic "rosette"-like rearrangement of the nuclear chromatin. Vaccinia virus produces eosinophilic Guarnieri inclusions in the cytoplasm.

An application of even greater importance than the identification of viruses already well recognized has been the discovery of over 50 antigenically distinct new viruses that grow only in tissue culture. These viruses fall into the families of adenoviruses, Echo viruses, croup-associated viruses, haemadsorption viruses, and several others (Table II).

Another important application of tissue cultures has been the mass production of virus-infected fluids for processing into vaccines. The method introduced by my colleagues at the Connaught Medical Research Laboratories, University of Toronto, early in 1953 is still in use, with modifications, in Canada and many other parts of the world, for the production of polio vaccine. Monkey-kidney tissue is finely minced and suspended in about 750 ml. of a synthetic nutrient such as Mixture No. 199 of Morgan, Morton, and Parker. The material is placed in a large diphtheria-toxin-production bottle and rocked; after a few days the fluid is replaced with fresh nutrient, and virus is then added. High yields of virus are obtained from the fluid a few days later.

Essentially the same simple technique has been used by Hilleman and by Huebner in the production of vaccines against the adenoviruses, and may well serve for the production of vaccines against measles, and some of the viruses which cause colds.

Advances in Study of the Arbor Viruses

Although I have stressed the advantages of tissue cultures in the study of viruses, about 20 new arthropod-borne (arbor) viruses pathogenic for man have been isolated since 1939 by the standard technique of inoculation of mice. The arbor viruses contain antigens that agglutinate red cells *in vitro*, and the nature and distribution of these antigens can be studied by tests in which this agglutination is prevented by antiserum. Casals and Theiler have found that the arbor viruses can be arranged in four antigenically distinct families by means of this haemagglutination inhibition test (Table II).

Laboratory Diagnosis of Virus Infections

The laboratory diagnosis of virus infections is made by two methods. In the direct method the causal virus is isolated from pathological specimens. In the indirect method evidence of infection is afforded by the demon-

stration that serum antibodies develop in convalescence. The modern trend is to concentrate on the direct method of diagnosis, by isolation of the causal virus from pathological specimens, if possible in tissue cultures. If the virus is one that produces specific cytopathogenic changes, identification is often possible within two to four days.

In the past few years facilities for virus diagnosis have been provided by university, hospital, and public health laboratories on a considerable scale.

In Canada, for example, many of our Provincial Government Public Health Laboratories now provide to practitioners a diagnostic service for virus infections. In several of the teaching departments of universities and in the larger teaching hospitals there are also laboratories where diagnostic tests can be done. The Virus Laboratories of the Federal Department of National Health and Welfare in Ottawa serve as a reference centre for identification of strains and for the preparation of diagnostic antigens and sera.

This development is remarkable when one recalls that ten years ago there were only two or three laboratories in Canada where virus diagnostic tests could be performed.

In the diagnosis of virus infections in the laboratory, main emphasis should be placed on isolation and precise identification of the causal virus as speedily as possible. The physician is offered a presumptive diagnosis based on the isolation of a virus from a patient. The interpretation of the significance of such an isolation must be made by the attending physician.

Subclinical infection with many of the new viruses is common, and isolation of a virus from a patient may merely represent excretion from an earlier infection some weeks before. The isolation of virus from blood, cerebrospinal fluid, organ suspension, or skin lesions is much more significant than isolation from throat or stool.

Virus infections usually occur in epidemics, and the isolation of the same antigenic type of virus from several acutely ill persons affords strong evidence for the aetiological role of that virus.

In the great majority of persons infected with a virus, antibody develops in the blood within 7 to 14 days of onset. This can be tested by the complement fixation, virus neutralization, or haemagglutination-inhibition techniques. The demonstration that antibody increases in level fourfold or more during convalescence is usually acceptable evidence for current infection. Though serological tests are essential in research studies, their value to the practitioner is limited because the tests are retrospective.

Poliomyelitis and Other Enteroviruses

Concept of the Enterovirus Family

Study of the biological properties of the poliomyelitis, Coxsackie, and Echo viruses, as manifested both in laboratory hosts and in human beings, has led to the concept of the "enterovirus family," the members of which proliferate readily in the gastro-intestinal tract of man. The fifty or so distinct antigenic types of poliovirus, Coxsackie virus, and Echo virus are now all classified into one family. Some of the common properties of enteroviruses are as follows: the members are of the same order of size (30 millimicrons), are nucleoproteins, and resist treatment with ether. Most of

the members of the family grow in tissue cultures and produce identical cytopathogenic changes.

Epidemiologically, the enteroviruses mainly cause infections in childhood, and are transmitted by close contact, droplets, and faecal contamination. One strain of enterovirus may cause several clinical types of illness even in individual family groups. In the temperate parts of the world infections are much commoner in the summer and autumn than in the colder months.

In any given year, one enterovirus type seems to predominate in each locality. For example, as shown in Table III, this has been the case in the Toronto area of Southern Ontario during 10 years of continuous study of epidemic enterovirus infections.

TABLE III.—*Enteroviruses Epidemic in Toronto Area, 1949–58*

	Polio	Coxsackie	Echo
1949	Type 1		
1950		B1	
1951	" 1	B2	
1952		B4	
1953	" 1		
1954		B2	
1955		B2	
1956			Echo 9
1957			" 9
1958		B5	

Pathogenesis of Enterovirus Infections

The enteroviruses have a common mechanism of pathogenesis, and studies in the past few years have led to a complete change in thought on the pathogenesis of infection with poliovirus, the most extensively investigated member of the family. Until not so long ago, poliovirus was regarded as a neurotrope, spreading to the central nervous system in nerve fibres, from the point of entry in the pharynx, olfactory mucosa, or gastro-intestinal tract. This concept was developed largely from study of the infected rhesus monkey. In this species the virus does indeed behave as a neurotrope and spreads to the central nervous system by nerve fibres. Rhesus monkeys cannot be infected by feeding virus, and are only susceptible by the intravenous route if very large doses are given.

A major step towards understanding the pathogenesis of polio in man was made when it was demonstrated that cynomolgus monkeys and chimpanzees can be quite readily infected with poliovirus administered in food. The chimpanzee constitutes a particularly close model for the study of the human infection. After the administration of poliovirus in food, these primates develop a gastro-intestinal infection and excrete virus in the throat and faeces. Only about one in ten infected animals becomes paralysed, but the others develop a subclinical infection.

The current concept of the pathogenesis of polio in man has developed from the work of Bodian, Howe, Horstmann, and Sabin on chimpanzees and man. According to this hypothesis, there are four distinct stages in the spread of virus through the body.

In stage 1, after ingestion or inhalation, the virus proliferates in the lymphoid tissue of the pharynx, especially the tonsil, and also proliferates in the intestine, especially Peyer's patches.

In stage 2, virus passes to the regional lymphatic nodes, where further multiplication takes place; some virus then enters the blood-stream to initiate stage 3, the stage of viraemia. During this blood-vascular phase, virus presumably multiplies in the internal organs. The

viraemia stage lasts for four to five days, and may be accompanied by fever and a non-specific illness.

Finally, in stage 4, virus enters the target organ—the central nervous system—attacking the ganglionic nerve cells.

Infection may be aborted at any of the stages, and it is probable that only in about one in every hundred infected persons does the central nervous system become attacked. Virus-neutralizing antibody in the blood will prevent the stage of viraemia, and therefore involvement of the central nervous system.

Killed (formalinized) vaccine exerts its protective role through the development of blood antibody, which neutralizes virus that enters the blood. Formalinized vaccine does not prevent stages 1 and 2, and when the vaccinated person is exposed to infection proliferation of virus takes place in the gastro-intestinal tract and lymphatic nodes, with some excretion of virus.

It now appears clear that the immune mechanism in those who have suffered a natural, usually subclinical, infection is different. Such "naturally immune" persons develop a relative state of resistance of the gastro-intestinal tract, so that exposure to virus does not usually lead to any significant degree of gastro-intestinal infection and faecal excretion of virus.

If this concept is correct, the attenuated strains of poliovirus now being widely tested by oral administration may have certain advantages, because they induce two barriers to reinfection—a state of local resistance of the gastro-intestinal tract, and a state of generalized resistance by the circulation of blood antibody.

Studies on the pathogenesis of the other enteroviruses are not nearly so complete, but it is reasonable to infer that the mechanisms are similar. The Coxsackie viruses proliferate in throat and gastro-intestinal tract, and have been isolated from the blood. Coxsackie viruses localize in more "target organs" than does poliovirus—for example, the throat, striated muscles, myocardium, pericardium, brain, and meninges. The target organs in Echo infections appear to be the meninges and skin.

Clinical Features of Enterovirus Infections

The three enteroviruses have a similar mechanism of pathogenesis, and some similarity of clinical features might reasonably be expected. As shown in Table IV,

TABLE IV.—*Spectrum of Clinical Features Caused by Enteroviruses*

Clinical Feature	Causal Agents			
	Polio	Coxsackie A	Coxsackie B	Echo
Paralysis ..	1, 2, 3	A7, 9	B3, 4, 5	2, 4, 6, 9, 16
Virus meningitis ..	1, 2, 3	A2, 4, 7, 9	B1 to 5	2, 4, 6, 9, 14, 16
Summer fevers ..	1, 2, 3	Several types	B1 to 5	Several types
Rubelliform rashes ..	—	A2, 4, 9, 16	B4	2, 4, 6, 9, 14, 16
Myocarditis ..	1, 2, 3	—	B2, 3, 4, 5	—
Pericarditis ..	—	—	B2, 3, 4, 5	—
Herpangina ..	—	Several types	—	—
Epidemic myalgia ..	—	—	B1 to 5	—
Diarrhoea ..	—	—	—	18 and other types

there are nine main groups of clinical feature now attributed to infection with the enteroviruses. It will be noted that paralysis, aseptic meningitis, and summer fevers may be caused by members of all the enterovirus families, and rubelliform rashes by all except poliovirus. It seems likely that further research will lead to a

recognition that myocarditis, pericarditis, and other features may sometimes be caused by additional members of the enterovirus family.

The enteroviruses are of considerable importance in these days of widespread immunization against polio. Laboratory investigations show that most of the illnesses reported in vaccinated persons are caused by Coxsackie and Echo viruses. Most of these illnesses are classified clinically as "virus" or "aseptic" meningitis. As shown in Table V, in the Ontario study of illness in vaccinated

TABLE V.—Ontario Poliomyelitis Vaccine Study, 1956.* Reported Cases of Polio and Suspect Polio in Patients Aged 18 and Under

Type of Illness	Cases of Illness in Groups Designated		
	Vaccinated (840,000)	Unvaccinated (960,000)	Total
1. Paralytic polio	5	71	76
2. Aseptic meningitis (caused by polio virus)	1	9	10
3. Aseptic meningitis (caused by Coxsackie-Echo viruses)	49	54	103
4. Aseptic meningitis (caused by unidentified viruses)	40	37	77

* Brown, W. G., et al., *Canad. med. Ass. J.*, 1958, **79**, 155.

and unvaccinated children, the Coxsackie and Echo viruses played a major part. The considerable protective effect of the vaccine against laboratory-proved polio infection may be noted.

It should be noted that a single strain of enterovirus may cause several different manifestations of illness in a given locality, even in one family. Thus, in 1958 in Toronto, Coxsackie virus B5 was responsible for cases of aseptic meningitis, Bornholm disease, myocarditis, and pericarditis.

The Adenoviruses

The tissue-culture techniques originally introduced for the study of poliomyelitis have been profitably used in the investigation of infections of the respiratory tract and conjunctival sac. In the past six or seven years, workers in Washington, D.C., have firmly established the pathogenic role of another new group of viruses that grow only in tissue culture (Hilleman; Huebner, Bell, and Rowe). These adenoviruses were first recovered from adenoids grown in tissue culture.

The adenoviruses, which occur in nearly 20 different antigenic types, grow particularly well in HeLa cell tissue cultures and cause a characteristic cytopathogenic change, with the formation of intranuclear inclusions. The viruses do not infect experimental animals.

In man, the adenoviruses proliferate in the conjunctival sac, cornea, and upper respiratory tract; occasionally they invade the lung to cause one form of virus pneumonia.

TABLE VI.—Clinical Entities Caused by Adenoviruses

Clinical Entity	Main Clinical Features	Type of Adenovirus Usually Involved
Acute respiratory disease of military recruits	Influenza-like illness	3, 4, 7, 14
Virus pneumonia	Pneumonia	1, 3, 4, 7
Pharyngoconjunctival fever of children	Fever, conjunctivitis, pharyngitis	3, 4, 7, 14
Minor respiratory illnesses (colds)	Often with pharyngitis	1 to 5, 7
Epidemic keratoconjunctivitis	Severe infection often with corneal opacities	8
Sporadic follicular conjunctivitis	Sometimes with transient opacities	2, 3, 6 to 10

The adenoviruses cause a wide range of clinical entities, as shown in Table VI, but there are common features, especially pharyngitis, fever, and keratoconjunctivitis.

Adenovirus infections are spread directly or indirectly by infected eye secretions or by droplet infection. There is good evidence that conjunctivitis and keratoconjunctivitis may be spread by contaminated eye solutions, eye instruments, and doctors' and nurses' hands.

Not all of the adenovirus types have yet been established as pathogenic, but several have been so accepted, especially types 3, 4, and 7 in respiratory disease and type 8 in epidemic keratoconjunctivitis.

Laboratory diagnosis is made by isolation of the causal virus from eye secretions, throat washings, or sputum, together with the demonstration of increase in virus-neutralizing or complement-fixing antibodies.

Vaccines have been produced by Hilleman and by Huebner by a method similar to that used in poliomyelitis. These vaccines have proved highly effective in military recruits.

Other New Respiratory Viruses

Continued investigation of cases of respiratory illness by tissue-culture techniques has led to the discovery of several new viruses, such as the croup-associated viruses, haemadsorption viruses, chimpanzee-coryza agent, JH virus, and the Uppsala virus. Two of these groups of virus are of particular interest, croup-associated and the haemadsorption viruses. These viruses are related to influenza virus and cause agglutination of red cells *in vitro*.

The croup-associated viruses were isolated independently in Toronto in 1955 by my colleague Beale, and in Cincinnati by Chanock. In our study, 10 of 15 patients with severe acute laryngotracheobronchitis (croup) yielded cytopathogenic viruses in tissue cultures of human amnion. The viruses produced a syncytial degenerative change. Chanock has shown that there are two major antigenic types of croup-associated virus, and that these are probably causal agents of some cases of croup and of milder upper respiratory illnesses.

The haemadsorption viruses, also isolated by Chanock, grow in tissue culture and produce a syncytial type of cell degeneration. The name "haemadsorption" derives from the fact that when a suspension of red cells is added to infected tissue cultures the cells are adsorbed to the areas where virus is growing.

The haemadsorption viruses appear to cause mild upper respiratory illnesses, bronchitis, as well as croup. It seems probable that many more viruses will be found which have the property of causing mild respiratory illnesses. Already claims have been established for the aetiological role of the chimpanzee-coryza agent, the JH virus, and the Coe virus in common colds and even more severe respiratory illnesses. Andrewes concludes that, though many of these viruses have been thought to produce cold-like symptoms, there is no evidence that they cause a substantial percentage of the "commonest type of common cold."

Recent Advances in the Arthropod-borne Viruses

During the period under review great advances in knowledge have been made in one of the largest group of viruses, the arthropod-borne or arbor viruses. Much

of the new knowledge has come from the Rockefeller Foundation's programme of planned search for these agents in man, mosquitoes, and animal hosts, being conducted vigorously in South America, Trinidad, East Africa, India, and South Africa.

To date, 27 antigenically distinct arbor viruses have been found in nature in South America, 24 in Eastern Africa, 15 in India, Malaya, China, and Indo-China, and 10 in North America. Many of these are of proved infectivity for man and often for wild and domesticated animals. It is certain that many more arbor viruses await discovery.

It has been known for many years that the arthropod-borne viruses can be arranged in various subgroups by standard serological methods, but only within the past few years has a reasonably satisfactory classification been achieved.

Most of the arbor viruses haemagglutinate, and Casals and Theiler have shown that the viruses can be classified into three main groups (A, B, and C) by the haemagglutination-inhibition reaction; there is also a fourth group of unrelated viruses. Casals and Theiler allocate to group A the following viruses: Eastern, Western, and Venezuelan equine encephalitis viruses and Mayaro virus of Trinidad. Members of group B include the viruses of yellow fever, dengue, St. Louis encephalitis, Japanese B encephalitis, Murray Valley encephalitis, West Nile fever, Ilheus encephalitis, and the members of the Russian spring-summer encephalitis subgroup (louping ill, Omsk haemorrhagic fever, bi-undulant fever, Kyasanur Forest, and the Powassan (Northern Ontario) viruses. The members of group C, isolated in South America, have not yet been established as important causes of disease in man.

Practically all of the arbor viruses produce encephalitis in mice, and are usually isolated by inoculation of these animals rather than tissue cultures. Though the tendency has been to regard many of the arbor viruses as being neurotropic for man, this property is in fact uncommon. Though certain of the arbor virus group cause encephalitis in man, subclinical and abortive non-specific febrile infections are much commoner.

Arbor viruses closely related antigenically may produce a wide range of clinical features. The Russian spring-summer complex of viruses in group B illustrates this point very well. The Russian spring-summer virus causes severe involvement of the nervous system in man, and louping-ill virus behaves likewise in sheep. Infection by the closely related virus of Omsk haemorrhagic fever causes in contrast a febrile illness with marked tendency to gastro-intestinal haemorrhage. Infection with Kyasanur Forest virus of India causes a similar illness to that caused by the Omsk virus, with prostration, myalgia, and gastro-intestinal haemorrhage, but the fatality rate is higher. In bi-undulant fever there are two paroxysms of fever, with an afebrile period of several days intervening; the illness is not fatal.

Conclusions

The past few years have witnessed a remarkable change in virology. Twenty years ago only two dozen viruses could be readily cultivated in the laboratory. Virology was largely an academic subject little concerned with applications to the daily practice of medicine. To-day, the list of viruses infecting man, and that can be readily studied in the laboratory, is about 150, and the

number is increasing rapidly. The introduction of new technical methods has led to the discovery of the new viruses, and the same methods are widely used for laboratory diagnosis, which is now almost as practical as bacteriological diagnosis.

Foremost in recent advances has been the introduction of new tissue-culture techniques which have enormously simplified the work of the virologist and extended his scope.

There can be little doubt that in most communities viruses are responsible for more illness than are bacteria. Research of the last few years has proved that many of the common ailments of man are virus infections. Further progress is confidently anticipated.

Summary

This paper reviews some of the advances in knowledge of virus infections, with particular reference to those new diseases discovered in the last few years, mainly by the widespread adoption of the techniques of inoculation of suckling mice and tissue cultures.

In 1939, only 36 antigenically distinct viruses causing human disease were known, and 14 of these could not be propagated at will in the laboratory.

During the period 1940-59, an additional 116 antigenically distinct strains were discovered, over 90 in the latter 10 years of the period.

Only five important known viruses infecting man have not been propagated in the laboratory—the viruses of molluscum contagiosum, warts, infectious hepatitis, serum hepatitis, and rubella.

The contribution of the newer tissue-culture techniques to the study of viruses is emphasized.

Recent advances in knowledge of poliovirus and the other enteroviruses are outlined. Attention is drawn to the similarity of the clinical features caused by the enteroviruses, including paralysis.

The paper includes a review of the prevalence of enterovirus infections in Ontario in the last 10 years.

Finally, recent advances in the knowledge of the arbor viruses, adenoviruses, and other respiratory viruses are described.

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VIRUSES, BACTERIA, AND
RESPIRATORY DISEASE IN CHILDREN

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The purpose of this investigation was to examine and correlate clinical, bacteriological, and virological information in acute respiratory infection in children.

Few comprehensive studies of this kind have been carried out. Morrison *et al.* (1957), in London, investigated children for both bacterial and virus agents; Graham (1958), in Melbourne, carried out an extensive laboratory investigation of laryngo-tracheo-bronchitis in children. These two groups of workers were unable to study their patients fully and examine them for the new types of parainfluenza viruses which have been isolated over the past six years and more fully classified in the last two years (Kuroya *et al.*, 1953; Chanock, 1956; Beale *et al.*, 1956, 1958; Gardner, 1957; Chanock *et al.*, 1958; Petersen and von Magnus, 1958; Vargosko *et al.*, 1959).

Numerous bacteria have been isolated from the respiratory tract in acute respiratory disease, but the exact role of these organisms is difficult to define, as many of them are found in the throats of apparently normal children.

The Investigation

The investigation included all children suffering from acute respiratory infection admitted to three hospitals in Newcastle—the Royal Victoria Infirmary, the Babies' Hospital, and the Newcastle General Hospital—they numbered 195. The period of investigation extended over six months from January 1, 1959, to June 30, 1959.

The clinical examination and records of the cases were supervised and co-ordinated by one person. The virological examinations were all performed at the Public Health Laboratory, Newcastle; the bacteriological examinations were shared between the Bacteriology Department of the Royal Victoria Infirmary and the Public Health Laboratory.

Methods

Clinical Assessment.—On admission the history was taken, usually from the mother, and the physical signs and the results of early investigations (x-ray examination, white-cell counts) were recorded on a special form. Treatment, day-to-day progress, condition on discharge, and final diagnosis were also recorded, and in each case a follow-up appointment was made for between 14 and 21 days after the onset of symptoms, if the child's

A bequest made by a West Australian doctor for the foundation of a medical research fellowship will become effective this year, 31 years after his death. The bequest was made by a former Chancellor of the West Australian University, Dr. Athelstan Saw, of Perth, whose son had died of diabetes. Dr. Saw, who died in 1929, directed in his will that the research should be conducted in association with a medical school when one was established (as it recently was) at the University in Perth.