VIRUSES OF HUMAN DISEASES*

BY

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Virology is no longer an offshoot solely of the science of microbiology, for viruses are of interest to the botanist, the entomologist, and the veterinarian as well as to those studying molecular biology and biochemistry. Yet it is a strange fact that no virologist understands the nature of the objects of his study or can define them. Past attempts to define viruses have been unsuccessful in separating these from other micro-organisms without involving anomalous groupings for pathogenic agents such as the rickettsiae. Definitions based upon particle size, physical structure, or chemical composition likewise fail in precision because of the range of properties possessed by viruses associated with different diseases. One is therefore forced to adopt a negative definition such as that viruses are incapable of multiplication except in an intracellular environment. The latter must, for animal viruses, be living vertebrate cells, within which the virus nucleic acid exerts its potentiality for replication and thus achieves multiplication of the virus as a whole.

Knowledge of the exact manner in which the virus particle enters a cell and undergoes replication is still awaited, and only a general picture of the mechanism of virus infection has yet been obtained. The essential basis of such infection according to Burnet (1957) is the interaction of the genetic system of the virus with that of the host cells into which the virus has penetrated. The multiplication of virus particles genetically similar to those originally present may or may not be accompanied by pathological consequences for the host. If they are, then symptoms and signs develop and the infection becomes manifest by the outward changes in the host; if not, then the infection is subclinical or inapparent. A virus infection is thus best regarded by those interested in human disease as a form of intracellular parasitism by specific agents incapable of independent replication apart from a host.

Growth of Virology

It is customary to date the science of virology from the early experiments on the filtration of tobacco mosaic virus by Ivanowski and Beijerinck and on foot-and-mouth disease virus by Loeffler and Frosch. Proof that the essential means of contagion of these diseases could be passed through filters which retained bacteria thus dates from the years 1892 to 1898. Yet twenty years before this Pasteur had carried out experiments on the transmission of rabies from dogs to rabbits with material in which he could not demonstrate bacteria, and a

hundred years earlier Jenner had similarly transmitted cowpox to man. Nevertheless many early transmission experiments designed to establish an experimental animal host for a human disease were unsuccessful, and even when they were successful progress was not assured. Poliomyelitis was thus transmitted to the monkey by Landsteiner and Popper (1909), but knowledge thereafter accrued very slowly. It is doubtful whether the early experiments of Smith *et al.* (1933) on influenza would have borne fruit at any faster pace than research on poliomyelitis had it not been for successful transmission of influenza virus from the ferret to the mouse, which permitted extensive experimentation and a rapid accumulation of knowledge. A major change in technique came through use of the chorio-allantoic membrane of the fertile hen's egg introduced by Goodpasture (1933) for virus cultivation and extended by Burnet (1940, 1941) to the amnion and allantois.

Up to the beginning of the second world war, however, human virology was limited by the difficulty of cultivating viruses in hosts other than animals, and even the fertile egg would only permit the study of a handful of agents such as influenza, herpes, vaccinia, psittacosis, yellow fever, and a few of the arthropod-borne encephalitis viruses. Though many other viruses capable of causing disease in man were known, most of them could not be propagated regularly in the laboratory. Andrewes (1954-5) has coined the phrase "the new look in virus research" for the present era, which dates from the pioneer studies on tissue-culture methods of virus cultivation by Enders and his colleagues at the Boston Children's Hospital. A modification of the technique of the suspended tissue culture introduced by Maitland and Maitland (1928) permitted Enders *et al.* (1949) to cultivate poliomyelitis virus in human tissue fragments in the test-tube. Later, by using trypsin to separate individual cells from tissues as suggested by Rous and Jones (1916), Scherer *et al.* (1953) developed the monolayer cell culture which has proved so flexible a tool for virus cultivation.

The virologist to-day prepares his basic experimental host by growing a monolayer sheet of cells from a trypsinized organ such as a monkey kidney, feeds it with suitable nutrients and antibiotics, inoculates it with the virus-containing specimen, incubates it at an appropriate temperature, and watches the cells at daily intervals by direct microscopy through the wall of the test-tube or bottle. The degenerative cell changes occurring in positive cultures known as the "cytopathic" effects of virus growth will also create a focus of translucency—the plaque—in a cell sheet covered over by agar (Dulbecco, 1952), thus providing a method

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of study analogous to that used in bacteriophage work. Moreover, the plaque, which represents the minimal quantity of infectious virus whose effects can be separately identified, is analogous to the bacterial colony. Subcultivation from plaques affords a method of purification of virus stocks for vaccine production similar to that used by the bacteriologist who picks a single colony for propagation as a "pure" culture.

These developments in the techniques of cultivation have been accompanied by progress in serological *in vitro* methods such as the complement-fixation and haemagglutination-inhibition tests and fluorescent microscopical staining techniques. The identification of many virus species by their serological behaviour has thus become relatively easy and is an essential sequel to cultivation. Serological methods are also needed when isolating viruses from specimens in order to obtain evidence that the agent was actually present in the specimen and was not derived from the cell culture or experimental animal. Unlike the bacteriologist, who is able to use lifeless media, the virologist is forced to use experimental animals or cultures from animal tissues themselves possibly containing viruses. If the latter were always peculiar to the animal species concerned they would be readily separable from the agents present in human materials. Unfortunately, mammalian tissues often contain viruses which may resemble or are related to human viruses. Thus much work may be required to prove the origin of a virus producing cytopathic effects in tissue cultures or lesions in experimental animals. A human serological response accompanying the illness under investigation, and demonstrated by one of the available neutralization, inhibition, or complement-fixation methods, is thus valuable collateral evidence that the virus was actually present in the human specimen concerned. It is an essential part of the fulfilment of Koch's postulates as applied to viruses. Caution is still needed, however, in interpreting the virus thus identified as the cause of the patient's symptoms and signs. This is because the occurrence of silent subclinical infection and the existence of a virus in a latent phase within the cells of a host raise problems of their own.

The Host-Virus Interaction

The outcome of the interaction between a host and an infecting virus is dependent upon the variable characters of both participants. From the standpoint of the virus, the major factor appears to be the question of competence to produce pathological effects. Viruses attenuated in this character are well recognized either in nature or as a result of manipulation in the laboratory. Yet virulence, as this character is usually termed, is an intrinsic property of the virus which can only be demonstrated by means of a host system. The manner in which that system reacts towards the virus is in fact the outward expression of virus virulence or avirulence.

Host factors must also often be dominant in determining whether infection is subclinical or clinically apparent because of the occurrence of both varieties side by side during an epidemic due to one and the same virus. Host resistance to virus attack is known to be complex. On the one hand, there is the complete form of genetic insusceptibility manifest towards viruses derived from a foreign animal species, and, on the other, the resistance towards viruses derived from animals of the same species, which is usually of the immunological variety. The animal rendered immune or partly immune

by a previous infection may not permit any further multiplication of virus or may limit such multiplication when an attempted reinfection occurs. The explanation for this is the presence of neutralizing antibodies in the host formed during the first infection. However, a non-serological resistance of the cells of the tissue concerned in virus infection is also known to exist. One variety of this depends upon the formation of the protein interferon (Isaacs and Lindenmann, 1957), which has broad antiviral properties, is secreted by cells exposed to living or inactivated virus, and diffuses from them extracellularly. At times when neutralizing antibody is not present in concentrations adequate to prevent a spread of virus to previously uninfected cells, interferon may block further virus multiplication (Isaacs and Hitchcock, 1960). In other words, interferon secretion is part of the defence by the host against virus infection and may be responsible for bringing such infection to an end.

There is yet a third possibility—namely, that a specific variety of cellular resistance is acquired during a first infection. The persistence of virus within the tissues in a latent perhaps masked form is a possible explanation of such resistance. For instance, lysogenic bacteria are immune to infection by bacteriophage homologous with that present within their cells in a latent phase. Thus both immunological and non-serological mechanisms are at work during the early phases of virus infection and in recovery from such infection. The fact that many virus infections pursue a normal course in children with hypogammaglobulinaemia emphasizes the importance of non-immunological host mechanisms in limiting the virus attack (Gitlin *et al.*, 1959).

The Enteroviruses

The three groups of viruses found in the alimentary tract and classified under this heading are the polioviruses, of which there are three serotypes, and the Coxsackie and E.C.H.O. viruses. In addition to sharing the habitat of the human intestinal tract, the enteroviruses are widely distributed throughout the world and have been found in outbreaks of infection from the Arctic to the Equator principally during the warm season of the year. There are counterparts to the enteroviruses in the alimentary tract of many mammalian host species.

The first of the enteroviruses to be found in the faeces—the polioviruses—were recovered from patients with poliomyelitis by Paul and Trask (1941). The Coxsackie viruses were first recovered by Dalldorf and Sickles (1948) and 30 serotypes are now known, 24 of which are classified in group A and six in group B. Group A viruses produce necrosis of striated muscle with flaccid paralysis in suckling mice, and group B viruses produce encephalitis and necrosis of the subcutaneous (brown) fat in mice. Few of the viruses produce any illness or lesions in adult mice. Robbins *et al.* (1951) recovered viruses which were cytopathic in tissue culture and non-pathogenic for suckling mice, but which were not polioviruses, from faecal specimens of patients with non-paralytic poliomyelitis. No fewer than 28 serotypes of these viruses are now known, and though E.C.H.O. stands for enteric, cytopathogenic, human orphans, only one type (E.C.H.O. 10) has been reclassified and withdrawn from the group even though definite relationships are now known between certain viruses and human illnesses. E.C.H.O. 10 was termed respiratory

enteric orphan (R.E.O.) virus by Sabin (1959) because of its size and association with symptoms in the respiratory and enteric tracts. The latest classification recommended by the Committee on Enteroviruses (1962) of the U.S. National Foundation has not been fully accepted, for it suggests a change in type number for many of the viruses whose serotypes are now familiar.

In spite of the seeming diversity of these various agents and particularly their serological specificity, there are justifications for regarding them as a natural group of viruses. All the enteroviruses are ether-resistant and consist of small particles 25 to 30 μ in diameter which pack together inside the cell into crystalline lattices. Electron-micrographs of poliovirus made by Horne and Nagington (1959) confirm a 20-sided particle (icosahedron) as suggested by x-ray diffraction studies (Finch and Klug, 1959). The individual particle probably consists of an internal core of ribonucleic acid and an outer protein shell composed of a finite number of protein subunits. The appearance of particles of Coxsackie and E.C.H.O. viruses within infected cells (Morgan *et al.*, 1959; Rifkind *et al.*, 1961) suggests a similar structure.

Clinical Effects of Enterovirus Infection:

(1) Poliomyelitis

A word of caution is first necessary in regard to the diagnosis of poliovirus infection. Direct recovery of virus from the stools is successful in only a proportion even of paralytic cases of disease. In the 1961 epidemic in Hull, Marmion (1962) found that the younger the patient the more readily was virus demonstrated. In a series of 358 paralytic cases in California, Magoffin *et al.* (1961) found that even complement-fixation and neutralization tests left many negative results, particularly in those with minimal weakness. As immunization becomes more effective the difficulty of distinguishing poliovirus infections from those due to other enteroviruses is likely to increase.

(a) Pathogenesis

Before 1940 poliovirus was regarded as a strictly neurotropic virus multiplying only within the brain and spinal cord which it was believed to reach along nervous channels from the portal of entry in the nasopharynx. The main site of virus multiplication in chimpanzees and cynomolgus monkeys infected via the mouth is now known to be the walls of the pharynx and the ileum. Virus spreads to regional lymph nodes and is later found in the brain and cord (Bodian, 1957). In man, virus is found in the pharynx just before and for a few days after the first symptoms of illness, and it is present in the faeces during the entire phase of illness and for days or weeks thereafter. It is believed that its main site of multiplication is in the pharynx and the mucosa of the ileum, and it has been found in the blood during the early phase of pharyngeal multiplication. This latter discovery (Horstmann *et al.*, 1954) suggested that the C.N.S. is invaded by virus travelling in the blood rather than along nerve fibres from the periphery. Nevertheless, it seems certain that once virus has reached the C.N.S. it can spread from brain to cord or vice versa along nerve tracts, and it cannot be denied that it may reach the C.N.S. both by nerve pathways and by the blood. Neutralizing antibodies are found in the blood by the time that paralysis has developed, but are not present in the pre-paralytic phase when C.N.S. invasion must occur.

The important fact is that specific antiviral antibodies preformed or introduced before infection can prevent paralysis. This was shown first when gamma-globulin was used in the prophylaxis of poliomyelitis (Hammon *et al.*, 1953). Later artificial immunization with inactivated virus vaccine (Salk) was found to reduce the incidence of paralytic poliomyelitis by stimulating the formation of neutralizing antibodies (Francis *et al.*, 1957). The potency of inactivated vaccine is, in fact, assayed in terms of its antigenic propensity, and policy concerning the dosage of vaccine is based on neutralizing antibody levels.

Antibodies are not, however, so clearly related to the resistance of the alimentary tract. Studies on persons who have been inoculated previously with two or more doses of Salk vaccine have revealed that infection of the alimentary tract either by natural poliovirus infection (Lipson *et al.*, 1956, 1960) or by live attenuated virus is as readily possible in them as in uninoculated persons. High titres of virus are found in the faeces of such persons and virus is excreted for many days. Thus the serum antibody in amounts ordinarily produced by commercial Salk vaccine is unable to prevent infection of the alimentary mucosa. There is some evidence that raising the antibodies to extremely high levels by very potent inactivated vaccine may reduce the titre of virus in the stools or the duration of excretion after deliberate infection (Dick *et al.*, 1961), and this agrees with the results obtained by Bodian and Nathanson (1960) in passively immunized chimpanzees. Deliberate or natural infection with poliovirus, on the other hand, renders the alimentary tract resistant to reinfection even though the level of serum antibodies may then be no higher than after Salk vaccine.

The duration of the resistance of the alimentary tract to reinfection is still unknown and so also is its mechanism. Artificial reinfection with living attenuated virus is, however, possible with a virus serologically different from that used for the first infection, so that antibodies must in some way be concerned in resistance. It has been shown also that attenuated virus may fail to become established in the gut after oral administration because an infection by some other enterovirus or even by an adenovirus is in progress. Probably the poliovirus infection is blocked by the production of interferon in the infected alimentary mucosa. There may indeed be other mechanisms, also of a non-serological character expressed by the words non-specific resistance, which enable the alimentary mucosa to defend itself from attack. Such local resistance has yet to be defined.

(b) Host-Virus Factors Concerned in the Neurological Consequences of Infection

At least five host-factors are important in determining the outcome of infection so far as the nervous system is concerned. The age of the host seems to determine the attack rate of the paralytic disease, which is highest in infancy, and immunological factors can explain most of this variation in susceptibility. However, the outcome of a paralytic attack is itself dependent upon age, as is shown by the rising mortality of poliomyelitis with increase in age (Martin, 1955). This suggests that either the ability of the host to localize the infection within the C.N.S., or more probably the ability of the neurone to resist infection, diminishes with age. Then there is the known higher incidence of paralytic disease in boys compared with girls and in pregnant compared with non-pregnant women (Rindge, 1957; Weinstein, 1957). This

may indicate the influence of the metabolism of the host upon the host-virus interaction.

An increase in the paralytic effect of poliovirus infection can be produced in experimental infection of hamsters with poliovirus type II when the animals are treated with cortisone (Shwartzman and Fisher, 1952). A similar effect of steroids in man cannot be ruled out but has not been described. Fatigue is a well-known factor established, particularly by the work of Russell (1947, 1949) in this country, as having a most important effect on the host during the preparalytic stage of infection. Extensive or fatal paralysis follows excessive fatigue in this phase. Finally, prophylactic inoculations (M.R.C., 1956), trauma, and surgical operations are all known to affect not only the likelihood of paralysis but also its actual location. Experimental work suggests that these factors act by an alteration in the permeability of the motor centres corresponding to the injured periphery, but there is no certainty concerning this.

The virulence of the virus inducing an attack of poliovirus infection is believed to be significant in determining whether lesions will or will not develop in the C.N.S. Virus virulence has been thought to be a biological character which is transmitted genetically, as are the other many characteristics of a virus species. Other biological characters, such as growth at 40° C. or in different cell systems, also define genetic markers, and some of these bear an approximate relation to neurovirulence. None are exactly equivalent, however, and virulence must still be measured directly by the pathological effects of the virus in a particular host-system. In the case of poliovirus, the only experimental measurement of virulence which can be used is the power of the virus to produce neurological symptoms and lesions after intracerebral or intraspinal inoculation into rhesus or cynomolgus monkeys. There is a wide range in monkey neurovirulence among various strains, but most viruses derived from paralytic cases are neurovirulent in relatively small doses. Those from healthy children (Sabin, 1956a) may or may not be as quantitatively neurovirulent as those from clinical cases of disease. The relative order of virulence of wild polioviruses for man cannot of course ever be measured, but there seems no reason to doubt that the virulence for man varies as it does for monkeys. Some of the vagaries of the epidemiological behaviour of poliomyelitis could be due to changes in this property of the virus rather than of the many complex factors of the human herd.

Laboratory manipulation of polioviruses either by tissue-culture cultivation (Enders *et al.*, 1952; Sabin, 1957) or by passage through rodents (Koprowski *et al.*, 1952; Koprowski, 1956) causes a loss of neurovirulence. There is suggestive evidence that such attenuated viruses stimulate the formation of interferon by infected tissues more effectively than do virulent ones (De Maeyer, 1960). The most highly attenuated polioviruses produce no lesions and fail to multiply even when large quantities (10^{10} T.C.D.₅₀) are inoculated intracerebrally in the monkey. When introduced direct into the cord they do not produce paralysis though small lesions may be found microscopically. It is these viruses which are the starting materials for the live attenuated virus vaccines now used in many countries. The attenuation in neurovirulence does not affect the ability of the virus to infect the alimentary tract, for virus can be recovered in high titre and for prolonged periods in the faeces after oral administration. Pharyngeal multiplication of virus and

of viraemia are both probably reduced in frequency compared with natural poliovirus infection.

The progeny of the vaccine virus recovered in the faeces show some return of the property of neurovirulence for the monkey and also of certain other properties found in natural wild viruses and defined by laboratory methods (Dick and Dane, 1957; Clarke *et al.*, 1958; Benyesh-Melnick and Melnick, 1959). The order of virulence of excreted viruses is, however, quantitatively still much lower than that of viruses recovered from paralytic cases of poliomyelitis. It is known that such excreted viruses can cause infection of susceptible contacts, and the inability to control the spread of vaccine virus could constitute a hazard if a full resumption of virulence ever occurred.

(c) Epidemiology

The remarkable changes in the prevalence of poliomyelitis in the twentieth century would remain inexplicable were it not for the light shed by extensive laboratory studies made in many countries. As in the U.S.A. and Scandinavia, the changes in Great Britain have been the emergence of epidemic poliomyelitis on a national scale and the shift in the age incidence from infancy to older childhood and to adults. About one-third of all cases of paralytic poliomyelitis in the period from 1947 onwards have been in children under 5, one-third in children between 5 and 15, and the remaining one-third in those over 15 (Freyche and Nielson, 1955). The explanation now generally accepted is that there has been a change in the age at which exposure to poliovirus infection is first experienced. Virus studies in so-called underdeveloped countries where poliomyelitis is a clinical rarity have revealed the existence of a high level of endemic infection. Where sanitation is primitive, enterovirus infections of all sorts are frequent and recurrent, and the rapid development of antibodies to all three types of poliovirus is accomplished before the age of 2 in a high proportion of infants (Paul, 1958).

In Western communities, and indeed wherever the infantile mortality falls below a certain critical level (Payne, 1955), epidemics of poliomyelitis appear to be correlated with a lack of infection by polioviruses in infancy. Serum antibodies develop gradually, and many children reach adult ages without having acquired antibodies to any of the three serological types of poliovirus (triple-negative individuals). As has also been shown, the risk of paralysis and death after infection increases with age, and this is one reason why exposure to infection for the first time in late childhood or adult age is far more hazardous for the host than when it occurs in infancy. If this interpretation is correct the effect of a gradual reduction in individual host-virus interactions is to prepare the way for a community epidemic. Community protection against paralytic poliomyelitis thus appears to depend essentially on the protective effect of neutralizing antibodies formed in response to alimentary infection. Its loss and substitution of clinical disease for inapparent infection is due to absence of exposure to virus at a sufficiently early age.

It is still too soon to speculate upon the ultimate consequence of the introduction of artificial immunization, though the figures for notifications for England and Wales (see Chart) tell their own story. There is no inherent reason why immunization with inactivated virus vaccine should affect the dissemination of the poliovirus in the community, though this is believed

by some to be the case. On the other hand, the mass use of attenuated viruses administered orally appears to result in at least a temporary disappearance both of these and of wild polioviruses from the community (Sabin *et al.*, 1960, 1961). A combination of both vaccines appears perhaps to offer the best chance of individual protection and a continuation of subclinical infection so necessary for the preservation of herd immunity. Certainly the failure of a significant proportion of children to acquire adequate levels of antibody to type I poliovirus after immunization with inactivated vaccine is a serious drawback. It has been found in

Total and paralytic cases of poliomyelitis notified for England and Wales from 1946 to 1961. Before 1950 non-paralytic cases were not notified separately.

some British children that as many as 20% who have received three doses of Salk vaccine may still be deficient in antibody response to type I virus (Kendall *et al.*, 1960), and though some may later respond to a fourth dose of inactivated vaccine there is no assurance that this will occur.

The controlled trials of live attenuated poliovirus vaccines in Britain have included attempts to throw light upon the immunizing properties of different combinations of vaccines in young children (Public Health Laboratory Service, 1961). It is clear that the vaccines prepared from Sabin's strains given by mouth cause the formation of antibodies in a high proportion of instances in young infants, particularly when trivalent vaccine containing all three types of viruses is used on more than one occasion. This action of live vaccine at an age when Salk vaccine is less effective in stimulating antibodies constitutes an important advantage. The boosting effect of live vaccines in children who have received previous injections of Salk vaccine has also been demonstrated (Public Health Laboratory Service, 1962). Infection by vaccine in children or adults previously immunized with inactivated vaccine has been found to depend upon the dosage of virus given as well as upon the initial level of serum antibodies (Hobson *et al.*, 1962b).

Studies concerned with the properties of the viruses excreted by those receiving attenuated strains by mouth have also been made in Britain (Dick and Dane, 1957 ;

Clarke *et al.*, 1958 ; Dane *et al.*, 1961 ; Hoskins *et al.*, 1962). These have shown an alteration in the biological properties of the excreted viruses with a partial return of neurovirulence for the monkey. The routine use of live vaccines in the populations of entire countries such as Czechoslovakia and the U.S.S.R. have, however, shown no hazard to unimmunized persons from the excreted viruses. The mass use of such vaccines in the control of epidemics of poliomyelitis, as for instance in Hull, has provided encouraging results (Ministry of Health, 1962) and further experience with these materials will provide the evidence needed concerning both safety and effectiveness.

(2) Clinical Manifestations Associated with Other Enterovirus Infections

Apart from poliomyelitis and the polioviruses, there is considerable obscurity concerning the full role of the enteroviruses in human disease. This is partly because the Coxsackie and E.C.H.O. viruses may be recovered from the faeces of healthy children undergoing a sub-clinical infection (Sabin, 1956b ; Gamble, 1962), or may be causing an infection simultaneously with that of some other virus. Proof of a causal relation between an enterovirus and a particular clinical state largely depends, therefore, upon the consistent recovery of the virus from the patients with an identifiable clinical picture. It has thus been shown that the Coxsackie and E.C.H.O. viruses produce minor or even trivial illnesses perhaps with local phenomena such as vesicular lesions in the pharynx or skin rashes of a rubelliform character, and yet at times cause neurological symptoms and signs and particularly the syndrome of aseptic meningitis. The role of the enteroviruses in diseases such as sporadic encephalitis or encephalomyelitis and infectious polyneuritis (Guillain-Barré syndrome) remains, however, in doubt, and in the case of the obscure syndrome of benign myalgic encephalomyelitis and the post-infective encephalitides, it seems unlikely that known enteroviruses are causative agents.

(a) Aseptic Meningitis and the Meningo-exanthematic Syndrome

The occurrence during outbreaks of poliomyelitis of cases of fever, headache, and stiff neck unaccompanied by paralysis was observed as long ago as 1907 by Wickman in Sweden. Non-paralytic cases of poliomyelitis similarly occurred during the localized outbreaks of poliomyelitis in England from 1911 onwards and became numerous during the epidemics after 1946. In 1950 the Registrar-General tabulated paralytic and non-paralytic cases separately, and Thomson (1954) observed that the ratio of the two sets of cases varied widely in the different outbreaks and during different seasons of the same year. He recalled the epidemic syndrome of acute aseptic meningitis, described by Wallgren (1925), which occurred in Gothenburg at a time when other diseases capable of causing irritation of the meninges were not prevalent. Thomson noted that some of the British outbreaks of non-paralytic poliomyelitis between 1951 and 1953 occurred in areas where no case of paralytic disease had been notified for some considerable time. The disease resembled Wallgren's syndrome. It was highly infectious and had an incubation period of about five days.

Light was thrown on these occurrences when tests for viruses in faeces and the C.S.F. were made with tissue culture and other techniques. It was then found that

polioviruses, Coxsackie, or E.C.H.O. viruses were sometimes present in the stools of patients with non-paralytic poliomyelitis but that often no viruses were found at all. Though mumps and lymphocytic choriomeningitis were also identified as occasional causes of the syndrome in all series of cases, many have remained aetiologically unsolved. Table I brings together the experience in recent years in Southern Ontario based on the findings in the research laboratory of the Hospital for Sick Children, Toronto (Clarke *et al.*, 1959; McLean, 1961, personal communication). The chronological variation in both predominant and other strains of enteroviruses is well shown. Karzon (1959) has summarized world experience of recent outbreaks.

TABLE I.—Occurrence of Virus Types in Southern Ontario, 1950-61

Year	Clinical Syndrome	Predominant strain. No. and type	Other strains. No. and type
1950	Aseptic meningitis	1 Cox B1	1 Cox B (untyped)
1951	" "	1 Cox B2	
1952	" "	9 Cox B4	E.C.H.O. (untyped)
1954	" "	4 Cox B2	E.C.H.O. (untyped)
1955	" "	13 (E.C.H.O. untyped)	1 Cox B2
1956	Aseptic meningitis with or without rash (94 cases)	58 E.C.H.O. 9	
1957	Aseptic meningitis (51 cases)	12 E.C.H.O. 9	1 Cox A9, 2 Cox B3, 2 Cox B4, 1 Cox B5, 3 E.C.H.O. 6, 15 untyped
1958	Aseptic meningitis (69 cases)	35 Cox B5	1 Cox A9, 1 Cox B3, 1 Cox B4, 1 E.C.H.O. 6, 5 E.C.H.O. 9, 1 untyped
	Pleurodynia (18 cases)	16 Cox B5	
	Pericarditis (18 cases)	5 Cox B5	
	Abdominal pain (11 cases)	5 Cox B5	1 Cox B3, 1 untyped
1959	Aseptic meningitis (75 cases)	9 Cox B2	2 Cox A9, 1 Cox B3, 2 Cox B4, 3 Cox B5, 1 E.C.H.O. 6, 1 E.C.H.O. 9, 1 E.C.H.O. 14, 9 untyped
	Pleurodynia (16 cases)	3 Cox B2	1 Cox B3, 2 Cox B4, 1 Cox B5, 2 E.C.H.O. 2, 1 untyped
	Pericarditis (3 cases)	2 Cox B5	
1960	Aseptic meningitis (46 cases)	3 Cox B2	2 Cox A9, 1 Cox B5, 4 untyped
1961	Aseptic meningitis (63 cases)	15 E.C.H.O. 9	4 Cox B5, 11 untyped, 1 E.C.H.O. 14
	Pleurodynia (3 cases)	3 Cox B5	

Information received from Clarke *et al.* (1959) and from McLean (1961, personal communication).

Until 1955, tests were conducted mostly during seasonal increases of poliomyelitis, but Archetti *et al.* (1956) reported the recovery of several strains of an unidentified cytopathic virus believed to be a Coxsackie group A virus during an epidemic of benign lymphocytic meningitis which occurred in Angola. Children were mostly affected, but there were cases in adults, and some of the latter showed more severe neurological symptoms and signs. It was subsequently found by Archetti *et al.* (1959) that the virus was serologically identical with E.C.H.O. type 9 virus, of which the prototype strains were recovered in 1954 in Cincinnati from the stools of healthy children by Ramos-Alvarez and Sabin (1956). The outbreak in Italy proved to be the first of a chain of epidemics of aseptic meningitis that occurred in 1956 in Britain, Germany, Switzerland, Belgium, Canada, and Iceland, all of which yielded the same serological type of E.C.H.O. virus.

The first British isolations of this virus from the throat and faeces of patients with aseptic meningitis were made in 1955 by McLean and Melnick (1957) in East Anglia. Some strains had, however, also been found in 1954 in London, and these came from children with a maculopapular rash and increased cells and protein in the C.S.F. but without meningism (Crawford *et al.*, 1956). The virus studies made by Tyrrell and Snell (1956) in Sheffield clinched the association of the E.C.H.O. 9 virus both with cases of aseptic meningitis and with cases in children exhibiting a macular rash. Some patients exhibited both meningitis and a rash, and such dual clinical manifestations due to an E.C.H.O. virus appear not to have been noted before 1956. Subsequently many reports of similar experiences were recorded in Europe in 1956 and in Canada and the U.S.A. in 1957. Again both meningitis and an exanthem were usually observed. In 1960, outbreaks of E.C.H.O. 9 infection of the same general character occurred in Glasgow (Landsman and Bell, 1962), Newcastle, and Sheffield.

Pattern of Infection with E.C.H.O. 9 Virus

The clinical picture of infection by this virus has been excellently described by Tyrrell *et al.* (1958), Lyle (1959), Sabin *et al.* (1958), and many other authors. It is generally agreed that the minor illness accompanied by an exanthem occurs predominantly in children and infants, and it is a short-lived febrile disturbance with headache, drowsiness, vomiting, irritability, and limb pains. Infants may show few signs of illness, and the rash, which may be fleeting, could easily be overlooked or attributed to teething or to a food upset. The rash appears on the first or second day of illness and is pleomorphic. Usually it consists of fine pink macular or maculo-papular elements on the face and trunk which could be mistaken for rubella or be termed roseola infantum. Sometimes it is petechial. On the face it is often blotchy, and lesions may persist for longer than those on the rest of the body. Mouth lesions consisting of vesicles or shallow ulcers on the pharynx or buccal mucosa occur but are inconstant. The cervical lymph-nodes may be slightly enlarged but there is no general adenopathy or splenomegaly. Convalescence is speedily established after two to three days except in adults, who may remain irritable and complain of myalgic pain and headache. Neurological signs are not found in children or adults with minor illness. There is no characteristic alteration of the leucocyte count.

In cases of minor illness the virus can be found in both throat and faeces for four to seven days, and it has been demonstrated in the blood during the incubation period up to five days before the onset of symptoms (Yoshioka and Horstmann, 1960). Studies in families suggest a minimum incubation period of five or more days. In view of the rash and viraemia it is plain that E.C.H.O. 9 virus invades the body generally and is not confined to the alimentary tract. It is perhaps surprising, therefore, that the nervous system is not involved more often. When, however, this happens there is a sharp rise in temperature, headache, and vomiting, and the child has a stiff neck with perhaps slight rigidity of the thoracic and lumbar muscles as well. Minor changes may be present in the reflexes. In spite of the mildness of the physical signs the C.S.F. shows a brisk pleocytosis with from 100 up to several thousand cells. These are mostly lymphocytes, but polymorphonuclear cells may be prominent in the earliest specimens. The cell count in

the C.S.F. remains abnormal for one to two weeks (Jamieson *et al.*, 1958) although fever subsides rapidly and the duration of illness is relatively brief. There seems no doubt that adults who suffer from E.C.H.O. 9 virus meningitis are more severely ill than children.

Patients with meningitis appear to develop a rash less often than those with minor illness, but a rash was nevertheless seen in nearly half the patients with meningitis in the Milwaukee epidemic (Sabin *et al.*, 1958). All possible combinations of clinical patterns have been described, so that there may be both pure examples of exanthematic illness or of meningitis, or a mixture of both. As in the cases of minor illness, virus is found in the throat and faeces of patients with meningitis during the acute stage of illness. It has also been demonstrated in the C.S.F. in from one-third to one-half of those yielding virus in the stools. An antibody response develops during the course of illness and can be demonstrated either by neutralization or by complement-fixation tests.

Epidemiology of E.C.H.O. 9 Virus Infection

Lyle (1959) estimated that some 3 to 5% of persons in his practice had E.C.H.O. 9 infection during the Lancashire epidemic. Yet the large number of trifling illnesses in infants including transient rashes which were seen in Sheffield in 1960 suggests that the attack-rate by the virus may easily be underestimated. Most observers agree that within families infection is widespread, and Sabin *et al.* (1958) consider that 85% of infections cause clinical illnesses. Hobson *et al.* (1962a) studied 60 families in whom some sort of rash occurred in at least one member at the time when cases of meningitis were occurring in another part of Sheffield. About one-quarter of the index patients from whom viruses were not recovered showed a similar clinical picture (Table II).

TABLE II.—Incidence of E.C.H.O. 9 Virus Infection in 60 Families with Rashes, Sheffield, 1960*

Index patients				Other Members of Same Family with Illnesses			Healthy†	
Type of Rash	No.	Viruses Isolated	No.	No.	E.C.H.O. 9 Isolated	Other Viruses	No.	E.C.H.O. 9 Isolated
Maculopapular	49	E.C.H.O. 9	15	21	9	0	20	1
		Other enteroviruses	1	1	0	1	5	0
		Nil	33	2	1	0	83	0
Morbilloform	4	Nil	4	0	0	0	11	0
Other	7	Nil	7	0	0	0	28	0

*After Hobson *et al.* (1962b).

†Five of 49 specimens from a nursery school for infants examined during this period yielded E.C.H.O. 9 virus though the infants were reported to be well.

E.C.H.O. 9 virus was also recovered from 5 of 49 healthy infants at a nursery school during the same period, so that the virus was undoubtedly prevalent in the area. Neutralizing antibodies were found in contacts of children with a rash, yet were often absent from the sera of adults collected at random.

In Sheffield few adults were affected clinically in 1960, yet in Glasgow (Landsman and Bell, 1962) about a quarter of the cases in 20 affected families were in adults; these usually experienced an influenza-like illness. Though adults can therefore be infected it is likely that a non-humoral form of adult resistance to infection exists in man which is analogous to that exhibited in mice. Unlike most other E.C.H.O. viruses, the E.C.H.O. type 9 will infect suckling mice, but these animals become insusceptible after the fifth day of life. The reason for the occasional involvement of the C.N.S. in man is, however, not apparent. Possibly infection occurring for the first time after infancy may be more

likely to cause involvement of the C.N.S., for the average age of those with rashes is below that of children with meningitis. In any case, it seems probable that an alteration has occurred in the pattern of infection with E.C.H.O. 9 virus in the past which is perhaps analogous to the change in poliovirus infection and which has led to the recurrence of epidemics of aseptic meningitis.

Other Causes of the Exanthematic Syndrome

The occurrence of an illness in children accompanied by a rubelliform rash had been recognized as an infection by a virus found in the faeces by Neva *et al.* (1954) in Boston. The children from whom this virus was recovered suffered from a mild fever and developed a maculo-papular rash on the face, trunk, and limbs. Adults who were affected developed severe headache, but no cases were described of meningitis with a rash. Enders (1956) likened the Boston rash to rubella, roseola infantum, or a heat rash. An outbreak similar to the one in Boston was experienced by Neva (1956) in Pittsburgh in 1954. The agent recovered from these outbreaks of so-called "Boston exanthem disease" proved, however, to be a serologically different virus from the one responsible for the outbreaks of E.C.H.O. 9 infection already mentioned. It is now classified as E.C.H.O. type 16. Illnesses in children exhibiting a macular or rubelliform rash have since been recorded in association with infection with other viruses, including E.C.H.O. types 1, 2, 4, 6, and 14 (Sanford and Sulkin, 1959), and Coxsackie group A virus type 16 (Robinson *et al.*, 1958), type 9 (Lerner *et al.*, 1960), and type 4 (Gear, 1959). Some of these viruses have also been found in the stools and C.S.F. of patients with aseptic meningitis. Thus it appears that many of the E.C.H.O. viruses and also some Coxsackie viruses can cause the meningo-exanthematic syndrome, though knowledge is lacking

concerning the frequency of this rather than of other clinical manifestations.

(b) Neurological Disorders Resembling Poliomyelitis

The realization that viruses of the Coxsackie or E.C.H.O. groups may cause illnesses of a paralytic character resembling poliomyelitis is comparatively recent. It is true that the original isolation of Coxsackie group A viruses was made from patients with typical paralytic poliomyelitis (Dalldorf and Sickles, 1948), but the frequent recovery of polioviruses from the same specimens of faeces has indicated a dual infection (Dalldorf *et al.*, 1959). Dalldorf and Wiegand (1958) have stressed this association of polioviruses with Coxsackie A viruses, and it has even been suggested that the latter might enhance the effect of the former. Paralytic illnesses resembling poliomyelitis were therefore not usually attributed to Coxsackie viruses alone until the description of a fourth type of poliovirus

by workers in the U.S.S.R. in 1956 (Chumakov *et al.*, 1956). This virus, which was recovered from patients with paralysis, was later shown to be Cocksackie A7 (Johnsson and Lundmark, 1957), an agent which after intracerebral injection produces lesions of the spinal cord in monkeys resembling those of poliomyelitis. This virus was also recovered from patients with a poliomyelitis-like disease in the U.S.A. (Ranzenhofer *et al.*, 1958) and in Scotland (Grist, 1960; Combined Scottish Study, 1961).

In contrast to the reluctance to regard Cocksackie group A viruses as being responsible for neurological lesions of the cord in man, Cocksackie B viruses have been frequently incriminated in sporadic cases with muscular paresis or actual paralysis (Curnen, 1960; Stern, 1961). The possibility of a simultaneous infection by both Cocksackie B virus and a poliovirus in such cases can probably be dismissed. It may, of course, be difficult to demonstrate poliovirus if the subject has been immunized with poliovirus vaccine or if serological evidence has to be depended upon. But Dalldorf (1951) has shown that Cocksackie B viruses interfere with poliovirus infection in experimental animals, thus contrasting with the action of Cocksackie A viruses. Nevertheless it would probably be incorrect to attribute much of the toll of paralytic disease resembling poliomyelitis to either groups of Cocksackie viruses, and a similar conclusion applies also to paralytic disease due to E.C.H.O. virus infections.

It has, however, been observed that transient muscular weakness is not uncommon in patients with aseptic meningitis, particularly during epidemic prevalence. This was noted during an E.C.H.O. 6 outbreak by Kibrick *et al.* (1957) and also by Sabin *et al.* (1958) during the E.C.H.O. 9 epidemic in Milwaukee. In the comprehensive study in California of 358 cases of paralytic poliomyelitis a total of 41 were found with evidence of infection by non-poliomyelitis enteroviruses only (Magoffin *et al.*, 1961). The paralysis was only mild or moderate in these cases and there was no fatal bulbospinal case such as that of the 2-year-old infant reported by Steigman *et al.* (1953) in whom E.C.H.O. 2 virus was recovered from the spinal cord. Steigman and Lipton (1960) have also reported a similar case in an infant due to E.C.H.O. 11.

Caution is needed, however, in attributing serious neurological lesions solely to E.C.H.O. viruses. The case of fatal encephalomyelitis recorded by Verlinde and Wilterdink (1958) yielded an E.C.H.O. 9 virus from the medulla, but subsequent study (Verlinde *et al.*, 1961) showed type II poliovirus as well in the same specimen. In conclusion, the full role of Cocksackie and E.C.H.O. viruses in relation to paralytic or encephalitic illnesses may well become more apparent as poliomyelitis is brought under control by immunization. The potentiality of a more widespread attack upon the C.N.S. than the superficial disease seen in aseptic meningitis undoubtedly exists for some at least of the enteroviruses and might at some time become a serious threat if a more virulent variant virus ever arose (Steigman, 1958).

(c) Non-neurological Syndromes

There remain for consideration certain relatively minor illnesses which form the opposite end of the clinical spectrum of enterovirus infection from that represented by neurological disease (Table III). These minor illnesses include herpangina (Zahorsky, 1924), in

TABLE III.—Known Association of the Enteroviruses with Disease

Syndrome	Viruses Concerned
Neurological : Paralytic poliomyelitis } .. Encephalomyelitis .. Aseptic meningitis ..	Polioviruses ; Cocksackie A7 ; other enteroviruses ? Polioviruses ; Cocksackie A and B ; E.C.H.O. viruses ; non-enteroviruses—mumps, L.C.M., herpes Cocksackie A and E.C.H.O. viruses
Meningo-exanthematic syndrome	
Non-neurological syndromes : Herpangina Pyrexial illnesses Bornholm disease (pleurodynia) .. Infantile myocarditis (adult pericarditis) Gastroenteritis Acute respiratory illnesses ..	Cocksackie A Cocksackie A or B Cocksackie B Cocksackie B E.C.H.O. 11, 14, 18 { E.C.H.O. 11, 20, 28 { Coe (Cocksackie A21)

which fever, sore throat, and vesicles on the palate constitute the clinical picture, and Bornholm disease or pleurodynia (Daae, 1872; Sylvest, 1934). Herpangina is seen during community outbreaks of Cocksackie A virus infections, but it may also occur in sporadic fashion or as family outbreaks. Subclinical illnesses are common in such outbreaks (Cole *et al.*, 1951). Clinical variants undoubtedly exist, as, for instance, the illness produced by Cocksackie A 16 which both in Toronto (Robinson *et al.*, 1958) and in England (Alsop *et al.*, 1960) was associated with a vesicular rash on the hands and feet as well as ulcers in the mouth. Minor illnesses due to Cocksackie B virus are often recognizably similar to pleurodynia in which myalgia is a dominant symptom, but a vague febrile illness with headache, vomiting, and abdominal pain has also been seen (Kenyon *et al.*, 1952). Community epidemics of Cocksackie B virus infection have been encountered in many countries, and during such outbreaks aseptic meningitis occurs in a small proportion of cases. A quite different clinical attack due to Cocksackie B viruses is seen in newly born infants. This is a severe fatal myocarditis which was first reported in South Africa (Montgomery *et al.*, 1955; Javett *et al.*, 1956). It has also been suggested that some cases of the syndrome of benign pericarditis are due to Cocksackie B infection in young adults (Fletcher and Brennan, 1957; Null and Castle, 1959).

The E.C.H.O. viruses present a more obscure problem from the standpoint of the clinical spectrum of illness. It may prove to be the case that a large proportion of the infections due to these agents are trifling undistinctive illnesses with a transient rash, but not enough studies of an epidemiological character have yet been made. A number of authors have sought to incriminate E.C.H.O. viruses in cases of gastroenteritis, and particularly in babies and infants. This was first suggested by Ramos-Alvarez and Sabin (1956), and a number of outbreaks have now been described involving E.C.H.O. type 11 (Klein *et al.*, 1960), type 14 (Lépine *et al.*, 1960), and type 18 (Eichenwald *et al.*, 1958). Most cases have been in very young infants, but the type 11 outbreak occurred in adult laboratory workers. When the habitat of the enteroviruses is taken into account it is extraordinary that gastro-intestinal symptoms are so relatively uncommon during enterovirus infection. It is true to say that studies on the commoner outbreaks of gastroenteritis of babies and the winter vomiting syndrome have failed to yield viruses of either the Cocksackie or the E.C.H.O. group.

Finally, there is the role of the enteroviruses in the production of acute respiratory illnesses. Until recently it was believed that Cocksackie viruses rarely caused outbreaks in which respiratory-tract symptoms were

dominant. However, Paffenbarger *et al.* (1959) drew attention to an outbreak of Coxsackie B virus in a boys' camp in 1957 which was characterized by upper respiratory as well as gastro-intestinal symptoms. Coxsackie B 2, 3, and 5 have also been noted as a similar cause of upper respiratory symptoms in naval recruits. The Coxsackie A viruses were also not regarded as a cause of respiratory symptoms apart from herpangina until the Coe virus recovered by Lennette *et al.* (1958) was recently identified as possessing the Coxsackie A 21 virus antigen. The Coe virus appears to cause primarily illness of the upper respiratory tract without rash or neurological signs. It produces in volunteers a "cold-like" illness (Parsons *et al.*, 1960). Yet the prototype strain of Coxsackie A 21 virus was recovered from stools.

Even more remarkable has been the uncovering of the role of certain E.C.H.O. viruses in producing respiratory illnesses. Excluding E.C.H.O. 10, which is no longer regarded as an enterovirus, three types, E.C.H.O. 11, 20, and 28, are now accepted as causes of respiratory illness. The diagnosis of their infection requires differentiation from that due to influenza and the adenoviruses. One (E.C.H.O. 28) behaves biologically in ways resembling the viruses recovered by Tyrrell and Parsons (1960) from typical common colds in adults. Thus the question which now exists is whether the latter viruses should properly be classified as enteroviruses even though they have not been recovered from faecal specimens. To say more would at this stage raise matters better dealt with in my second lecture on various respiratory syndromes due to viruses. I have, however, said enough to indicate the great difficulties which exist in the classification of the array of new viruses discovered by means of the technique of tissue-culture cultivation.

It is perhaps sad to reflect that virologists interested in taxonomy have rejected clinical manifestations from consideration when attempting to define the characteristics of the various new viruses. This is because of the variable characters of the human infection and the frequent similarity in clinical picture of illnesses due to quite different viruses. Size of virus particles, type of nucleic acid, and properties such as ether-resistance or ability to form haemagglutinins are preferred to pathological effects. When one remembers the complex characters of host and virus which combine to produce the latter, one is compelled to acquiesce. Perhaps it is fortunate that we do not have 59 different illnesses to correspond with the 59 enteroviruses.

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GASTRO-INTESTINAL-SPECIFIC ANTIGEN: AN IMMUNOHISTOLOGICAL AND SEROLOGICAL STUDY

BY

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Fine histochemical differences between the tissues of the body may be demonstrated by immunohistological methods. Thus immunofluorescence offers a means of studying microscopically certain characteristics which distinguish one organ from another, so-called organ-specificity, and the technique has already been used for this purpose by several workers (reviewed by Nairn, 1962). The present investigation is concerned with such a demonstration of an antigenic component specific for gastro-intestinal mucosa; the antibody employed for its detection was produced by immunizing rabbits with microsomal material from human colon mucosa (Nairn *et al.*, 1961).

Methods

The same general methods of cell fractionation, anti-serum preparation, and immunological study were used as in previous investigations of organ-specific antigens in human skin, rat liver, and hamster kidney (Nairn *et al.*, 1960). The antigen was obtained from mucosa scraped from normal areas of fresh specimens of human colon removed at operation for carcinoma. The scrapings were suspended in chilled buffered physiological saline (0.01 M phosphate, pH 7.1) and homogenized for four minutes at about 10,000 r.p.m. in an M.S.E. homogenizer; further cell disruption was obtained by two to three minutes' treatment with a 60-watt M.S.E.-Mullard ultrasonic disintegrator. Sucrose was added to the suspension to make a 0.25 M solution, and the cell components were separated by differential centrifugation. The washed microsomal fraction, resuspended in buffered saline at a concentration of 4 mg. of nitrogen per ml., was used as antigen throughout.

Antiserum was prepared in three 2.5-kg. rabbits by intravenous injection of 0.5 ml. of antigen at intervals of two or three days for three weeks. The antigen, which was toxic, caused *in vivo* coagulation of the blood and was capable of killing a rabbit at the dosage used for immunization; to avoid this hazard it was mixed with heparin (500 I.U./ml. antigen) and given slowly. The total dose of antigen N given to each rabbit was 20 mg. The animals were bled six days after the last injection and each produced antibody of high complement-fixing titre: 1/600 in two and 1/1,200 in the third, which was used in the main experimental study. Complement fixation was carried out against 8 µg. of antigen N with 3 minimum haemolytic doses (M.H.D.) of complement in the test and 2 M.H.D. in the controls. Fixation was carried out at 4° C. for one and a half hours followed by one hour at 37° C.; the haemolytic system was then added and the results

The Research Defence Society helps in various ways to combat attacks by antivivisectionists against medical and veterinary research workers. It has recently produced a pamphlet entitled "The Use of Vaccines in Controlling Disease in Domestic Animals." By means of simple questions and answers this describes for the layman the reasons why domestic animals need to be vaccinated, the diseases against which they need protection, and the methods of vaccination. It is obtainable (6d. net) from the Society at 11 Chandos Street, Cavendish Square, London W.1.