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VIRUS DISEASES*

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At the beginning of this century the term "virus disease" was hardly known: to-day a mass of knowledge has been acquired in regard to virus infections, even if, for the moment, it is impossible to give any precise definition of a virus. Man is the victim of at least thirty-five proved virus diseases, while the list of viruses affecting bacteria, plants, insects, fish, amphibia, birds, and mammals other than man runs into several hundreds. McKinney (1937) estimates that there exist nearly a hundred variants of tobacco mosaic virus, while those producing potato diseases are also very numerous.

Properties of Viruses

Despite the range of hosts affected, all viruses possess certain properties in common. Viruses were first recognized because they passed through porcelain filters which retained the majority of bacteria. By means of collodion filters, such as the gradocol membranes prepared by Elford (1929), in which the size of the pores is graded and uniform, it is now possible to estimate the size of infective virus particles with considerable accuracy. The size of these particles ranges from that of the smaller bacteria to something only a little larger than protein molecules.

The second characteristic property of the viruses is their failure to grow except in the presence of living cells. They appear, in fact, to be obligatory intracellular parasites. The organisms isolated by Barnard (1935), Laidlaw and Elford (1936), and Seiffert (1937) from sewage, earth, humus, and similar materials, and grown on simple media, in certain phases resemble virus particles, but are more closely related to the causal agents of pleuropneumonia and agalactia, and must be classed as bacteria among the Actinomycetaceae (Ledingham, 1933). Many viruses are very resistant to chemical agents such as glycerol and to desiccation. A strain of yellow fever virus dried for seven and a half years was still highly pathogenic for rhesus monkeys. Viruses, both those affecting animals and those affecting plants, show considerable powers of variation. Such variations may occur spontaneously or may be brought to light by change in the environment (Findlay, 1936a). The appearance of new strains is of importance in the epidemiology of virus infections.

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The Nature of Viruses

Two theories are now current as to the nature of viruses: the first regards viruses as minute organized living entities, one generation being directly descended from the previous generation; the second regards viruses as autocatalytic substances which when introduced into susceptible cells alter their metabolism in such a way that the cells produce further amounts of the autocatalytic substance. The origin of the autocatalytic substance is uncertain, but it is thought to be due to some non-specific stimulus applied to the cell.

The first evidence that viruses are granular particles is usually credited to Paschen, who in 1906 described, in material from vaccinia and small-pox, corpuscles which he believed to be the causal agents of these diseases. Similar bodies had, however, been described in 1887 by Buist of Edinburgh. In addition to the animal-pox diseases, a number of virus infections are now known in which elementary bodies have been demonstrated of such dimensions as to be visible with the highest powers of the ordinary microscope. Among such virus infections affecting man are psittacosis, trachoma, inclusion conjunctivitis, molluscum contagiosum, lymphogranuloma inguinale (poradenitis venerea), varicella, zoster, herpes, and influenza, while Steinmaurer (1938), using fluorescence microscopy, claims to have demonstrated the elementary bodies of rubella and mumps. The aetiological significance of elementary bodies is based on the following findings: (1) Their demonstration in tissue lesions and exudates by staining methods, ultra-violet light, or fluorescence microscopy. (2) Production of the disease in susceptible animals by the injection of suspensions of elementary bodies. (3) Multiplication of elementary bodies outside the body by growth either in association with tissue cultures, in flasks containing serum, Tyrode's solution, and embryonic cells, or on the chorio-allantoic membrane of the developing chick embryo. (4) The infectivity of suspensions of elementary bodies can be neutralized by the serum of animals or men recovered from the disease; the elementary bodies may also be specifically agglutinated or may fix complement with immune serum. (5) The elementary bodies of psittacosis, lymphogranuloma inguinale, trachoma, inclusion conjunctivitis, and very probably variola and vaccinia, pass through a developmental cycle recalling that of certain

protozoa. The evidence that at least certain viruses are minute corpuscles is thus practically conclusive.

The autocatalytic theory of viruses, dating back to a suggestion put forward by Beijerinck (1898), received little attention till Stanley (1935, 1936) salted out from the tissues of tobacco and cucumber plants infected with tobacco mosaic virus fine needle-shaped rods which, though composed of chemically pure protein, were capable of inducing infection even after repeated redissolving and reprecipitation. There is doubt whether the rods are true crystals, but from tomatoes infected with the bushy stunt virus Bawden and Pirie (1938) have recently obtained twelve-sided protein crystals which continue to be infective even after repeated recrystallization. The virus proteins isolated from three strains of tobacco mosaic (Bawden and Pirie, 1937a), from two strains of cucumber mosaic virus (Bawden and Pirie, 1937b), and from tobacco ringspot and potatoes infected with X virus (Stanley and Wyckoff, 1937), whether appearing as true crystals or not, belong to the group of heavy proteins, and are almost certainly nucleoproteins. Eriksson-Quensel and Svedberg (1936) calculate the molecular weight of tobacco mosaic protein as between 15 and 20 million. Heavy proteins may, however, be isolated from both normal plant and animal tissues, but in plants infected with tobacco mosaic the amount of the heavy protein is very considerably increased. It was at first believed that the proteins acted merely as carriers of the virus, but as anything which denatures or destroys the proteins also destroys the infectivity of the virus, it is probable that virus activity is inherent in the protein. In birds and mammals no conclusive evidence has yet been obtained that viruses are heavy proteins and nothing more, although the viruses of equine encephalomyelitis and papilloma of rabbits are closely associated with heavy proteins. In silkworms, however, there exists a virus infection—polyhedral disease—in which, in addition to minute infective granules, large mono-refringent crystals appear in the blood and certain cells. An antiserum which agglutinates the infective granules also agglutinates the crystals even after repeated washing, suggesting that the crystals are very closely related to the virus granules (Gratia and Paillet, 1938).

The demonstration that paracrystalline or crystalline proteins can produce disease raises questions of great interest. It has been suggested that these virus nucleoproteins are themselves enzymes—proteases—but no enzyme is yet known to be a nucleoprotein, and the molecular weight of the virus proteins is considerably greater than that of the known enzymes. On the other hand, there is evidence to suggest that, at any rate in the case of the tobacco mosaic protein, the virus is a submicroscopical elongated structure composed largely or entirely of liquid crystalline nucleoprotein, these structures tending to become orientated by streaming or standing to produce the double refraction which is observed (Rawlins and Takahashi, 1938). The question has also arisen whether such virus proteins arise by "spontaneous generation" as a result of some error in cell metabolism: no conclusive evidence has been brought forward in favour of such a view (Dale, 1935; Laidlaw, 1938), nor with any profit is it possible to discuss whether virus proteins are living or dead. Life can only be defined as a summation of a number of activities, and of these activities virus proteins possess the powers of assimilation, reproduction, and variation. Similar activities are, it may be added, possessed by the genes (Gowen, 1937).

It is thus obvious that under the general term of virus are included agents varying in size, structure, and complexity: nevertheless all are capable of causing infectious diseases and have a life history of their own.

The Spread of Virus Infections

Virus infections may be spread either by direct contact, as in the case of infective warts and molluscum contagiosum, or during coitus, as in the case of lymphogranuloma inguinale and coital exanthema of cattle. Droplet infection plays a part in the spread of diseases such as influenza, the common cold, poliomyelitis, and measles, while inhalation of dried virus particles is also probable, especially in association with fomites. The role of contaminated clothes, especially furs, in the epidemiology of small-pox was recognized certainly as early as the sixteenth century. Dried faecal material from parrots and possibly fulmar petrels (Rasmussen, 1938) transmits the virus of psittacosis, as shown by the occurrence of the disease in persons who have never come in contact with parrots but have cleaned their cages. Infection with swine fever virus is carried by infected carcasses, while viruses may remain active in animal tissues used for other inoculations. A few years ago an outbreak of foot-and-mouth disease occurred in Detroit the origin of which was finally traced to vaccinia virus imported some years previously. Rabid animals transmit rabies by their bites, the species usually infected being dogs, hyenas, and meerkats; in Trinidad the vampire bat *Desmodus rotundus murinus* acts as a carrier of the virus without showing symptoms (Pawan, 1936). Many viruses are transmitted by the bites of insects (cf. Findlay, 1936b). Under natural conditions yellow fever and dengue are the most important diseases transmitted by aedine mosquitoes, but laboratory and epidemiological evidence suggests that in the United States of America both the eastern and western strains of equine encephalomyelitis are also transmitted to man by the bites of aedine mosquitoes (Eklund and Blumstein, 1938; Wesselhoeft *et al.*, 1938). The same insects very probably play a part in the spread of St. Louis encephalitis (Casey and Broun, 1938) and Japanese Type B encephalitis (Inada, 1937). In this country the only virus disease carried by an insect is louping-ill, a disease of sheep, which is transmitted by the tick *Ixodes ricinus*, L.; man is occasionally infected. A number of plant viruses are transmitted by insects such as aphides and thrips. The fact that the climate of Scotland is too cold to allow *Myzus persicus* to flourish is the explanation why Scottish seed potatoes are not infected to the same degree with viruses as those grown in England. In the case of plant viruses transmitted by insects there is as yet no conclusive evidence that the viruses multiply in the insect: in *Aedes* sp. infected with yellow fever and equine encephalomyelitis multiplication of virus undoubtedly takes place.

Action of Viruses on Cells

Since viruses are obligatory intracellular parasites their action on the cells is especially noticeable. The cells affected by a particular virus are not, however, necessarily those present at the portal of entry of the virus. The portals of entry include the skin, mucous membranes, and placenta. Certain viruses, such as those of warts, molluscum contagiosum, herpes, and lymphocytic choriomeningitis, may enter through the lightly scarified skin. Other viruses, such as rabies and B virus, are introduced by the trauma caused by the bites of animals. All the viruses transmitted by biting insects also, of course, enter

by the skin. Mucous surfaces are readily permeable to viruses, even in the absence of trauma. The viruses which enter by these portals are: conjunctiva—trachoma, inclusion conjunctivitis, herpes, lymphocytic choriomeningitis; oral mucosa—dog and rabbit warts, mumps; nasopharyngeal mucosa—measles, acute coryza, variola, varicella, and the common cold (poliomyelitis appears to infect the olfactory nerve endings or to enter through traumatic wounds such as those caused by tonsillectomy and tooth extraction); respiratory mucosa—influenza, psittacosis, and laryngotracheitis of fowls; genital mucosa—warts, lymphogranuloma inguinale, herpes, and exanthema pustulosum coitale of cattle. Infection through the alimentary canal is uncommon. The viruses of polyhedral and wilt diseases of caterpillars, however, enter through the alimentary tract, as does swine fever virus. Unpublished observations show that rhesus monkeys can be infected with yellow fever by passing the virus direct into the stomach through a rubber catheter. Although by direct inoculation of virus into the lumen of the intestine it is possible to infect monkeys with poliomyelitis, it is generally believed that the acidity of the human gastric juice is sufficient to destroy the virus. Placental transmission to the embryo may occur in variola, varicella, Rift Valley fever, and rinderpest. A point of interest is that a virus pathogenic by one portal of entry may be relatively avirulent when injected by another. The fixed strain of rabies virus, for instance, is innocuous when injected subcutaneously, but fatal if injected intracerebrally.

When once viruses have entered the body they may infect the cells at the point of entry, as in warts and molluscum contagiosum, or may travel to particular cells for which they are highly pathogenic. The more usual paths of spread involve the blood stream, as in yellow fever; the lymphatics, as in lymphogranuloma inguinale; the nerve fibres, as in herpes, Borna disease of horses, poliomyelitis, and rabies. Certain viruses, such as those of warts and molluscum contagiosum, spread on skin or mucous surfaces by auto-inoculation.

The fact that rabies virus passes along peripheral nerves to the brain probably accounts for the lengthy incubation period of the disease, sometimes as long as eight months. Very long incubation periods have, however, been noted in other virus diseases. In man, for instance, the incubation period of infective warts may extend to eight months (Findlay, 1930). After experimental infection of animals with Borna disease the incubation period in the rabbit may be as much as 161 days, and in the guinea-pig 350 days (Nicolau and Galloway, 1928). Perdrau (1938) reports the onset of a fatal herpetic encephalitis in rabbits which were immunized six months previously with living virus, while in mice injected intracerebrally lymphogranuloma inguinale virus may not produce clinical symptoms for ninety-four days (Findlay, 1933). Scrapie, a virus disease of sheep, has recently been found to have an incubation period of at least sixteen months (Cuillé and Chelle, 1938). Neither the exact location of the virus during the latent period nor the cause of its sudden activity has yet been investigated. Very short incubation periods have also been noted in virus infections. The common cold appears to have an incubation period of forty-eight hours; while, in mice, Rift Valley fever virus may induce symptoms in thirty-six and death in forty-eight hours after intraperitoneal inoculation. Physiological conditions to some extent influence the length of the incubation period both in mammalian and in arthropod hosts. In the hedgehog infected with foot-and-mouth disease during hibernation the period may be considerably increased (Hulse and

Edwards, 1937), while in the mosquito *Aedes aegypti* infected with yellow fever the interval before it becomes able to transmit the virus by bite is dependent on the external temperature.

When a virus becomes active within a cell it produces one of two lesions: either the cells may be stimulated to divide so that the tissues become hyperplastic, or cell metabolism may be so severely damaged that rapid necrosis is inevitable. Instances of intense necrosis are to be found in the liver of man and animals infected with the viruses of yellow fever and Rift Valley fever or in the anterior horn cells of the cord in poliomyelitis. Instances of hyperplasia are the papillomata caused by infective warts of man and many animals, the skin nodules produced in birds by the fowl-pox viruses, and the fibromatous growth induced in rabbits by the filterable fibroma virus. In many hyperplastic conditions the primary hyperplasia is eventually followed by necrosis, but in certain cases the hyperplasia is indistinguishable from that of a true neoplasm. The Rous sarcoma of hens and the other filterable tumours of this species, as well as fowl leukaemia, simulate closely neoplastic formations in man, except that they can be produced by the injection of cell-free material. In frogs, also, malignant adenocarcinomata in the kidneys appear to be due to a virus (Lucké, 1938), while the filterable papillomata of rabbits may not infrequently undergo malignant changes: when, however, a malignant change has occurred it is no longer possible to obtain the virus from the tissues. It is obvious that although viruses may be one of the exciting causes of malignancy, their exact relation to the neoplastic change is still obscure.

Specific Action of Viruses

Many viruses exhibit considerable specificity in regard to the tissues in which they produce lesions. Thus warts affect only the skin and squamous mucous membranes; poliomyelitis attacks only nervous tissues, and is therefore said to be neurotropic; others, such as herpes and yellow fever, produce lesions in tissues derived from all three embryonic layers, and are said to be pantropic. The specificity displayed by many viruses, not only in regard to their hosts but also as to the particular tissues they parasitize, is in striking contrast to the ease with which the majority of viruses can be cultivated outside the body in association with embryonic cells, derived from animal species which ordinarily may be quite insusceptible. Since viruses grow within cells, it is pertinent to inquire whether specific cytological changes occur as a result of their growth. At an early stage in the study of virus diseases bodies distinct from the ordinary cell structures were observed in both the cytoplasm and nuclei of infected cells. Certain cytoplasmic bodies, such as those occurring in psittacosis, fowl-pox, vaccinia, and molluscum contagiosum, are now recognized as virus colonies, made up of masses of elementary bodies. The status of other cytoplasmic inclusions, such as the Negri bodies in rabies, is still uncertain, while no definite conclusions can yet be drawn as to the nature of the amorphous red-staining bodies formed within the nuclei in many virus infections. One view regards them as agglutinated masses of submicroscopical elementary bodies, but the fact that similar inclusions may be formed by the injection of chemicals such as lead suggests that they are more probably caused by flocculation of the nuclear colloids.

In association with the action of viruses on particular cells clinical symptoms develop, their severity depending partly on the rapidity of action of the viruses, partly on the number of cells invaded. Thus an infection with the virus of warts produces no clinical symptoms, but an

infection with the virus of yellow fever associated with necrotic changes in liver and kidney causes acute toxæmia. It should, however, be emphasized that the severity of all virus infections varies within wide limits and "inapparent" infections are extremely common. The importance of these subclinical cases in the epidemiology of virus diseases is very great.

Excretion of viruses from the body follows the rupture and breakdown of infected cells. In those diseases that are transmitted by biting insects the virus is, of course, picked up from the blood stream while the insects are feeding, but in the majority of infections the viruses are actually excreted from the body. Thus in variola and varicella the viruses are present in the skin lesions; in poliomyelitis, influenza, the common cold, and measles, in the secretions of the nasal and respiratory mucosa; in rabies, mumps, herpes, and virus B, in the saliva; in psittacosis, fowl pest, and possibly poliomyelitis, in the faeces; in lymphocytic choriomeningitis, in the urine of mice.

Immunity in Virus Infections

Virus diseases such as lymphogranuloma inguinale, which produces rectal stricture and elephantiasis of the vulva, run a chronic course; the majority are more acute, and end either in death or in the development of immunity. Immunity may be life-long, as in yellow fever, or of a few weeks' duration, as in herpes and the common cold. The mechanism of virus immunity is still in dispute. In most virus infections, virucidal immune bodies, agglutinins, and complement-fixing antibodies may be demonstrated, but in some diseases serum-immune bodies are either undemonstrable or are present only in very small amounts. Nevertheless a strong immunity exists. This immunity, it is thought, must reside actually in the tissue cells, since in many conditions immunity appears to be linked with the persistent presence of virus in the tissues. In birds that have recovered from psittacosis the virus is excreted in the faeces certainly for eighteen months (Berry and Rivers, 1935); the virus of poliomyelitis has been found in the nasopharynx of convalescents four months after an attack (Lucas and Osgood, 1913); and herpes virus may be present in the saliva and occasionally in the cerebrospinal fluid of apparently healthy individuals. Vaccinia virus has been obtained from the testicles of rabbits 114 days after inoculation into the skin (Olitsky and Long, 1929); lymphocytic choriomeningitis virus remains for 120 days in the brains of mice; and lymphogranuloma inguinale virus has been isolated from the genital tract for one and a half years (Caminopetros, 1935). Other records of virus carriers are swine fever ninety-four days, rinderpest 177 days, and laryngotracheitis of fowls 741 days (Gibbs, 1933). The vampire bat may carry rabies virus in its saliva for some months; while in infectious anaemia of horses the blood, after recovery from an acute attack, contains the virus certainly for fourteen years (Schalk and Roderick, 1923) and probably for life. Virus carriers obviously play an important part in the spread of infection. On the other hand, it must be confessed that in some diseases with a strong immunity attempts to demonstrate residual virus have so far failed. Further knowledge of the mechanism of immunity in virus diseases is essential, for in only two virus infections, lymphogranuloma inguinale and trachoma, is there evidence that specific drugs are of value: once viruses have entered cells they cannot be destroyed by the use of specific immune serum. Prophylactic immunization is therefore the best method of protecting against virus diseases, but so far the only infections against which methods of active

immunization are available include variola, rabies, and yellow fever among human diseases; rinderpest, canine distemper, louping-ill, feline enteritis, and fowl-pox among animal diseases.

In this short summary it has obviously been impossible to touch on all the problems involved in virus diseases: the role of animals in transmitting infections to man; the encephalitis which may follow vaccinia, mumps, measles, varicella, rubella, or zoster; and the nature of virus variations.

It must be remembered that in addition to the known virus infections of man other diseases occur in which there is presumptive evidence that a virus may be the cause, such as rheumatism, infective hepatitis, epidemic myalgia, encephalitis lethargica, pemphigus, erythema multiforme, glandular fever, and aphthous stomatitis. In the elucidation of these conditions the general practitioner must play an important part, for he it is who alone sees these diseases in their early stages, when, if ever, viruses may be successfully isolated.

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The annual malaria control course for laymen (engineers, planters, etc.) will be held at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, W.C., from June 26 to 30, under Sir Malcolm Watson and the staff of the Ross Institute. The course includes instruction on mosquitos and their habits, drainage, and other measures for the prevention of malaria. The course is free, and applications to attend should be sent in as early as possible to the organizing secretary at the Institute.