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LATENT INFECTIONS WITH VIRUSES AND RICKETTSIAE*

BY

M. G. P. STOKER, M.D.

Huddersfield Lecturer in Special Pathology, Department of Pathology, Cambridge University

[WITH SPECIAL PLATE]

The laboratory diagnosis of virus diseases is to-day somewhat complicated by the embarrassing number of new agents which have been isolated through increasing use of human and monkey cells in tissue culture. One of the principal problems is to establish a causal connexion between these new viruses and particular clinical syndromes. A number are still unattached and continue to deserve the name "orphan virus" originally used by Melnick (1955).

Some of the newly discovered viruses are clearly transient visitors, whether or not they are associated with disease. Certain types, however, appear to persist as latent infections in the tonsils and adenoids (Rowe *et al.*, 1953). Latent infection raises special problems, not only in its relationship to illness, but also in the mechanism by which obligate intracellular organisms can survive for long periods in one host. A review of this topic should not be limited to latent virus infections of man, however, because there is much to be learnt from similar conditions of animals, birds, and even bacteria.

In this paper "latent infection" will be used to mean the persistence of a virus or rickettsia in the tissues for long periods, during most of which there is no obvious manifestation of disease. This will not be taken to include the incubation period even when it is long, as, for example, in infective hepatitis.

Our knowledge of latent viral and rickettsial infections of man is, of course, limited by our inability to search the tissues for the hidden organism. We are dependent upon the reappearance of virus in the blood or vesicle fluid or in some other situation where it can be isolated and detected by laboratory methods, or where its presence can be deduced through secondary infection of contacts. An inevitably different approach has, however, revealed the presence of latent viruses in the tissues of wild and laboratory-bred animals.

Latent Infections of Animals

Attempts to isolate the causative organism from suspected virus diseases of man often involves inoculation of experimental animals by various routes with a number of so-called "blind" passages of tissue suspension from one group of inoculated animals to the next. After one or more such passages the animals may sicken

and a filterable agent be obtained from the tissues which will cause reproducible disease in other animals of the same species. A great many organisms isolated in this way have turned out to be viruses lying dormant in the stock animals, which are activated by passage of tissue suspensions. Sometimes salt solutions or other simple diluents will activate these viruses, rather like the non-specific stimuli which activate herpes simplex in man. These latent infections of laboratory animals naturally add to the difficulties of virus research. Eaton (1950), in his attempts to isolate the causative agent of primary atypical pneumonia by intranasal inoculation of cotton-rats and hamsters, identified three different latent viruses in the lungs alone of his stock animals. A number of latent virus infections have also been found in the lungs of mice and guinea-pigs. Such infections are not confined to the respiratory tract, however. There is, for example, spontaneous encephalomyelitis of mice, sometimes called mouse poliomyelitis, and originally investigated by Theiler (1937).

Mouse Encephalomyelitis

Theiler's virus is almost universally present in the intestinal wall of laboratory mice between the first and sixth months of life and sometimes longer; in fact it is difficult to obtain for experimental work a line of mice which are free of the virus. Infection apparently occurs through exposure of the baby mice to faecal contamination, and not *in utero* or through the milk. When carrying Theiler's virus the mice are healthy, and it may be regarded for the most part as a harmless commensal infection, but occasionally the virus invades the central nervous system and produces a paralytic disease.

Lymphocytic Choriomeningitis

Another condition of mice which has considerable bearing on our problem is lymphocytic choriomeningitis, which, of course, can occasionally spread to man. Traub (1939) at the Rockefeller Institute studied a stock of mice in which this virus was originally absent but gradually became established naturally over several years. At the end of the period of study all the mice, including the newborn, carried the virus in large numbers in their tissues and, except for an occasional illness in young animals, remained perfectly healthy. Infection was transmitted to the young *in utero* and persisted throughout life. Of special interest was the complete absence of antibody development in animals infected with the virus *in utero*, although antibodies appeared in the usual way after infection of older virus-free mice from another stock. It was suggested by Burnet

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and Fenner (1949) that this was due to failure to recognize as antigenically foreign substances previously present in foetal life. The work of Billingham *et al.* (1953) with skin grafts in mice has led to the full confirmation of this important principle of "immune tolerance."

The adaptation of lymphocytic choriomeningitis virus to the mouse is the most complete that has been identified. The discovery depended, however, on the availability of other stock mice which were free of this virus and susceptible to infection. If such an agent was universally present in mice, and if no other host were susceptible, it might never be detected even if it occasionally produced obvious disease.

Latent infections are not confined to small rodents. The virus of swine influenza, whose complicated life history would surprise even the students of parasitic worms, was shown by Shope (1941) to lie dormant in a lungworm in the pig tissues, and it is only activated by a stimulus such as a superadded bacterial infection with *Haemophilus influenzae*. Monkeys are known to suffer from their own variety of herpes which persists in the same way as human herpes, while the recent large-scale use of monkey cells for growth of poliomyelitis virus has yielded a number of other viruses from the tissues of apparently healthy animals.

Rickettsial Infection

Rickettsiae, which resemble viruses in their intracellular habitat, characteristically persist in their natural or laboratory hosts long after the original infection. When rats or mice are inoculated with typhus rickettsiae the organisms can be re-isolated from the brain for periods up to at least a year despite the presence of antibody.

Rickettsia burneti, the causal agent of Q fever, also remains alive in the tissues of animals for long periods after the initial acute infection, and this has considerable bearing on the natural history of Q fever. Unlike other rickettsiae, this organism is maintained in large domestic animals such as cows and sheep. Its spread between these animals and to man depends not on ticks or other arthropods, but upon massive excretion at parturition in the placenta and birth fluids, and then in the milk. Animals are susceptible to infection at any time whether pregnant or not, but after a short generalized infection the organism becomes quiescent. Although it can be isolated from the spleen and kidneys, no transmission occurs. This latent state continues perhaps for years, until the animal becomes pregnant. Towards the end of pregnancy the infection is activated and the rickettsiae multiply in enormous numbers in the placenta until at parturition they are shed and are potentially able to infect other animals and occasionally man.

Psittacosis

This virus also persists as a latent infection in a great variety of wild and caged birds in a rather similar way: shedding of virus in the excreta occurs at certain periods of physiological stress, and particularly at nesting time, thus causing infection of the young birds. Some of these die, but the survivors develop latent infection and the cycle is continued. Many of the latent viruses in the lungs of laboratory animals, which have been mentioned above, also belong to the psittacosis group.

The Tumour Viruses

It is not the purpose of this paper to consider the part played by viruses in the aetiology of tumours. In certain neoplasms of mice, however, a virus-like agent clearly plays some part in a fashion which implies long-continued latent infection. In strains of mice with a high incidence of cancer of the breast the milk factor, or Bittner factor, is transmitted to the offspring by suckling and carries with it the predisposition to develop cancer of the breast in middle life. The agent is filterable and particulate, and present in the breast and other tissues as well as the milk from mice of the high-cancer strain before the age at which cancer normally develops. It has many of the characteristic properties of viruses, but like lymphocytic choriomeningitis

it remains quiescent in the cells of the mouse throughout most of its life, in the meanwhile infecting its offspring.

According to Gross (1952), mouse leukaemia is transmitted in a similar fashion. He found that leukaemia could be transmitted to a strain of mice which was free of the disease, by inoculating filtrates of tumours from high-leukaemic strains. As with the milk factor, transmission was possible only in the first few days of life. Thereafter the mice developed normally, but by the end of a year about half developed leukaemia. The leukaemic factor was subsequently found at all ages in other tissues of healthy mice from the high-leukaemic strain, and it is thought to be continuously transmitted from generation to generation, possibly *in utero*.

This work on animals suggests that there are a large number of viruses which lie dormant in the tissues, perhaps gaining entry when the animal is very young and persisting throughout its life. In some circumstances the balance between host and parasite is upset, cells are damaged, and clinical manifestations may result, perhaps long after the initial infection. It should, however, be noted that, purely on clinical grounds, there would be little to suggest that such an illness was due to an infectious agent.

Latent Infections of Man

When a patient recovers from a virus disease such as measles or smallpox the virus disappears from the tissues. It has admittedly been suggested that the subsequent life-long immunity implies continued survival of the virus, but there is as yet no clinical, epidemiological, or experimental evidence to support this hypothesis. In other conditions such as poliomyelitis the cells of the alimentary tract support the virus for several weeks, but prolonged carriage has not been reported.

Herpes Simplex

The only common disease of man which is generally acknowledged to be due to life-long persistence of a virus is, of course, herpes simplex. The vesicular eruption or cold sore which appears on the lips or nostrils during acute fevers is one of the most familiar of the minor nuisances of medicine. Many individuals are subject to recurrent attacks throughout their life, and these develop in response to non-specific stimuli such as sunburn, fever, or trauma, and not through exposure to another source of herpes virus. There is no doubt that the virus is present during each of these recurrent attacks, because it can invariably be isolated in the early stages by inoculation of vesicle fluid into some susceptible tissue such as that of the rabbit cornea or chick embryo. Individuals who are prone to repeated herpes have specific antibody in their blood between attacks, which neutralizes the virus in laboratory tests, and probably prevents wide extension of the lesions; but the antibodies do not seem able to prevent either the life-long persistence of the virus or the onset of the attack. Paradoxically, the individuals who do not have herpes are the ones without herpes antibody.

This is only one aspect of this disease, however. With increased facilities for virus isolation it has become clear that in infants and children, and occasionally young adults, several entirely different illnesses are caused by the same herpes virus. The most common is herpetic stomatitis, a mild vesicular eruption of the oral mucous membrane with fever and constitutional disturbance. More severe, but rarer, examples are eczema herpeticum, a type of Kaposi's varicelliform eruption, and meningoencephalitis due to herpes virus. These conditions as such do not recur, and they follow the more usual pattern of an acute infectious disease. They affect individuals who have no antibodies initially but who develop them during the attack, and it is often possible to show that infection was exogenous through contact with another patient suffering from herpes.

Burnet and Williams (1939) suggested that these infections in childhood constituted the primary attacks due to first exposure to herpes virus, but that the virus established

itself permanently in the cells in some latent form, causing the usual recurrent manifestation in later life. It was supposed that the virus remained within the cells, dividing with them, normally causing no damage, and unexposed to antibody. This comfortable symbiosis was presumably upset by a number of non-specific stimuli which led to cell breakdown with the release of a large number of virus particles.

This idea of the natural history of herpes, which was later more fully developed by Burnet (1946), is now widely accepted, but a good many unsolved problems remain. Where, for example, does the latent virus reside? The recurrent attack is most often in a place contaminated by saliva, and herpes virus is known to be present in the saliva of carriers, both between and during attacks. Furthermore, Stalder and Zurukzoglou (1936) have shown that areas of facial skin subject to recurrent herpes no longer develop the disease if transplanted to another part of the body. This suggests that the virus persists in some other cells, perhaps those of the buccal mucous membrane at the site of the primary attack, passing in the saliva or nasal secretion to the site of the recurrent attack. If this were the sole explanation, however, it would imply some rather curious dribbling habits. The recurrent attack usually occurs in roughly the same place every time, and this is not the only place likely to be contaminated with saliva. It is therefore necessary to postulate that one particular area of skin is sensitive to reinfection. This brings us back to the other idea that the virus lies dormant at the site of the recurrent attack and is activated by saliva. Others favour the central nervous system, dorsal root ganglia, or cutaneous nerve endings as the permanent home of the virus, but there is little supporting evidence.

Herpes virus from humans does not cause a similar recurrent infection which can be studied in animals, although Good and Campbell (1948) showed that rabbits given an anaphylactic shock long after recovery from an acute herpetic infection may die of herpes encephalitis. Perhaps investigation of natural monkey herpes might give a further lead in the study of human herpes.

Brill-Zinsser Disease

There are other, less familiar but well established forms of long-continued infection, and a notable example is Brill-Zinsser disease. This is a sporadic form of typhus which occurs principally in the Eastern United States, but it has also been recently reported in Britain (Hawksley and Stokes, 1950; Steel and Lawy, 1956). For many years it was assumed that it was contracted locally, but it was difficult to explain why it was chiefly confined to Jewish immigrants to the U.S.A. from South-Eastern Europe. It was then shown that the rickettsia from patients with Brill's disease was not of the endemic variety present in the United States but was of the classical epidemic type from Eastern Europe, and Zinsser (1934), after a prolonged study, was forced to the conclusion that the disease was a recrudescence of epidemic typhus contracted in the typhus foci of South-Eastern Europe. Some of the patients had not visited Europe for many years, and the rickettsia can presumably persist in the tissues throughout this period even in the presence of antibody. Price (1955) has recently confirmed this by isolating the organism from the lymph nodes of healthy individuals who have previously had typhus. These observations are of wide importance because they also explain how the epidemic typhus rickettsia can survive in the intervals between typhus epidemics in Eastern Europe and Asia.

Other pathogenic rickettsiae can sometimes persist in man. Smael *et al.* (1952) isolated *R. tsutsugamushi* from the lymph nodes of a patient one year after scrub typhus which had been treated with chloramphenicol. Q fever can be a long-continued relapsing illness, and Huebner has found *R. burnetii* in the blood and urine one year after an attack (see Marmion *et al.*, 1953). Psittacosis virus has been isolated from the same patient over a period of eight years by Meyer and Eddie (1951).

Herpes virus, pathogenic rickettsiae, and the psittacosis virus grow well in a variety of laboratory animals and eggs, and so are relatively easy to isolate and identify. We come now, however, to other viruses which are apparently host-specific and able to grow only in man. These are, of course, much more difficult to study, and depend upon the use of volunteers or, alternatively, cultures of human cells *in vitro*.

Serum Hepatitis

One virus which has been discovered unintentionally by using man himself as an experimental animal is, of course, the virus of serum hepatitis. It is now known that a proportion of apparently healthy adults carry in their blood a virus capable of causing hepatitis in non-carriers. Transmission occurs by parenteral inoculation alone during blood transfusion or from inoculation of blood products such as serum or plasma. The large outbreaks which occurred during the last war were due to human serum used as a preservative in yellow-fever vaccine. The amount of virus in the blood of carriers must be quite appreciable, because transmission may occur with minute amounts of blood such as that contaminating the needle of an imperfectly sterilized syringe, and large pools of serum and plasma can be infected from one contributor to the pool. Potentially the virus might be transmitted by blood-sucking insects simply by contamination of the proboscis, although there is no evidence that this in fact occurs. Little is known about serum hepatitis virus because it has been studied only by inoculation of human volunteers, and the substantial mortality rate does not exactly encourage this means of investigation. Attempts to use human cells in culture from liver or elsewhere have not been successful. In recent months Richtsel *et al.* (1956) have described a strain of human cells apparently susceptible to a similar virus, that causing infectious hepatitis, but it is not yet known if serum hepatitis will also infect these cells.

It is difficult to see how the virus gains access naturally, and it has even been claimed that this is an example of spontaneous generation from the cells of the carrier. The account of hepatitis in newborn infants by Dible *et al.* (1954), however, suggests the possibility that transfer may normally occur from mother to child *in utero* with consequent development of immune tolerance—that is, loss of ability to develop antibodies to the virus. This might then lead to a permanent latent infection, persisting for life, in the same way as lymphocytic choriomeningitis in mice, and, like the latter, detectable only by inoculation of susceptible non-carriers.

Serum hepatitis may not be the only latent virus which manifests itself in this way by parenteral inoculation. Beutler and Dern (1955), when working on the survival time of transfused red cells, discovered that the blood of one healthy donor produced an acute feverish illness in nearly all recipients about 12 days after inoculation. The agent causing this illness was filterable and could be transmitted from these recipients to others during the acute stage and up to at least nine weeks later, causing similar symptoms. The fever, lasting about a week, was accompanied by headache and muscle pain, sometimes with nausea and vomiting or respiratory symptoms, and 6 patients out of 38 developed arthritis one to five weeks after the end of the acute illness. The agent producing this disease was present in the blood of the donor in repeated tests over a 13-month period, during which he remained perfectly well. The authors point out that this rather mild illness could easily be missed after the usual transfusion of a single recipient from a donor who was a carrier.

The Adenoviruses

The development of tissue-culture methods for the study of viruses, stimulated by Weller *et al.* (1949) in their work on poliomyelitis, showed the great potential advantages in the use of human and monkey cells growing *in vitro*. This has not unnaturally led to the uncovering of new viruses previously undetected through lack of pathogenicity for laboratory animals and eggs. These viruses are in roughly

two groups: (1) a heterogeneous collection from faecal specimens mostly isolated during attempted isolation of poliomyelitis virus; and (2) a single family of related viruses largely from the upper respiratory tract and eye, now known as the adenoviruses or A.P.C. viruses (adenoido-pharyngo-conjunctival viruses). Rowe *et al.* (1953) isolated the first of these agents when growing cultures of tonsils and adenoids removed at operation. They found that epithelial cells from these tissues grew well for about two weeks, but degeneration then appeared in some of the cultures. A virus could be isolated from the degenerating cells, which would then cause similar changes in the remaining adenoid cultures which had remained normal, as well as in certain other cultures of human epithelium, such as the HeLa strain of carcinoma cells. The virus could not so easily be obtained from swabs or even ground suspensions of adenoids, although it regularly became unmasked after growth of the cells in culture. This could perhaps be due to the elimination of antibody or other inhibitory substances originally present in the extracellular fluid.

Since then at least 14 closely related viruses have been isolated, some from adenoids removed at operation and some from patients with acute respiratory disease, while two members of the same group have been found in monkey and chimpanzee tissues respectively (Rowe and Huebner, 1956).

Some members of this group of viruses—notably Types 3 and 4, 7 and 8—cause acute diseases such as pneumonia, pharyngitis accompanied by conjunctivitis, and other upper respiratory syndromes in patients of all ages. It is not known if these viruses persist after the initial infection. Certain other adenoviruses, however—Types 1, 2, and 5—are found only in association with febrile respiratory illnesses in young children, but they are present in the majority of adenoids removed at operation from both children and adults, and antibodies against one or other type are also present in the blood of nearly all adults. This suggests that widespread infection with these types occurs at an early age and is associated with the typical hyperplasia of tonsils and adenoids seen in young children. Despite the development of antibodies after first infection, the virus apparently persists, perhaps indefinitely, in the lymphoid tissue.

This resembles latent herpes except that adenovirus may be more widespread. The latent herpes virus, however, at intervals damages the cells and gives rise to disease. The similarly latent adenovirus would have practical importance only if there was also some clinical manifestation of its presence after the initial infection. There may be a number of unexplained illnesses due to recrudescence of latent viruses in lymphoid tissue. It is tempting, for example, to suspect some of the recurrent spontaneous afflictions of the throat and nose, especially in children, and lymphoid hyperplasia caused by a virus of this sort might even initiate attacks of acute appendicitis.

Proof of a causal connexion is not likely to be an easy matter, however. If such illnesses were accompanied by release of virus from damaged cells the virus would probably not be detectable in practice through immediate neutralization by antibody already present. In recurrent herpes the virus conveniently presents itself in an easily recognized superficial vesicle from which it can be isolated before antibody filters in as well. Apart from the difficulty of sampling the lymphoid tissue sufficiently early in an illness, neutralization of adenovirus is likely to be much more rapid in what is the probable site of antibody production.

Demonstration of a rise in serum antibody after the illness might be suggestive, but there is little alteration after recurrent herpes. Perhaps it would be possible to show an increase in viral antigen (even if infectivity was neutralized) by injecting exudates, or tissue suspensions where available, into rabbits, and comparing the antibody rise with that resulting from injections of control material; but the variability of antibody response in different rabbits might be prohibitive. Nevertheless, a fresh approach of some sort will be needed to study this type of aetiological relationship.

Mechanism of Latent Infection

Having considered some examples of latent viral and rickettsial infection in man and animals, it would now be appropriate to discuss the mechanism by which these parasites can survive so successfully in their hosts. This review is confined to the viruses and rickettsiae, but bacteria and even larger parasites can persist for long periods as well. Most of these organisms live on an internal surface of the host, however, and they avoid the immunological problems involved by prolonged sojourn inside the tissue. Furthermore, most of the larger organisms, even inside the tissues, retain their morphological identity, whereas some viruses probably develop a much more intimate relationship with the cells of the host.

Once a virus or rickettsia has gained access to the susceptible cells initially, one may suppose the following requirements if it is to establish a persistent latent infection: (1) the virus must produce no damage to the cells, or at least the rate of damage must be less than the rate of production of new cells; (2) it must have some mechanism for getting from cell to cell, particularly if the cell concerned has a short life; and (3) it must not be affected by the immunity mechanism of the host.

It is admittedly possible to imagine an equilibrium with a low-grade immunity, in which a small proportion of virus released is always left unneutralized and is thus able to infect a small number of new cells and so on. One could hardly expect such a system to remain stable for long, however, without some sort of over-all control mechanism. Our consideration of some examples of latent infections indeed suggests that there may be more than one way in which viruses persist. After all, there are certain infections, such as lymphocytic choriomeningitis in mice and possibly serum hepatitis in man, in which antibody never develops and virus can pass unhampered throughout the body. On the other hand, herpes virus is presumably maintained in cells which are surrounded by antibody.

It would be convenient, at the risk of oversimplification, to consider three main groups of persistent viruses, which probably have different mechanisms for survival.

1. *No Development of Antibody.*—In lymphocytic choriomeningitis of mice, mouse mammary cancer, mouse leukaemia, and possibly serum hepatitis of man, infection occurs *in utero* or within the first few days of life. Antibodies probably never develop against these viruses even though they persist for life. This is presumably due to the phenomenon of immune tolerance. Lymphocytic choriomeningitis virus, and probably the agent of mouse leukaemia, are widely scattered in various tissues and present in high concentration, while it is also probable that serum hepatitis virus is plentiful at least in the blood of human carriers. The only limitation to the growth of these viruses is the supply of susceptible cells. Since a large number of cells are infected, one would assume that these are not damaged during virus multiplication. This immediately leads to difficulty in explaining why serum hepatitis virus causes necrosis of liver cells when transmitted to non-carriers. Perhaps the liver damage in non-carriers is connected with a normal immune reaction to the virus. This might help to explain the very long incubation period, allowing for growth of the virus and development of immunity before onset of clinical manifestations.

Theiler's virus may fall into this first group, but, since infection of young mice is sometimes delayed for two or three weeks, the immune tolerance is not complete and latent infection generally persists for only about six months, after which antibody appears and the virus is eliminated.

2. *Antibody Present but Ineffective.*—In persistent infection of man and animals, or of birds, with rickettsiae or viruses of the psittacosis group, antibody to the organism is normally present, and can be detected by various *in vitro* methods such as agglutination or complement fixation. These antibodies will neutralize the toxic effects of the organisms

and prevent fever or other clinical signs of infection in animals, but it is difficult to show any effect on the actual growth of the organisms. For example, *R. burneti* grows just as effectively in eggs in the presence of high-titre immune serum as with normal serum. Growth of smaller viruses is probably neutralized because antibody prevents their entry into the cells. Perhaps the large viruses and rickettsiae have a different method of entry and one which is not blocked by antibody. Some grow in phagocytic cells, and here the opsonic action of antibody might actually help. Anyway, it seems likely that the long persistence of these organisms in the tissues of naturally infected individuals is connected with a similar failure of antibody to prevent multiplication and dissemination.

Several of us at Cambridge have investigated this phenomenon with *R. burneti*, the causal agent of Q fever. Dr. Fiset and I have found that organisms freshly isolated from animals, either in the acute stage or after long persistence, fail to react in certain *in vitro* tests even with their homologous antibody (Stoker and Fiset, 1956). This was subsequently found to be due to a polysaccharide of low antigenicity on the surface of the naturally occurring rickettsiae which prevents the main protein antigen of the organism from combining with its antibody (Fiset, unpublished). It was suggested that the surface layer allowed *R. burneti* to survive in the host in the presence of this antibody. An *in vivo* neutralization test developed by our colleagues Abinanti and Marmion (1956) added some confirmation, but there is not yet sufficient evidence to conclude that this phenomenon is entirely responsible for long persistence under natural conditions.

Morgan's (1956) studies with psittacosis suggest that, with this virus at least, other factors may also be involved. Thus the psittacosis virus remains latent in chick cells cultured on deficient medium, but multiplies normally when the cells are given certain amino-acids. Studies of this sort with other latent viruses and rickettsiae are now needed.

3. Antibody Present and Effective, but No Contact with Virus.—In the third group of latent infections, which includes herpes and the adenoviruses, antibody is also present in the circulating blood of the host. Unlike the rickettsiae and psittacosis group, however, growth of the virus can easily be neutralized by antibody. When herpes virus or adenoviruses are mixed with antibody in laboratory tests and brought into contact with susceptible cells they are prevented from entering the cells and multiplying. This seems to imply that under natural conditions the latent virus, once established, can pass from cell to cell without exposure to antibody. How could this take place?

If there is no damage to the cells during virus growth there would, presumably, be no inflammatory reaction with exudate of plasma protein, including antibody globulin, in the extracellular fluid. Thus a virus such as herpes could pass from cell to cell quite freely, provided it remained outside the blood stream, particularly where the cells are closely packed as in the basal layer of epithelium. In lymphoid tissue, where the adenoviruses persist, however, antibody may be in much closer contact with the infected cells.

An alternative is passage from cell to cell along cytoplasmic connexions. Black and Melnick (1955) observed that when sheets of monkey cells in culture are infected with B virus, the monkey herpes, spread was at first confined to cells adjacent to the initial focus of infection and was not affected by antibody in the medium. With human herpes virus growing in human carcinoma cells (HeLa strain) a similar focal spread is observed. Under ordinary experimental conditions the cells release virus into the surrounding medium, secondary foci appear, and the lesions soon become confluent, with the development of giant cells (Special Plate, Figs. 1 and 2). If antiserum is added to the medium a few hours after first infection the change does not become confluent. The primary foci continue to enlarge, however (Plate, Fig. 3), suggesting that the virus is passing to contiguous cells without coming into contact with antibody.

One of the most striking changes is the development of a web of cytoplasmic connexions between cells, but these are most easily seen in unneutralized cultures in the neighbourhood of giant cells (Plate, Fig. 2), and are not visible between the tightly packed cells of the foci which develop in the presence of antiserum. My colleague Dr. Ross, who has stained herpes-infected cells with fluorescent antibody by the technique devised by Coons and Kaplan (1950), has found some fluorescence in these cytoplasmic extrusions, but it is not certain if this is due to the presence of the infective virus particles themselves.

An alternative would be the transfer of the virus to the descendants of the originally infected cells at mitosis. This is unlikely in the cultures of carcinoma cells, because we have found that they stop multiplying immediately they are infected, but we must seriously consider this possibility under natural conditions, where absence of histological change in the quiescent phase implies a lack of damage to be expected if cell division were proceeding normally. It is perhaps noteworthy that herpes and adenovirus are the only two animal viruses for which there is unequivocal evidence of multiplication in the nucleus. If such viruses could divide at the same rate as the host cell, the progeny might pass with the chromosomes to the nuclei of the daughter cells and might almost be considered cell constituents. It is in the latent virus infections of bacteria, however, that this type of relationship has been most fully investigated, largely following the work of Lwoff and Gutmann (1950).

Lysogenic Strains of Bacteria

When two closely related strains of bacteria—for example, two strains of staphylococci—are grown together, one strain may grow normally but cause lysis of the bacteria in the other strain. The apparently normal strain is called lysogenic, and the property is due to the presence of latent virus in each bacterium. The virus does no damage to the bacterial host, and divides with it so that the strain is permanently infected but its physiological functions are unimpaired. A small proportion of the cells release virus, which has no effect on the remainder of the infected bacteria, but which will cause lysis of other susceptible strains of bacteria not already carrying the latent infection. These uninfected strains which undergo lysis can therefore act as indicators of virus from the apparently normal carrier strain. Provided one can find a suitable indicator, it is now possible to show latent virus infection in a large number of strains of various bacterial species.

This symbiotic relationship between the virus and bacterial cell can be upset by certain physical stimuli and chemical agents. If a lysogenic strain is irradiated with ultraviolet light, for example, all the bacteria undergo lysis and release large amounts of virus. One is immediately reminded of the transformation of latent into active herpes by sunlight and other apparently non-specific stimuli.

State of the Latent Virus

It is well to remember that the mature virus particle, as shown in electronmicrographs of purified virus suspensions, is probably only one stage in the life cycle, allowing transport to and penetration of the virus into new cells. The work of Hershey and Chase (1952) on bacteriophage has led to the conclusion that the characteristic tadpole-shaped particle does not enter the bacterial cell, but acts as a sort of syringe to introduce nucleic acid, which is apparently the organizer of new virus and which carries its genetic characters. Work on human and animal viruses, and especially influenza virus, also suggests that the virus particle, after penetration of the cell, is broken down into its constitutive genetic elements, or, at least, completely altered into a vegetative state during multiplication.

If in some latent infections the virus lies permanently within the cell and its descendants, it may consist solely of the genetic material of the virus dividing in time with the hereditary constituents of the parent cell. It is in this form, called prophage, that the virus persists in lysogenic bacteria.

This adds to the problem, because if latent animal viruses exist in this state they would not be detected by ordinary isolation procedures, because virus material would not be in a form which could infect new cells. Some inducing agent would be needed which would upset the equilibrium and allow the virus to mature to a fully infectious form, as in the induction of lysogenic bacteria by ultra-violet light. There is no clear evidence at present, however, that latent virus infection of human or animal cells resembles that of bacterial cells. Further progress awaits a suitable model of latent infection in tissue culture which could be studied by the quantitative techniques which have been so fruitful with lysogenic bacteria.

A more or less undetectable latent virus which persists indefinitely without damaging the cell in the ordinary sense may anyway seem rather an academic nicety. Absence of recognizable histological change, however, does not mean that the continued presence of viral material in a cell will not alter some of its functions. For example, the tumour viruses change the potentiality of the cells they infect without necessarily causing necrosis. Latent virus infection of a bacterium may confer entirely new characters, such as the development of flagella or toxin production, on the otherwise normal host. We have yet to see whether viruses may cause long-standing alterations of cell function in man.

Conclusion

We have now indulged in a good deal of speculation and have come some way from practical medical problems, but, judging by the numbers of latent viruses found in animals, our own tissues may harbour more unobtrusive guests than we imagine. For the survival of a virus or any other parasite it is, of course, the most satisfactory arrangement. No damage to the house—no trouble with the landlord.

When symbiosis is complete, especially if early infection leads to immune tolerance, we may expect few medical problems. These will arise only when artificial procedures, like blood transfusion, carry the virus to someone who has escaped early infection. Nevertheless, this risk will call for great care if virus vaccines are ever made from human cell cultures, which are more likely than blood to carry these hidden agents.

With other infections, like herpes and Brill's disease, the balance may occasionally be upset with a sudden acceleration of virus production and cell damage, causing illness in the carrier. Some sporadic afflictions of tonsils and adenoids, or even other lymphoid tissue, may eventually prove to be due to recrudescences of infection with adenoviruses. Such conditions lack many of the usual attributes of an infectious disease, and their recognition as latent virus infections will involve subtle problems for the virologist as well as the clinician. It is to be hoped that investigation of the more fundamental problems of the mechanism of latency, as outlined, may ultimately be of practical value in the elucidation of these diseases.

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ADULT HYPERTROPHY OF THE PYLORUS

BY

A. M. DESMOND, M.B., F.R.C.S.

Surgeon

AND

B. F. SWYNNERTON, D.M., M.R.C.P.

Physician and Research Assistant

St. James's Hospital, London

[WITH SPECIAL PLATE]

Pyloric hypertrophy in adults has in the past ten years been found 25 times during laparotomy at St. James's Hospital. Fewer than 200 cases have been recorded in the literature since the condition was first described by Cruveilhier over 100 years ago, and many of these were discovered only at necropsy. Horwitz *et al.* (1929) found that the normal thickness of the pyloric muscle varied from 3.8 mm. to 8.5 mm.; in all our cases it has been 1 cm. or more (see photograph). Furthermore, in all cases the hypertrophy was mainly confined to the circular muscle.

Only once has a confident pre-operative diagnosis of the condition been made, but increasing experience has enabled us to form a strong suspicion of its existence from the radiographs after a barium meal in a few

Resected stomach from Case 6, showing incised thickened pylorus (1.3 cm.).

M. G. P. STOKER: LATENT INFECTIONS WITH VIRUSES AND RICKETTSIAE

FIG. 1.—Culture of uninfected human carcinoma cells (strain HeLa). All preparations $\times 320$, stained haematoxylin.

FIG. 2.—Cytopathic change five days after infection with herpes virus. Note giant cell and cytoplasmic processes between cells.

FIG. 3.—Cytopathic change five days after infection with herpes virus; herpes antibody added to medium two hours after virus. Focal distribution of affected cells surrounded by apparently normal cells.

A. M. DESMOND AND B. F. SWYNNERTON: ADULT HYPERTROPHY OF PYLORUS

FIG. 1.—Elongation and narrowing of pyloric canal with surrounding soft-tissue shadow and duodenal bulbar impression. (Case 18.)

A. F. ROBINSON *ET AL.*:
HAMARTOMA OF BOWEL

FIG. 2.—Elongation and narrowing of pyloric canal with proximal and distal impressions. (Case 14.)

Photomicrograph of tumour. ($\times 5$.)

R. B. THOMPSON AND S. G. M. MacKAY: ALEUKAEMIC MYELOBLASTIC LEUKAEMIA

Sections of ribs to demonstrate (a) fibroblastic proliferation; (b) reticulin fibrils. ($\times 225$.)