The Troubles of a Virus

The Seventh Marjory Stephenson Memorial Lecture

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The subject of my lecture concerns an aspect of microbiology rather different from that upon which Marjory Stephenson's great reputation was built. However the Marjory Stephenson lecturer may, I gather, deal with any side of the subject. I believe that the matters I shall discuss would have interested her and I hope also that my remarks may contain a few crumbs of interest for you.

Most people regard viruses simply as noxious parasites to be destroyed or at least thwarted, though perhaps virologists take a more lenient view, since upon the viruses their livelihood depends. But either way the viewpoint is distressingly anthropocentric; the only criterion seems to be their effect, direct or indirect, on that great hulking mammal, man. Nobody looks at matters from the point of view of the poor little virus, so many times smaller than man himself. After more than forty years' study of virology, I feel that I may try to get under the skin or capsid of a virus and from my seat in its nucleoid see how the world looks from a virocentric point of view.

It can too easily be imagined that a virus's life is an ideal one. A virion is equipped to penetrate the cell of its choice. It has the trick of divesting itself of the paraphernalia which helped it in that penetration and thereafter it can compel the cell it has entered to devote its resources to make more virions like itself. Within a short time—an hour or two in the case of some phages—it can become not only a mother but a grandmother and great-grandmother and that without any necessary introduction of the joys and sorrows associated with sex. Its progeny can go and do likewise, doubtless supposing that they can continue and duly inherit the earth. In fact, of course, they are limited, as all parasites are limited, by the supplies of susceptible hosts and by the ability of those hosts to react against them.

The first trouble they meet with is that they find themselves inadvertently stimulating the production of a hostile substance called interferon and this even while they are supposing themselves quite safe inside a cell. In consequence the cellular environment becomes much less favourable and their replication may be slowed down or even halted. They can, however, themselves react. Strains of viruses may develop, having less tendency to evoke interferon production and less susceptible to its action. I shall be suggesting later in this talk that variation in susceptibility to interferon plays no little part in affecting a virus's prospects.

The next trouble is that when enough virions have been shed into the environment they begin to stimulate antibody production. This reaction comes along a little later than the interferon production, but it is a menace which persists and increases

and may soon end the virus's prospects so far as the originally infected host is concerned. Here again is a trouble which viruses have to some extent learnt to overcome. First of all, of course, they can escape and manage in one way or another to reach fresh hosts. Before they have to do this, however, they may exploit the local possibilities a little further. They may penetrate the blood-brain barrier or reach the cornea or some other foothold where antibodies cannot so easily reach them. They do better in fact anywhere where they are superficially placed, in the skin or on mucous membranes. Antibodies may indeed be found in mucous secretions, but they are lower in titre and less effective than where there is readier interchange with the fluids of the blood.

Viruses may dodge antibodies in another way. Those of the pox and herpes groups may pass from cell to cell along fine intercellular channels and so extend the area of their dominion without having to leave the shelter of a cell and brave the antibody menace without. The virus of varicella spreads readily in such a manner in many sorts of tissue culture, but when one tries to demonstrate free virus in the culture fluids one commonly fails to do so. In the case of the virus of malignant catarrh of cattle the phenomenon is even more striking. Though it was long suspected that this was a virus infection, it was until recently impossible to demonstrate a free filterable agent. There is a bit of a mystery to be unravelled here. Both varicella and malignant catarrh readily infect fresh hosts; how, if they are so closely cell-bound, do they manage to do so? Some viruses of the myxovirus group are haemolytic *in vitro* and this haemolysis seems to be merely a manifestation of a cytolytic property. This seems to serve the purpose of allowing adjacent cells to become fused and to form a syncytium in which viruses can multiply. Here we have another ruse by virtue of which more virus can be built up in a focus in the body despite the presence of circumambient antibody.

But now suppose that our virus can make no more headway in its original host and is seeking fresh victims in ways to be discussed later. A time will come when those in its immediate environment are either dead or immune—in either case unavailable. How is this trouble to be surmounted? There is the geographical escape. An epidemic may be a migrating one. Outbreaks of fatal yellow fever amongst South American monkeys can be followed and have been found to spread as fast as 200 km. a month. In Africa, too, though yellow fever is an inapparent infection among the local monkeys, there is evidence that waves of spreading infection occur. Widespread epidemics among human beings, due to the Chikungunya and O'Nyong-nyong viruses, show a similar pattern of movement. Nor, to come nearer home, do I need to remind you how influenza A virus travels from country to country.

The influenza virus exhibits two other features of interest. One may see phase-variation, the so-called P-Q variation, wherein less avid forms are produced, reacting poorly with specific antibody. Isaacs *et al.* (1954) have suggested that though the total antigenic composition of the less avid or Q phase may be unchanged, the components have been somehow shuffled, the virus being partly turned inside-out so that the antibody-reacting constituents are not so readily available at the virus's surface. The virus may well find this helpful to it, but of far greater significance for its future is the phenomenon known as 'antigenic drift'. Observations over the past thirty, and more conclusively, the last fifteen years, have shown that, after a period in which influenza has been virtually absent, new strains have appeared,

antigenically distinct from those prevalent earlier. As a rule they have been more closely related to their immediate predecessors than to those from further in the past. It has been shown experimentally that influenza passaged in partly immune mice or in the presence of amounts of antiserum inadequate for complete suppression, will encourage the emergence of antigenically novel strains. It was hoped at one time that if we knew the antibody-composition to be found in a human population, we could passage virus in the presence of similar antibody and see what turned up; thus it was hoped to predict what sort of a variant would appear with the next outbreak. I need hardly say that the influenza virus was smarter than we were and did not behave as had been guessed. What I have described as the 'flu virus's behaviour was true of the years up to 1957. Since the Asian or A2 virus appeared it has not varied as much as it had done before. Post-1957 strains do differ from each other but not very greatly: the A2 virus seems relatively more stable. Perhaps it is as a consequence that its visitations have been rather less frequent and widespread of late. Possibly production of the 1957 variant was a mistake from the virus's point of view.

Let us now consider what means are at a virus's disposal for reaching a new host. Though viruses are non-motile, they are equipped with homing devices which help them to attach to appropriate cells. These take the form of chemical substances with affinity for receptors on the cells' surface. The mechanisms involved have been particularly well studied in the case of influenza; here the readily observed reaction between the virus and receptors on the surface of red blood cells serves as a model of what is happening when the virus reaches a susceptible cell on the epithelium of the respiratory tract.

The homing devices require, however, the assistance of some outside agency to to bring virus into fairly close contact with the cell to be invaded. Often the host itself provides the mechanism; a sufferer from a cold sneezes and projects virus towards the next victim; a mad dog bites; a child's fingers pick up contaminated material; or infection is carried by the movement of wind or water. One of the most primitive and still highly successful methods depends on an alternation of hosts, especially when both of them are mobile. We are probably wise to consider so-called arthropod-borne infections of vertebrates as being essentially vertebrate-borne infections of arthropods. Overt disease in the arthropod is unknown in the case of arbovirus infections of birds and mammals; so it is more likely that the association of virus and arthropod is the older one. The virus then regards the mosquito- or tick-bitten vertebrate as a convenient means of finding its way to a new tick or mosquito. Much the same is probably true in the realm of plant-viruses, for the aphid-vectors of plant viruses (if we look at it that way round) are commonly unaffected. The matter is more doubtful in the case of some cicadellid-borne viruses, which may lead to harmful results in both plants and insects (Maramorosch & Jensen, 1963).

The arthropod-vertebrate cycle may not always be a sure and certain matter for the virus. Mosquitoes have a short life and may well perish before the ingested virus has had time to mature within them and reach their salivary glands ready for introduction into a new host. A mosquito which normally bites birds may at times take a meal from a horse or a man; a virus such as one of the North American equine encephalomyelitis viruses may then find itself in a host in which it can only multiply to a low titre; it has got into a blind alley. And what if one of the members of the

cycle disappears, as mosquitoes do during winter months? There has been much discussion as to how these equine encephalomyelitis viruses manage to overwinter, to carry on till mosquitoes re-appear in the spring. It has been suggested that migrating birds carry them south and then north again, or that they persist in occasionally hibernating mosquitoes or in bats or snakes. The most recent suggestion is that there is a hitherto unsuspected cycle in small rodents and their ectoparasites, for the viruses have been recovered from mice in New Jersey during winter months (Sussman, private communication).

A virus might be pardoned for thinking that this arthropod transmission was too chancy an affair and that it might be safer to try to reach one's next host directly by air transport, riding upon a sneeze. But here more troubles are encountered. Virus carried on the smallest droplet nuclei is exposed to the inactivating influences of dessiccation and exposure to light. It must reach its objective very quickly or perish. It is more stable if associated with a large particle which can somewhat protect it; but then it can easily fall too quickly to the ground. To succeed, it has to manage to board a particle of such intermediate dimensions that it avoids both these dangers. It must then be carried by the air current of an inhalation round the corner of the nostrils into the nose; to land prematurely on the external nares is to court destruction. Once within the nose it might expect to find safety at last. But not so; it probably lands upon a moving sheet of mucus upon which it gains an insecure foothold and which carries it inexorably backwards and then downwards to meet its doom down the oesophagus and into the acid cauldron of the stomach.

One inevitably wonders just how a respiratory virus ever manages to avoid these hazards; yet it certainly can do so. A local chilling and desiccation within the nose may temporarily halt the flow of mucus and give the virus its chance. Or perhaps the stream of mucus turns over and over as it goes, so that a few virus particles can seize an opportunity to snatch at a near-by cell before they are whisked away. Experiments on volunteers with rhinoviruses show that one cannot dilute virus-containing nasal washings very far and still produce colds. Rather large doses are necessary. The amount of virus which leads to a cold in nature must be much less than this. I shall try to account for this anomaly later.

The rhinoviruses which cause colds are labile in the presence of acid and cannot therefore survive passage through the stomach. Their relations, the enteroviruses, can withstand this and therefore have no trouble in reaching their habitat in the intestine. One can only guess whether they are acid-stable derivatives of the rhinoviruses, adapted to a life in the gut, or whether it is they who are the ancestors of the rhinoviruses which have discovered how to lodge in the nose and multiply there. The troubles of the enteroviruses are in any case different ones. For them faecal contamination is the key to success. Here they may fall between two stools. In an environment, let us say in a tropical village, where hygiene is practically non-existent, they can spread so readily that all children are infected at an early age. The enteroviruses then cause only infections of infants and are dependent for their continual existence on a continuous supply of fresh susceptibles. On the other hand, in a modern highly civilized community, faecal contamination cannot be relied on as a means of cross-infection and as our hygiene improves the enteroviruses will spread with greater and greater difficulty. Their victims will, however,

always include the visitor from civilization who penetrates into less hygienic parts of the world. On the whole, the enteric viruses seem to thrive pretty well.

Three of them, however, have made a grave error. The three serotypes of poliomyelitis have, apparently quite fortuitously and with no benefit to themselves, found themselves able to invade the central nervous system of man and cause paralytic disease. So hurtful has this been that man has been inspired to develop an effective polio vaccine which will, we hope, get rid of the paralytic disease. I do not know how a virulent poliovirus regards the prospect of being eliminated in favour of an anaemic avirulent man-made vaccine strain. With much the same disdain, I imagine, as a wolf might regard a Pekingese dog.

Passing over some less important routes of transfer of infection we come to vertical and transovarial transmission. This must seem an easy way out of a virus's troubles. Some viruses, in ticks, may be passed to a new generation in the ovum, never having to battle with the dangers of the world outside. The same is true of some viruses which are pathogenic for the larvae of Lepidoptera. In the case of lymphocytic choriomeningitis of mice, the phenomenon of immunological tolerance permits the virus to persist indefinitely in the blood and tissues of mice infected *in utero*. Infection of the young may be through the ovum, or, as with Bittner's virus causing mammary cancer in mice and in some other tumours and leukaemias of mice, it may be passed in the maternal milk. Some plant viruses, too, are transmitted in seed. Such vertical transmission is doubtless helpful to a virus, but notably so only when it supplements some other method of transmission. Unless the host species is increasing or extending its range, a pair of animals will on the average only give rise to two descendents in the next generation. Viruses transmitted only in a vertical way can therefore rarely hope to do more than maintain their numbers: they cannot conquer new worlds.

Some viruses have in the course of evolution found new ways of reaching fresh hosts. A few of these changes can be observed today or can be assumed to have taken place quite recently. The tick-borne encephalitis virus of Central Europe is at times transmitted by the milk of infected goats. The virus of Eastern equine encephalomyelitis, normally carried by mosquitoes, spreads among captive pheasants, probably as a result of pecking. The pox viruses seem to be adaptable creatures for some, such as smallpox, spread by the respiratory route, while the closely related ectromelia, at least sometimes, passes out in the faeces, and others—for instance, fowl pox and myxomatosis—are mechanically carried by insects. It is a reasonable hypothesis that many changes in routes of transmission have occurred in the past.

We now come to the greatest trouble which a virus must cope with. While it has to be sure of spreading to fresh hosts in the ways we have considered, it must not be too efficient or all its potential hosts will be eliminated by death or by becoming immune. Either it must spread only indifferently well, by a calculated inefficiency, or it must find some way of remaining latent until a new supply of susceptible hosts has become available. The virus's problem is of course the same as that of any parasite. The virtues of self-restraint may have to be learnt in a parasite's own interests.

'Inapparent infection' in the virus field has been defined (*Symposium*, 1957) as covering 'the whole field of infections which give no overt sign of their presence' and 'latent infection' as a special instance of inapparent infection in which 'the

infection is chronic and in which a certain equilibrium between host and parasite has been established'. Both truly latent and transient inapparent infections come into our story. It is a familiar idea that parasites and their hosts in the course of evolution become mutually tolerant. Instances of inapparent virus infection are accordingly far more numerous than those in which viruses cause overt disease. Arbovirus infections are always inapparent in arthropods and are usually so in their normal hosts—monkeys, rodents or birds as the case may be. They only come to our attention when fortuitously spreading to strange hosts. Enterovirus infections of man are usually inapparent; only the exceptional one 'goes beyond its bill' and by invading nervous or muscular tissues leads to overt disease. Polyoma virus in mice, and others which potentially give rise to leukaemia, are completely harmless in the ordinary way: it is man's deliberate interference with nature in his laboratory which permits them to cause malignant disease. One can pass other virus families in review and observe the same sort of thing. Those viruses are successful which have learnt not to damage their hosts and to spread, usually among young animals, wisely yet not too well. There are of course others which cause disease and use that disease as a means of spread: the encephalitis of rabies causes mad dogs to bite and the 'coughs and sneezes' caused by rhinoviruses and others 'spread diseases'.

A number of viruses, however, do not seem to have achieved the peaceful co-existence of the quietly successful ones, and these have gained their ends in other ways. For some a periodically activated latent infection has marked the road to success. The classical example, of course, is afforded by Herpes simplex. Primary infection usually takes place early in life or not at all; it often takes the form of a widespread stomatitis in children. Thereafter the virus settles down as a latent infection, often in the corner of the mouth or external nares, to be activated ever so often as a crop of blisters by various stimuli; these are different in different people, perhaps a common cold, menstruation or eating cheese. A little virus may be recovered at times from the saliva between episodes of fever blisters, but it seems fairly certain that it is the virus shed at the time of these episodes of activation which acts as the main source of infection.

Something of the same sort is seen with the related varicella virus. After an attack of chickenpox, virus may remain latent in the central nervous system, awaiting activation in the posterior root ganglia and a centripetal spread to cause the eruption of Herpes zoster. This may be the starting point of more varicella cases. Hope-Simpson (1965) has lately suggested that the whole sequence may have evolved for the benefit of the varicella-zoster virus; it can thus initiate a new chain of infection if a child-to-child infection-series has petered out.

New light has been thrown lately on the ecology of rabies. No longer can it be considered as an invariably fatal disease. In vampire bats it may exist as a latent infection, virus being present in salivary glands but not necessarily in the brain. There may also be virus in the brown fat and this may persist there during a bat's hibernation. Insectivorous and fruit-eating bats may also carry the virus. Recently 5% of bats in New Jersey were found to harbour the virus, though no cases of rabies in dog or man have been seen in that state for almost a decade (Sussman, private communication). At times the virus in insectivorous bats may cause encephalitis and they may then bite the human hand that touches them, or even attack unprovoked. In that the rabies virus does not necessarily kill infected bats, they

might seem to qualify as the original hosts of the virus. Quite a different view has been put forward by Johnson (1965). He regards bat rabies as part of an aberrant cycle and considers that the true hosts in North America are mustelids—skunks and ermines. The small spotted skunk was apparently known to backwoodsmen of the west as a vector of hydrophobia and was accordingly called by them ‘phobey-cat’. From them the virus may spread to foxes and wolves in which it may cause devastating epizootics and may thus actually serve to eliminate the mustelids’ competitors. Whichever view is correct, a sequence of alternating quiescence and activity is indicated and this might appear well suited, as with herpes simplex and zoster, to help in the perpetuation of the virus. The relative importance of bats, mustelids, foxes and dogs and other creatures in the ecology of rabies is, however, still under discussion.

Alternating quiescence and activity is of course a familiar feature of influenza. Over periods of a month or two it can cause outbreaks affecting millions and then, perhaps for two years, it may be hard to find. In between whiles, virus is only recovered from an occasional sporadic case, or perhaps serological evidence of its activity is detected. It seems that the virus’s activity is at such a low ebb then that it can only infect the occasional unusually susceptible person. Then after a while a useful antigenic variant turns up or waning of herd immunity helps it to get going again.

We are beginning to get glimmerings of ideas concerning the periodical activity of colds—why they are more prevalent in winter and in certain climates. Despite efforts to produce some, there is no good evidence that rhinoviruses and epidemiologically similar agents spread any better in winter than in summer. Rather does it seem that an infection in winter is more likely to be clinically manifest. Simultaneous occurrence of colds over wide areas points to some activating stress which is likely to be of a meteorological nature. I say this in spite of our own failure at Salisbury to show that chilling increases susceptibility to colds in individuals. It does not seem that one can explain matters on the supposition that some people carry a particular virus, as herpes is carried, and that this is periodically activated; for in one individual observed by Dr D. Hamre five successive colds were caused by five different rhinovirus serotypes. In a recent discussion of the subject (Andrewes, 1964) I have put forward a tentative hypothesis. According to this, cold viruses in small doses are constantly being passed back and forth between people, obtaining only temporary footholds, perhaps in quite small foci on the respiratory mucosa. These may be insufficient to cause generalized immunity and may be prevented from spreading widely on the mucosa by rapidly mobilized interferon or some other local defence. Only when this defence is breached as a result of some stress does a cold result. There is evidence that stress may inhibit interferon production. In a particular instance which has been published, interferon production was inhibited when mice were stressed by subjecting them to loud noise for 3 hr a day (Chang & Rasmussen, 1965). It is also clear that stresses of several kinds play a part in causing outbreaks of respiratory infections among service recruits (Pierce, Stille & Miller, 1963). If such an explanation should be correct it would indicate that cold viruses have evolved an ingenious mechanism for self-perpetuation. Despite the troubles I discussed earlier they seem to get around without much difficulty. If every happy landing led to a miserable cold, too many subjects would acquire

immunity, as they do after a cold—though only to that particular virus. But if it is only the people who chance to be in a receptive state who develop overt and therefore immunizing colds, the virus will find susceptibles available over long periods.

We do not yet know whether rhinoviruses are antigenically labile as influenza A is and whether such lability is a further aid to them. If, as now appears, there are at least eighty serotypes and probably many more, they may not need to vary. The ingenuity of a mechanism whereby they spread only to certain individuals may be an expression of their subtle efficiency rather than being calculated inefficiency such as I suggested earlier.

An alternation of activity and quiescence seems to be useful to a number of viruses and this may be helped by quite small variations in their properties. If, for example, environmental factors could switch them between states of greater and lesser susceptibility to interferon they might be able either to grow and spread more actively or, when it suited them, to go into temporary retirement.

An ideal virus could of course be one which directly benefited its host; and such viruses may actually exist. The maize hopper, *Dalbulus maidis*, normally feeds only on maize and a related grass species; on asters it dies. But when fed on asters already infected with aster yellows virus it thrives, and after a while is able to thrive also on uninfected asters, on carrots or on rye. The effect seems to be on the ability of the hopper to digest previously indigestible foods. Now aster yellows virus can be killed by moderate heat and such heat-treatment cures infected hoppers of their infection; but then, behold, their abnormally fine powers of digestion have gone also. Maramorosch & Jensen (1963) have suggested that we may be overlooking something of wide importance. If there were lots of beneficial viruses in the world, should we ever suspect that they existed?

I shall now re-assume my normal anthropocentric view of my subject. We have, I think, to admit that though viruses encounter many troubles, they are remarkably successful in overcoming them. If, however, we can manage to be temporarily virocentric and see where a virus's troubles lie, we may better be able, in the case of truly harmful viruses, to see that those troubles become even greater, so that we can become their masters.

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