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How Do Viruses Invade Mucous Surfaces? [and Discussion]

D. A. J. Tyrrell and R. F. Sellers

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How do viruses invade mucous surfaces?

By D. A. J. Tyrrell, F.R.S.

M.R.C. Common Cold Unit, Harvard Hospital, Coombe Road, Salisbury, Wilts SP2 8BW, U.K.

One can express this subject in terms of the 'strategy' that a virus 'uses' to survive by ensuring passage from host to host. Firstly, viruses are shed into the environment by a route and in sufficient quantities to ensure access to the mucosa of another host. In this connection spread may be more successful if symptoms are produced; if, for instance, an intestinal virus produces diarrhoea or a respiratory virus causes sneezing.

The virus has next to survive exposure to adverse circumstances such as drying in the air or exposure to light in order to retain sufficient viability when it reaches the mucosa: very labile viruses, such as genital herpes, are usually transmitted by close contact of mucosa to mucosa.

The mucosal secretions, and in some areas the ciliary apparatus, form a barrier to all particles, including viruses. However, viruses do traverse them and reach the plasma membrane of susceptible cells in which they then initiate infection. It is possible that viruses may be trapped by phagocytic cells and be carried by them through the mucosa.

Introduction

I have been allotted a very wide and general topic and one that could therefore be tackled in a number of different ways. It is included in a review by Burrows (1972) and in many ways there is little new to say but I shall try to build up a picture of how a virus particle approaches, reaches and then passes into or through the cells of a mucous membrane, and to think about the possible obstacles in its way. Before doing this it is probably worth while correcting a few possible misunderstandings. In the first place viruses cannot invade at all, in the sense that they are completely non-motile. Of course, so are many bacteria, fungi and microparasites, but viruses are totally unable to push themselves in any direction but are moved entirely passively, though when in free solution they can be agitated by Brownian movement and progress very slowly even on a microscopic scale, by diffusion processes. It has become fashionable to speak of the 'strategy' of the virus genome, in the sense that viruses show different combinations of nucleic acid structure and enzyme synthesis and activity as a result of which faithful copies of the viral nucleic acid are eventually produced. Nevertheless, their other properties and behaviour are also highly integrated and appropriate to their way of life, and so it is also possible to talk of a virus having a 'strategy' for invading mucous surfaces.

The term 'strategy', of course, is teleological and implies that the organism has thought out a scheme for reproduction, which is not really meant. It nevertheless makes for convenient exposition of the natural history of viruses at the molecular level. I am going to write mainly at the level of descriptive biology and natural history and although I should like to be able to take the description and the analysis to the molecular level this is not usually possible. However, I shall point out from time to time where, in my view, there are opportunities for

this kind of approach. The distance between a virus in an infected host and the mucosa of another is one of the most effective barriers to successful infection, so I start my consideration of a strategy by considering how the virus arranges to be released and to find its way up to the mucosal surface.

Shedding into the environment

Viruses only invade mucous surfaces if they are shed into the environment in such a way that in due course they encounter the mucous membranes of an appropriate host species: this phase of the life cycle is therefore closely determined by the cells in which the virus can multiply freely and to which it can gain access. Viruses that multiply mainly in bloodstream-related cells are usually transmitted directly from blood to blood by arthropod vectors.

On the other hand, various viruses of the intestinal tract are shed in large amounts into the lumen of the intestinal tract and so are shed automatically in faeces (Tyrrell & Kapikian (eds) 1982); polioviruses would be an example. It is interesting to speculate that the shedding may be more effective if the virus also produces symptoms, for it is known that excreters of *Shigella sonnei* are more likely to infect others while they have diarrhoea. It may well be that those viruses, such as rotaviruses, that often produce diarrhoea are more likely to be successfully disseminated that those that do not. Although apparently both strategies can be effective there must be important trade-offs between the strategy of being shed in very large amounts in a fluid motion or being shed over a longer period at a lower concentration. Indeed, it should be possible to analyse these optimum situations by mathematical modelling including the frequency, duration and closeness of direct or indirect contact with the next host in the chain of transmission. Normal nasal and trachobronchial secretions are passed into the pharynx and down into the gut without being expelled into the exterior; indeed, most of them would be inactivated there. It may therefore be of great importance to survival that many of the respiratory viruses produce symptoms in the form of sneezing and coughing, which expel an increased amount of nasal secretion in droplets of various sizes, without making the host so ill as to withdraw from the vicinity of other potential victims.

Whether a virus replicates in a particular cell type in a particular surface is thus a crucial element in its natural history and whether it can reach a mucous membrane. This important subject is being studied at the molecular level and dealt with elsewhere, but in general terms it is true that virus is passing from the mucosa of the one host in which it replicates to the same mucosa of the next, and particularly from respiratory tract to respiratory tract or from one alimentary tract to another. However, there are other routes: in mumps the virus is shed as infected saliva and probably enters by the upper respiratory tract, and rotaviruses could become aerosolized from fluid faeces. Herpes viruses may well spread usually by direct contact between stratified epithelial surfaces of the oropharynx or the genitalia, but the varicella zoster virus passes through a very successful cycle in which it becomes airborne from skin lesions and is presumably trapped in the upper respiratory tract.

It is therefore clear that the question of invading a mucosa cannot be separated from the question of what cells are susceptible to infection and from what cells viruses are shed. Indeed, it might well be that of the total number of virus particles shed by a patient with a common cold more reach the skin of the face or hands, or the pharyngeal or even the gastric mucosa of another subject than reach the nasal epithelium (Bynoe *et al.* 1961). Yet, just like the failure

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of most sperms to fertilize an ovum, none of this matters as long as one infectious virus particle gets to the layer of cells that it is exquisitely adapted to parasitize.

Once shed, a particle is more likely to infect the longer it persists in the environment. It seems that there are considerable differences in this respect. Respiratory viruses are usually relatively unstable, but, on the other hand, the aerosols in which they are expelled are probably rapidly dispersed and precipitated. Enteroviruses are relatively stable, especially in humid conditions, and this may be linked with their transmission, which usually occurs by the faecal–oral routes in contaminated water or food.

Transfer to the new host

Once virus has been shed, the transfer to the mucosa depends on two factors: whether physical forces are available to move it to the vicinity of the target mucous membrane, and secondly whether the particle will still be infectious when it arrives there.

Normal excretion and secretion may, in fact, provide these forces; it may be significant, however, that viruses do not in general leave the body in urine, though rodents that are urinary carriers are notorious as the reservoir of some of the viral haemorrhagic fevers. It may be significant that the urinary tract mucosa is normally only accessible via the bloodstream, so a urine–urine transmission is not readily set up. Large numbers of viruses are shed in the faeces, presumably because the intestinal tract is, in a sense, an exterior or open mucosa constantly exposed to anything that enters via the mouth particularly in the way of food or drink. The respiratory tract is slightly different because normal mucosal secretions are largely swallowed and then eventually reach the exterior via the faeces. This may well be a 'dead end' because even if the viruses survive, the faeces are not readily spread, at least as an aerosol.

Physiological mechanisms are 'exploited' by various viruses to enhance the efficiency with which they are shed, particularly the way in which symptoms are produced in the upper respiratory tract. They are an inconvenience to us but the increase of nasal secretion means that more fluid is found in which a virus can be rendered airborne. In addition, reflexes such as sneezing or coughing are triggered and produce airflows that scatter pools of fluid into small airborne droplets and expel them forcibly into the surrounding air and sometimes towards unsuspecting hosts. Very little is known about exactly how the presence of virus stimulates these reflex responses, though the loss of functioning cilia is probably part of it (Anderson *et al.* 1977). More work could also be done on the abnormal physiology of diarrhoea in virus infections. Similarly, some intestinal viruses alter the balance between absorption and secretion in the intestine and so give rise to fluid faeces in which they are more likely to reach another host.

The return of a virus to the cells of a respiratory tract is assisted by the so-called 'air-conditioning' mechanisms of the respiratory tract. About 10 l min^{-1} of air are respired for 24 h a day, and that air is cleared of particles (and also warmed and humidified) by contact with the ciliated mucus-secreting surfaces of the nose and tracheobronchial tree on which particles are impacted and from which water is evaporated. Virus particles are thus picked up and incorporated into the surface mucus: in which area this occurs depends largely on the size of the physical particles of which they form part, roughly speaking those less 5 μm in diameter in the lower respiratory tract and greater than in the nose (Druett 1967).

It is customary to divide routes of transmission simply into airborne or faecal–oral, but, in fact, it is unlikely that such a sharp division corresponds to reality. If an aerosol of faeces is

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produced it can be inhaled and subsequently swallowed and reach the alimentary tract just as well as if it reached the water or food supply by failures in good hygiene. On the other hand, recent work has indicated that viruses expelled as airborne droplets may land on objects such as domestic utensils and be transferred by finger contact to the nose or conjunctiva (Hendley *et al.* 1973; Reed 1975). In some cases this rather 'abnormal' route of transmission can play a role in producing what seems to be a 'new' disease; an example is the way in which adenoviruses are spread by inadequately sterilised instruments in epidemics of adenovirus keratoconjunctivitis or 'shipyard eye'; without this mechanism the viruses usually infect the pharynx and intestinal tract.

If we are to understand at a basic level either the usual or unusual routes by which viruses reach the mucosa we need to consider how they survive and remain infectious for susceptible cells.

Survival of infectivity

It is surprising at first sight that certain viruses that in the laboratory are very unstable or of limited infectivity can be transferred from host to host in a cycle of mucosal infection. Further thought shows that some of these viruses have particular elements in their 'strategy for survival' that counteract this apparent disadvantage. Members of the herpes virus group exemplify this. Type I herpes simplex is usually spread to a child from the mother or another child; nevertheless the improvements in hygiene and child caring of recent decades seem to have delayed considerably the age at which subjects acquire type I herpes viruses either from cases of herpes or from individuals who silently shed the virus in saliva. Herpes types I and II can produce recurrent mucosal lesions that enable them to infect a new host many years after successfully establishing an infection, but we do not understand why in ordinary circumstances type I is usually successful in invading the oropharyngeal mucosa while type II succeeds better in the genital tract. Type II herpes viruses are usually transferred by direct contact between mucosal surfaces during sexual activity, and EB virus infection, in which small amounts of virus are found in mouth secretions, has been christened colloquially 'the kissing disease'. Thereafter, these viruses produce life-long infections by various molecular mechanisms, and frequent successful transfers are not required for the organism and its genome to survive. The varicella-zoster (V-Z) virus is different. In the laboratory it produces less infectious virus than the herpes simplex viruses, and more than EB viruses. However, in skin it produces infectious particles, and clinical experience shows that it spreads most successfully by the airborne route. Then, later in life, infection may recur and produce a skin lesion, shingles; virus latent in the nervous system moves to skin epidermal cells, which produce large amounts of infectious virus, and this can become airborne on a sufficient scale to set off an epidemic of chickenpox. It is presumed that virus is inhaled, infects the mucosa and then disseminates to produce the characteristic generalized skin lesions from which more virus is shed. By contrast, EB virus shed in the respiratory tract is believed to be often in the form of infected cells.

The molecular mechanisms that lead to the formation of infectious V-Z virus in skin or in tissue cultures are ill-understood, and so, to my knowledge, are those that determine how much of the EB virus genome is expressed and whether it generates infectious virus particles. It would thus be of great interest to bridge the gap between our general understanding of the herpes viruses and the molecular biology of their replication, by further work in this area.

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The respiratory viruses form an even more heterogeneous group, many of them differing profoundly in structure, some being DNA, e.g. adenoviruses, and others RNA of various types, e.g. rhinoviruses and the myxoviruses. However, it is probably of even more importance for our present discussion that some are enveloped while in others the viruses consist simply of nucleic acid and protein. This and other differences must explain why there are profound variations in their survival under varying conditions of temperature and humidity. This has been examined by measuring the decline of infectivity of virus contained in aerosols of controlled size maintained in atmospheres of known humidity and temperature. The rate of inactivation is found to be higher at higher temperatures, as might be expected, but to vary quite widely with humidity. Although the suspending medium clearly plays a role it seems that some viruses are more stable at lower relative humidities while others survive better when r.h. is higher. Some rough generalizations can be made, namely that lipid-enveloped particles survive better in lower r.h. but unenveloped at higher (Buckland & Tyrrell 1962). Furthermore, viruses that survive at low r.h. like influenza (and probably coronaviruses) are said to be more prevalent, or at least tend to increase in incidence, in the depth of winter when r.h. indoors is at its lowest, whereas unenveloped viruses like rhinoviruses increase in prevalence earlier, in the autumn, when there is little heating and indoor humidities have not fallen. However, these relations are not solidly documented. More information about the seasonal incidence of infections shows that at least one enveloped virus, e.g. parainfluenza 3, is prevalent throughout the year, but that may indicate that others of its properties, such as transmissibility and shedding pattern, are such that its temporal distribution is controlled by something other that the effect of temperature and humidity, perhaps the entry of new susceptibles into the population.

Some work has been done on the mechanisms by which infectivity is lost in stored aerosols; for instance, it may be that the virus nucleic acid is still infectious when extracted from the particle, so the protein coat is presumably damaged, but we do not know which viral peptides are altered, or in what way. Nor do we know whether it is cell attachment, uncoating or some other function of the particle that is impaired. This could be attacked by a direct study of the defects in the infectivity of stored aerosols, or perhaps by comparing the inactivation rate of parental strains with that of mutants with lesions in defined elements of the genome. There is epidemiological evidence that weather has an effect on the efficiency of transmission of colds. It is therefore an attractive theory that this is because changes of temperature and humidity affect the survival of virus in the air, but it could be due to quantitative differences in the efficiency with which the respiratory mucous membranes trap or dispose of viruses. I know of no detailed experiments on this possibility.

The transfer of intestinal viruses is rather different. It is generally found that in laboratory conditions most of these viruses are more stable than most respiratory viruses. I suspect that this is correlated with the fact that most natural aerosols are rather short-lived so that there is limited selection pressure to encourage long-surviving virus particles. On the other hand, faecal–oral transmission may take a long time and the faecal environment may be quite harmful to most biological material; this may be why viruses such as enteroviruses and rotaviruses are generally found to survive well both in the environment and in the laboratory. Enteroviruses do not well withstand drying, but rotaviruses have a curious biomodal survival pattern with variations in relative humidity (Moe & Shirley 1982); this may explain why they can be passaged freely in humid conditions, for instance, in farmyards or in village conditions in some tropical countries, whereas in temperate zones they are found most commonly in the early part

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of the year, just when the upsurge in influenza virus activity often takes place and they are probably travelling by the aerosol route. There is apparently a further refinement in the 'strategy' of some intestinal viruses. It is likely that modifications that increase the survival of the virus particle in the environment are likely also to increase the stability when it enters the cell and so impair uncoating and the inactivation of infection. But it has been found that certain viruses require exposure to trypsin to become fully infectious: this has been shown with reoviruses in some detail, but is apparently also true of rotaviruses (Sato *et al.* 1981; Kurtz & Lee 1982) and I should not be surprised if it turns out to be true of others (and this might explain why at least some of them have proved difficult to cultivate *in vitro*). Thus they are stable until they reach the environment in which susceptible cells are also found, just as a tough seed germinates and becomes vulnerable when it encounters the right temperature and humidity. Perhaps some 'difficult' viruses are activated by low pH or by deoxycholate. For many respiratory viruses acid, bile salts and proteolytic enzymes are lethal, but in terms of natural history this is irrelevant.

Traversing the mucous barrier

Mucous membranes by definition all contain mucus-secreting cells in or under the surface epithelium, and the mucus they secrete serves to keep the surface moist and also acts as a protective barrier. In addition, they vary enormously in their structure and function. The oral and pharyngeal mucosa is a non-keratinized squamous epithelium, as is the vagina; the intestine is covered with villi the surface of which is largely composed of enterocytes; and the respiratory mucosa is lined with very active cilia that beat in a layer of mucus secreted by small glands or non-ciliated goblet cells. It is clear that these cells vary greatly in their susceptibility to viruses; Hoorn & Tyrrell (1966) showed *in vitro* and *in vivo* that a rhinovirus did not replicate in mucosa from the mouth but did do so in nasal or tracheal epithelium, so we must bear in mind that the fact that a virus fails to infect or produce disease by a particular route does not necessarily mean that it has failed to penetrate the defences: rather, no defences may be needed because the cells are fundamentally insusceptible. The mucus is moved over the surface either by movements such as swallowing or peristalsis, in the alimentary tract, or by the action of cilia, most characteristically in the respiratory tract. The mucus is said to form a sticky gelled surface in which particles may be trapped, and this 'blanket' is moved forward by the cilia moving in a fluid layer immediately beneath it. However, this blanket may not be uniform or continuous.

We should first consider the role of the mucus. The functioning of the postulated gel phase in trapping virus particles has never, to my knowledge, been examined specifically and in detail. Bang & Bang (1969) showed that chicks that were dehydrated and whose mucus became thickened were less susceptible to infections with Newcastle disease virus. Attempts were made to modify susceptibility by damaging the muciciliary apparatus in human volunteers by getting them to inhale SO_2 about the time of virus inoculation (Andersen *et al.* 1977). This did not increase susceptibility, but rather reduced the severity of the disease. This might be the result of the inoculum's being inactivated because of the low pH produced by the SO_2, but it is also possible that the virus exploits the cilia in getting to the cell surface. Dourmashkin & Tyrrell (1970) showed that influenza virus can react specifically with cilia (e.g. particles adhere to cilia of vertebrate but not of protozoal cells). However, whereas a paramyxovirus can fuse with the

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cilia membrane, and the nucleic acid so released might initiate infection, the morphological evidence that we obtained suggested that influenza virus entered non-ciliated cells first, though later on ciliated cells were also infected and destroyed. I therefore developed the rather fanciful picture of viruses exploiting the cilia which are constantly 'combing through' the mucus sol layer. Thus virus would be trapped, free from the risk of being carried away in the mucus flow, and would later release itself, via neuraminidase action, in the vicinity of an adjacent non-ciliated cell towards which it had been carried by the action of the cilia. The mechanism of the interaction of the virus haemagglutinin with the cell membrane is now being unravelled at the molecular level. However, modern methods have still not been used to understand how virus particles are handled after they impact into the mucus. Studies with particles of plastic, etc., are not adequate because they would not interact with the mucus and the cell surface as a virus does. It should be possible to study the fate of isotope-labelled virus particles.

However, there has been interest in the possibility that mucus might react more specifically with viruses. Secretions and serum are known to contain 'non-specific' (really 'non-antibody') inhibitors, for influenza viruses (Burness 1981). These are really receptors in solution or, to use another image, 'decoy' molecules. They can be readily detected because they inhibit virus haemagglutination and they apparently contain terminal neuraminic acid residues that can be eliminated by neuraminidase, just like those on cell surfaces. Although in a model system one can show that these inhibitors prevent the influenza virus from getting to the cells it is often necessary to manipulate the system to show a big effect: for instance to use a low temperature for the reaction or to heat the virus to inactivate the neuraminidase or modify the haemagglutinin. It is generally felt that there is little or no protective effect if inhibitors are added to the medium in systems *in vitro* with the aim of reducing the chance of infection of target cells. It may be that a really high local concentration, such as is found at a mucous surface, might have an effect, and certainly Soviet workers were at one time impressed with the importance of these and other inhibitors in resistance to infection. Serum contains so-called β inhibitors, which actually neutralize virus infectivity and viruses may become resistant to these as they are adapted to growth in mice, but this is not a regular phenomenon and the inhibitors are not found in the mucus secretions whose function we are considering, so it is unlikely that they constitute an initial barrier. Because they can be destroyed by virus neuraminidase it is unlikely that these mucin receptors can prevent infection with myxovirus, and for other viruses for which they do not act as receptors it is unlikely that they do anything but trap them mechanically.

Serum and secretions such as saliva and milk also contain another inhibitory substance (or substances) that appears to be glycopeptide that is not fully characterized but is not antibody (Matthews *et al.* 1976). *In vitro* it can prevent the infection of a monolayer of susceptible cells by an indicator virus such as VSV, probably by competition for a receptor. However, when the hypothesis was tested that the concentration of such inhibitor in breast milk might modify rotavirus infection in the newborn, no evidence of such an effect was found (Totterdell *et al.* 1982). There is thus little to support the idea that there are barriers of this sort, though more work is needed. It is much better documented that mucous secretions contain specific antibodies, particularly IgA, and not only do these neutralize virus infectivity *in vitro* but their presence can be shown to be associated with resistance to infection by the corresponding virus. However, this aspect of the entry of viruses into mucous membranes is excluded from the present discussion. The mucous secretions also contain phagocytes and other cells that may play a protective role although they have been studied relatively little. It is clear that neutrophils can

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take up influenza virus particles and not be infected by them. Eventually, particles would be inactivated in lysosomal vacuoles. Macrophages are present and certain viruses, for instance human coronaviruses, can replicate in such cells. A virus in a macrophage may not be released to infect a mucosal surface; it may be carried through the mucous membrane. In some systemic virus infections in which the organism enters by the respiratory tract it appears that it multiplies silently in the mucosa before disseminating, but in others it is possible that a virus is picked up by a cell in the mucous layer, that this cell then migrates actively between the cells of the mucous membrane and enters the circulation, and that virus particles released there will have ready access to susceptible cells in other parts of the body. In this way, being taken up by a phagocyte can be an essential step in breaching the mucosal barrier (Mims 1972). It is important to realize that it may be difficult to do a critical experiment to study such a process; indeed, if a virus enters a cell and begins to replicate but is then quickly and efficiently eliminated, because the cell is destroyed by a cytotoxic lymphocyte, progeny virus is neutralized by an inhibitor, or because adjacent cells are rendered resistant by interferon, then it might be very difficult to distinguish this from a simple failure to penetrate the defences of the mucosal cells.

Final contact with cells

This is a subject in which research is difficult and established facts are few. It is clear that under favourable conditions a very small dose of virus, a single plaque-forming unit or a few t.c.i.d.$_{50}$, representing a small number of physical particles, can infect an organism. However, the dosage response curve is often 'flat', implying that in many animals large amounts of virus are required to infect. This may be due to variations in the method of administration of the virus or to variations in the resistance of the target cells perhaps genetically determined; but it could also or alternatively occur because there are random obstacles to the virus's reaching the target cells.

The simplest situation to analyse might be exactly how viruses that invade squamous mucous membranes get to the cells. In the skin it is known that an abrasion is needed to break the barrier of the keratinized cells, but it could be of interest to study the progress of the early stages of infection, to determine perhaps by immunofluorescence exactly which cells are invaded first. Of course, oral lesions may be due to infections carried to them in the bloodstream, as in the characteristic ulcers of measles and some enterovirus infections. It has been suggested that viruses enter the epithelium of the intestinal tract by the processes that take up inert mineral particles from the lumen, but it seems unclear how this operates and whether organisms such as rotaviruses, which invade absorptive epithelium, are taken up by the same mechanism as enteroviruses, which are characteristically found in the lymphoid tissue of Peyer's patches. Are they moved to the cells by peristaltic movement? Could we calculate the probability of a virus's being carried to the surface of a cell in this way? Respiratory tract cells deserve closer study, and both they and squamous epithelium could be investigated *in vitro* as organ cultures. It would be worth studying the interaction of various viruses with the mucous layer and cilia, and indeed to find out whether ciliated or goblet cells are primarily infected.

It may be that contact with a cell may not initiate infections. A myxovirus might be eluted by neuraminidase if it is not rapidly engulfed, indeed this may be advantageous if it attaches to a sialic acid residue but does not trigger the formation of a vacuole.

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Conclusion

At the request of the organizers I have deliberately cast this paper in a form to highlight the number of unanswered questions that I can see in this aspect of pathogenesis of virus infections. I cannot say that the answers will be of great practical value, though some might be, but I do not think we can say we understand how a virus causes an infection if we take for granted that it can reach and gain access to cells of a mucous membrane. No, there is still much to do and to learn.

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Discussion

R. F. Sellers (*Animal Virus Research Institute, Pirbright, U.K.*). In infection by the respiratory route, virus is presented in the form of airborne particles or droplets 3–6 μm in diameter. As respiratory viruses are much smaller, is there any information on how such viruses become available to infect cells of the respiratory tract?

D. A. J. Tyrrell. No, not really.

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