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Mechanisms of Virus Pathogenicity

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INTRODUCTION

This review stems from a previous report (143) in which the biochemical mechanisms of bacterial pathogenicity formed the main subject and were discussed in a manner which prompted the following questions regarding the pathogenicity of other, less-studied microbes including viruses. Can differences in virulence be detected and measured in vivo? Are reasonably stable virulent and avirulent strains available so that virulence markers and determinants can be recognized (i) by comparing the behavior of the strains in vitro and in vivo and (ii) by observing the effects of the products of the virulent strain on the behavior of the avirulent strain? How far can avirulence be due to any inherent inability to grow in host tissues (as distinct from an inability to combat host defense mechanisms)? What host defense mechanisms act against the microbe and what aggressions inhibit them? Are the pathological effects of the disease due to production of toxins (acting intracellularly or systemically, or both), depletion of nutrients, mechanical blockage of vital tissues, or evocation of hypersensitivity or auto-allergic reactions? Can host and tissue specificities be explained either by differential distribution of microbial inhibitors or differential suitability of tissues for microbial growth?

At the time, only incomplete answers to these questions could be provided for viruses due to lack of space and knowledge. Now more information has accumulated (148), and the object of the present review is a more comprehensive discussion of these and other questions relating to the pathogenicity of viruses. The review is written from the standpoint of one who has entered the field of virus pathogenicity from studies of bacterial pathogenicity, and whose predominant impression is that the broad aspects of pathogenicity of the two microbial types are similar despite the obligate parasitism of viruses. In particular, viral-like bacterial pathogenicity is not determined solely by biochemical ability to replicate in host tissues; virulent and attenuated strains of viruses replicate in host cells in vitro, yet they differ fundamentally in behavior in vivo, presumably—as for bacteria—due to different capacities to counteract host defense mechanisms and to damage tissues. Also, the fact that the viral factors responsible for virulence mechanisms are induced within host cells does not confer uniqueness on viral pathogenicity. Although replicating by different processes, many pathogenic microbes (including bacteria) are intracellular parasites, and often bacterial virulence factors that are produced extracellularly enter and act within host cells, e.g., the interference with protein synthesis by the toxin of *Corynebacterium diphtheriae*. Even the latency of viruses has its parallel in the carrier state of bacteriology. Finally, animal experiments with viruses are as essential as those with bacteria, for many aspects of pathogenic behavior in vivo are missing in tissue culture experiments (142).

It is hoped the reader will use the previous review (143) as a basis for what follows. The terms "pathogenicity" and "virulence" are nearly synonymous and mean the capacity to produce disease; as suggested by Miles (93), virulence is used here mainly with respect to comparisons of the disease-producing capacities of strains within a species. Tumor viruses have been largely excluded from the review since their pathogenicity is a special case requiring separate treatment. Also, latency has

not been specifically discussed, because the phenomenon has been described many times, there is little real knowledge of the mechanisms involved, and the speculations on their nature are well known (98).

QUANTITATIVE COMPARISONS OF VIRULENCE: PROPERTIES OF VIRULENT AND ATTENUATED STRAINS

Although mechanisms of pathogenicity can be investigated by using a single strain of virus, the existence of stable strains of differing virulence greatly increases the experimental scope in the search for virulence markers and determinants. Different virus strains exist, and advances in viral genetics (48) will increase the number available, but quantitative comparison of their virulence is difficult because of the low efficiency of viable counts. The effects in animals (LD_{50}, lesion size, or mean death time for the "same" dose) must be related to amounts of virus particles indicated by plaque counts or egg infection. The latter detects only a small proportion of the total virus particles and therefore may not measure all the particles (which could vary for different strains) capable of multiplying in experimental animals. For example, plaque counts on chick embryo fibroblasts detected less infectious particles of Semliki Forest virus than infection of suckling mice (20), and in this system the proportion of total virus particles detected by the plaque counts was fairly high (approximately 1 in 10) compared with many other virus systems. Thus, for this and other reasons, comparisons of the virulence of virus strains are often imprecise. Burrows (24), for example, has described the imprecision of virulence comparisons between strains of foot-and-mouth disease virus and the lack of correlation with behavior in the field.

Only virus strains for which conventional tests have indicated large differences in virulence should be compared to recognize virulence markers and determinants. Comparisons of such well-tested and well-separated strains have been rare (14, 164) but informative. Attenuated strains of poliovirus had less affinity than virulent strains for the cell receptors of primate central nervous system (65). Virulent strains of ectromelia virus had a greater capacity to infect mouse macrophages than attenuated strains, although they had equal ability to infect hepatic cells (95). Well-tested strains of Semliki Forest virus of widely different virulence (20) have been examined in mice (121). Surprisingly, in view of the wide difference in virulence, and in contrast with studies on less well-defined strains of Japanese B encephalitis virus (70), muscle replication and systemic infection of the attenuated strain was at least as high, if not higher, than that of the virulent strain; only in the brain was virus replication higher for the virulent strain than for the attenuated strain (121). Because of the ease of virulence comparisons in chickens (164), well-established virulent and attenuated strains of Newcastle disease virus are available. Early work showed virulent strains to have a greater capacity to replicate in chicken brain and penetrate the tissue of eggs (14, 164). Recently, the virulence of strains was associated with an increased capacity to produce cell-fusion effects and plaques in chicken embryo fibroblasts (125); there were no major differences between strains in the kinetics of replication, either in timing or amount of virus released (127), but virulent strains produced more cell-associated hemagglutinin and neuraminidase (1, 126).

ENTRY OF THE HOST: SURVIVAL ON AND PENETRATION OF MUCOUS MEMBRANES

Some virus diseases are more communicable than others. Communicability depends on the factors (24) determining virus release from one host, survival in the intermediate environment, and entry into another host. Only the latter process is discussed here. Some viruses enter host tissues directly by trauma or insect bite, but most infections start on the mucous membranes of the respiratory and alimentary tracts. To initiate infection, virus particles must first survive on these mucus-covered membranes in the presence of viral and nonviral commensals. Subsequently, to replicate, the virus must enter host cells either in the mucous membrane itself or in tissues farther afield after penetration through the surface membrane. Replication in mucous membrane cells can produce the disease effects directly as in respiratory diseases, but sometimes it provides a staging post for subsequent damaging replication in another site, e.g., poliovirus replicates first in the alimentary tract cells and ultimately in anterior horn cells. Detailed knowledge of the factors influencing the early stages of virus disease is almost completely lacking, mainly owing to lack of techniques for observing the behavior of a few, highly dispersed virus particles on mucous surfaces with their indigenous microbial populations. Respiratory-tract infections provide most of our present information, and they are the main

examples in the following discussion of early virus attack. Descriptions of the mucus-producing cells and the clearance action of the mucociliary movement have been provided by others (42, 80, 118, 161) and need not be repeated here.

What factors determine the initial site of virus lodgement? In the respiratory tract, the size of the droplet to which the virus is attached is the prime factor (42), but some differences in deposition occur in different animal species due to different size, anatomy, and posture (24). Within one species, small differences in anatomy and breathing pattern can influence deposition of virus (134). Apart from these mechanical factors, chemical factors which might determine tissue specificity (16, 17) may play a role in primary lodgement if present in the pathway of the incoming virus. The site of virus deposition can influence the subsequent pathogenesis of the disease. A recent reinvestigation (24) of the pathogenesis of foot-and-mouth disease following the discovery that the disease can be aerosol-transmitted showed differences in disease development according to whether initial infection occurred through the natural site, the pharyngeal region, or through tongue epithelium formerly thought to be the natural site.

How do viruses penetrate the moving mucus blanket which sweeps particles towards the pharynx? Mucus depth and speed of flow are obviously important in this process. Little is known of the depth and nature of mucus in different parts of the respiratory tract, although it appears that the cilia beat in a watery layer underlying a stiffer particle transporting layer, and in the lower respiratory tract mucus thickness decreases and the composition changes (37, 80, 118). Mucus flow rates are better understood. There is a velocity gradient from small to large airways (4) and individual rates can vary widely, for example, 4-fold in chickens and 10-fold in humans (11, 122). Obstructions in the tract such as bronchial junctions and protrusions can produce local changes in flow rate (42). Many factors affect mucous secretion and ciliary action (11, 118, 166), including ion concentration in the air, temperature, and humidity; the last two affect nasal epithelium rather than lower parts of the tract where the air has been preheated and humidified. Clearly, the thinner the mucus and the slower the rate of flow, the more likely the occurrence of epithelial infection or penetration. This has been demonstrated experimentally. Reduction of nasomucociliary activity of chicks by exposure to low temperature or by injections of cocaine or pilocarpine increased infection rates with a standard exposure of Newcastle disease virus (11, 13). What happens in natural infection is still a matter of conjecture. Viral infection should occur more easily in the lower than in the upper respiratory tract because of a slower movement of a thinner protective film. Mucus flow might be almost stationary at respiratory tract obstructions, thus providing foci for virus attack (42); or gaps in the mucus coat might occur, exposing the underlying tissue (118). However, in epidemics, infection occurs in too many individuals to implicate simultaneous impairment of mucociliary action. The penetration mechanisms operating in many virus infections may have little connection with variations of the mucous blanket and may reside in the properties of the virus itself, such as an extra affinity for the surface receptors or phagocytic action of susceptible mucosal cells against which they brush during the mucociliary movement. Better techniques for observing the behavior of small numbers of virus particles on mucous surfaces will be needed before these possibilities can be investigated.

If mucus contains virus inhibitors, how do virulent viruses counteract them? There is little doubt that mucus can inhibit or kill some viruses. The slightly alkaline *p*H inactivates some viruses, such as foot-and-mouth disease virus (24), and nonspecific inhibitory substances have been found in mucus and homogenates of mucosae (123). Perhaps the major antiviral activity in respiratory and alimentary tract mucus resides in specific immunoglobulins (largely immunoglobulin A) arising from previous infections or immunization (74, 159). How virulent viruses overcome these nonspecific and specific virus inhibitors is unknown. Local concentration of virus particles at one site might saturate the inhibitory materials, allowing active virus to penetrate the mucus. But this is pure speculation. Comparisons of virulent and avirulent strains as regards resistance to mucus inhibitors might be revealing.

Clearly, some viruses, such as influenza virus, attack the mucosal cells, but do others pass through the mucosa without establishing infection in the membrane itself? Simple penetration appears to occur in infections with African swine fever and rinderpest where there are rapid recoveries of virus from the lymph nodes draining the respiratory tract (24). The mechanisms of penetration are not clear, but carriage by nondestructive macrophages may occur as for ectromelia virus by mouse alveolar macrophages (98).

How are any inhibitory effects of commensals overcome? Although tissue culture and animal experiments show that different types of commensals could, and probably do, interfere with early virus attack by such mechanisms as interferon production or usage of essential metabolites (like arginine by mycoplasmas [157]), there is no real proof that such interference occurs in natural infection or that viruses counteract it. Some experiments are suggestive; for example, reduction of commensal bacteria might have accounted for the increased susceptibility of oxytetracycline-treated pigeons to Venezuelan equine encephalitis virus (94).

When mucosal infection occurs, which surface cells are attacked? Differential susceptibility of the upper and lower respiratory tract has been detected by applying the same dose of virus to the nasal epithelium and administering it as a small-particle aerosol. The upper respiratory tract of man was more susceptible to infection with coxsackievirus A21 and rhinovirus NIH 1734, the lower to adenovirus type 4, and both tracts were equally infected with influenza virus (34, 35, 81). But the initial target cells were not recognized. Histological and immunofluorescent studies have shown influenza virus in ciliated intermediate, basal, and possibly goblet cells of human nasal epithelium (43), but again the initial site of attack was not clear. In Newcastle disease of chickens, however, the acinous mucous cells appeared to be the target cells (13). Organ cultures of relevant parts of the respiratory tract may allow a closer study of early events, for example, the close adhesion between influenza virus and the cilia and microvilli of nasal epithelium that precedes virus replication (41, 56).

REPLICATION IN VIVO

Although ability to replicate in host tissues is not the only factor in virus virulence, it is essential, and the more rapid the rate of replication the more likely the success of the virus in producing its disease syndrome. At present, a method for measuring the absolute rate of virus replication in vivo comparable to that in bacteriology (92) does not exist. However, sequential determinations of virus contents of tissues, the resultants of virus replication, destruction, and removal, indicate in some instances a more rapid replication of virulent strains than avirulent strains either in the tissues generally or in a vital site (14, 121). Also, some support for a connection between the replication rate and virulence comes from tissue culture studies. For strains of some (65) but not all viruses (138), there is a relation between plaque size and virulence, and, although the size of the plaque can be determined by cytotoxic factors (3, 125, 127) and not by rate of replication, in some cases the latter seems the dominant factor (132, 164).

Ability to proliferate in vivo depends on an inherent ability to replicate in the biochemical conditions of the host tissues, coupled with a capacity to resist or not to stimulate host defense mechanisms which would otherwise kill or remove them. Distinguishing between these effects is not easy for any type of microbe (147), but it is particularly difficult for viruses because of the absolute parasitism involved. The cellular factors required for virus replication are complex (104), and it is almost impossible, with present techniques, to distinguish clearly the influence of their absence in a particular situation from the influence of host factors (defense mechanisms) which destroy virus or interfere with replication. Nevertheless, the distinction has been made occasionally, for example, when lack of replication has been shown to be due to the host defense mechanism, interferon (15, 31). And further attempts to distinguish "replication factors" (147) from host defense factors seem worthwhile, since they may lead to the recognition of virus-induced products which inactivate or resist host defense factors (e.g., interferon antagonists). Ability to replicate and interference with host defense factors are discussed in this and the following sections.

The ability of a virus to replicate in a particular cell depends on inherent features of the cell (104) as well as the virus. These features can be involved in one or more stages of replication: attachment, penetration, uncoating, provision of energy and precursors of low-molecular-weight, nucleic acid and protein synthesis, assembly, and release (104). Cell culture experiments have shown that "replication factors" vary from cell type to cell type. Thus, by comparison with cells supporting full virus replication, certain cell types appeared to lack such factors at the receptor stage for poliomyelitis virus (65), at penetration for feline virus (155), at uncoating for mouse hepatitis virus (136), at viral nucleic acid synthesis and maturation for some PK-negative mutants of rabbit poxvirus (48), and at maturation or envelopment for KB-negative mutants of adenovirus type 12, some DK mutants of herpes simplex virus, and Sendai virus (48). Also, it is abundantly clear from experiments in culture that virus replication is influenced by changes in the environment of the cell. These include

temperature, pH (65), and small molecular materials such as arginine (18), leucine (50), yeast extract materials (27), and some fatty acids (82). Furthermore, replication in some cell types can be promoted by extracts of others (139).

In animals, virus pathogenicity will be affected by variation of the availability of replication factors in particular hosts or tissues and under different environmental conditions; and attenuated viruses may have a decreased capacity to use the factors. Experiments comparable in depth to those conducted in tissue culture have not yet been accomplished in animals or organ culture. Nevertheless, there are signs of the influence of replication factors in the following studies. With regard to receptors, the ability of homogenates of some primate tissues to bind poliomyelitis virus paralleled their susceptibilities to virus replication and damage in infection; also, virulent strains adhered to susceptible nerve tissues more strongly than avirulent strains (65). The effect of temperature on virus virulence (14, 150) probably reflects temperature sensitivity of processes needed for virus production rather than an influence on host defense mechanisms, and the protective effect of fever on virus infection may result from a similar mechanism. Leucine enhanced vaccinia infection of mice as well as that of cell cultures (50), but similar animal experiments with arginine and appropriate viruses have not been done. Lack of the complete set of replication factors may result in production of defective virus particles which can influence virus infection by inducing host defenses against infective particles (69). The spread of a virus in a host may be prevented by a layer of insusceptible cells (i.e., those lacking replication factors), barring entry to a target organ; thus, a blood-borne virus unable to grow in vascular endothelium may not enter the brain, placenta, or skin tissues (98).

In organ cultures, the defense mechanisms of the reticuloendothelial system and the inflammatory response are largely absent. Hence, the influence of replication factors on virus growth can be studied in a system removed to some extent from the influence of host defense factors without complete destruction of the in vivo character of experiments. Infection patterns in organ culture may parallel those in whole animals, for example, in the replication of rhinovirus in different human tissues (67), infectious bovine rhinotracheitis virus in different bovine tissues (137), and influenza virus in different ferret tissues (16, 17). In these cases, host defense mechanisms (other than those present or induced in infected cells) may not be as important in infection as replication factors. On the other hand, when the pattern of infection is not repeated in organ culture, for example, in growth of trachoma-inclusion conjunctivitis agents in baboon and guinea pig conjunctiva (115), host defense mechanisms may be more important in pathogenicity in vivo. Comparisons of virulent and attenuated virus strains in organ cultures might reveal some aspects of the influence of replication factors on pathogenicity. Thus, in bovine pharyngeal epithelium, virulent strains of foot-and-mouth virus multiplied more rapidly and to a higher titer than attenuated strains (24). Such investigations are rare. They should be extended to strains of other viruses and pressed deeper by using modifications of tissue culture methods, for example, one-step growth curves (56, 68).

COUNTERACTING HOST DEFENSE MECHANISMS

Although much has been written on host defense mechanisms against viruses (2, 15, 57, 65, 95, 98, 164), little concrete information exists on the ability of viruses to counteract them (98, 105, 164). In particular, there seem to be few studies of the early stages of virus infection (95), where comparisons of the behavior of virulent and attenuated strains might reveal viral invasive mechanisms as they have done in bacteriology (143). In this section, host defense mechanisms are summarized to form a background against which virus counteraction is described, and possible determinants of it are discussed.

Nonspecific defense against virus infections is stronger in adult than young hosts (14, 20, 63, 102, 128), is reduced by treatment with cortisone or X-rays (106, 150), and contains both humoral and cellular factors. Humoral factors include the low pH of inflammatory exudates (65, 95, 164) and nonspecific virus inhibitors in tissues and serum. These inhibitors may be present before infection (103, 150) or be induced by it (156, 162). Cellular factors include those present or induced in any tissue which the virus attacks, such as host nucleases capable of destroying virus nucleic acid (104) or interferon (15, 98). There are probably also factors present in the phagocytic and other cells of the reticuloendothelial system (95) which can prevent the spread of viruses into susceptible tissues such as influenza virus into liver parenchyma (95) or neuroviruses into the brain (75). Macrophages ingest and destroy some

viruses (57, 63, 73, 95, 105, 130, 152), and anti-macrophage serum has enhanced infections with yellow fever virus, vesicular stomatitis virus, and herpes simplex virus (62, 114, 170). The role of polymorphonuclear leukocytes in defense against virus infections is not as clear as that of macrophages and has not been investigated thoroughly (57, 95, 98, 105). Although present, neutrophils do not figure so prominently in early inflammatory lesions as they do in bacterial infections. Nevertheless, they may play some part in aborting virus infections since, in the few available studies, they phagocytosed virus particles and either destroyed them or inhibited replication (60, 98, 113, 130, 167).

In a preimmunized host, or a few days after primary infection, a virus must contend with the specific defenses of the host. Here, neutralizing antibodies supplement the nonspecific inhibitors in body fluids, and cellular viricidal mechanisms are strengthened by influence of these antibodies and immune lymphocytes (119). Antibody may combine with surface components of the virus essential for host-cell penetration or opsonize the virus leading to phagocytosis and destruction by the macrophages (57, 95). The virus-antibody combination can be reversed, and, although complement potentiates some combinations, it does not necessarily have a viricidal action (98). In primary infection, immunoglobulin M may be more important than immunoglobulin G because of its earlier appearance. As regards cellular mechanisms, there is no clear evidence yet that macrophages from immune animals are more viricidal than normal macrophages (98). But there are indications from the influence of antilymphocyte serum and from other experiments (72, 170) that immune lymphocytes are important in defense against some but not all virus infections; they may react with viral antigens and stimulate the infiltration and activity of macrophages. In some virus infections, such as those with cytomegalovirus and herpes simplex virus, there appears to be a dynamic equilibrium between virus replication and destruction by specific defenses, because immunosuppression can result in clinical disease (36, 101).

Viruses break through host defenses to cause disease and, as for bacteria, this process depends not only on the strength of the defenses and the microbe's capacity to counteract them, but also on the number of invaders. A sufficiently large infecting dose can overwhelm the initial defenses of a susceptible host and cause irreparable damage before the induced defenses can be mounted. Thus, morbidity and mortality in Newcastle disease of chickens is directly related to dosage (164), and Rift Valley fever virus will kill mice in only 6 hr if a high dose is given intravenously (98). However, in natural disease and most laboratory experiments, a small infecting dose is involved. This must be built up to a population sufficiently large to cause damage against the activities of the host defense mechanisms that initially are heavily weighted against the few invading microbes. In the nonimmune host, defense mechanisms act at three overlapping stages. First there are those preexisting in the tissues. Then there are those induced fairly quickly and nonspecifically, such as interferon production and the inflammatory response. And finally there are the specific responses. Since many virus diseases are self-limiting (as regards pathological effects but not necessarily as regards virus elimination), it appears that even virulent strains of these viruses cannot withstand the specific responses which probably determine recovery from disease. Hence, in acute virus disease, the virulence mechanisms are first those which interfere with the two primary stages of host defense and then those which delay and possibly reduce the specific response to which viruses appear especially vulnerable.

Little is known about the resistance of viruses to the nonspecific viricidins in body fluids. Virulent strains of influenza virus appeared to resist these factors in serum more than avirulent strains (150), but possible reasons for the differential resistance, such as subtle differences in the envelope proteins of the strains, were not investigated. Similarly, alimentary tract viruses such as enteroviruses withstand a low *p*H, but the biochemical basis for this resistance is unknown.

Virus species and strains within species differ both in the amount of interferon they induce and in their susceptibility to it. Most of the work has been done in tissue culture. In many cases, virulent strains of viruses induce less interferon or are more resistant to it than attenuated strains, but there are instances when this is not so (14, 15, 25, 65, 89, 100, 120). However, virus virulence is almost certainly determined by more than one mechanism. These mechanisms may be additive in their contributions to virulence rather than interdependent (cf., bacteria [140]). Thus, a strict correlation between virulence and one factor such as induction of or resistance to interferon would not be expected. The role of interferon in virus infection is still not clearly estab-

lished, owing to the difficulty of dissociating its action from more specific processes. Nevertheless, interferon is produced in vivo, and it is reasonable to assume that a capacity to reduce its production or resist its action would be an advantage to an invading virus. How could a virus achieve these ends? Strains which induce early inhibition of host cell ribonucleic acid (RNA) and protein synthesis would depress interferon production, and a blocker of interferon production in vitro has been reported (31, 71). Also, there are persistent reports of viruses producing in vitro antagonists of interferon—termed variously as stimulators, enhancers, and anti-interferons (29, 53, 61, 78, 135, 160). The chemical nature of these substances is unknown, and whether they are produced in infection and play any role in virus invasion has yet to be assessed. Nevertheless, these interferon antagonists have been recognized, and some of them may prove to be viral counterparts to bacterial "aggressins" (143). Similarly, if virus internal proteins can be proved to prevent the destruction of viral nucleic acids by host nucleases, as speculated by Newton (104), they might also qualify as viral "aggressins."

Some viruses are killed after ingestion by macrophages and others are not (57, 95, 98). Ability to resist the killing mechanisms of monocytes and possibly to replicate within them appears to be one of the main virulence mechanisms of some viruses. The Hampstead mouse strain of ectromelia has an increased ability to grow in mouse macrophages compared with the Hampstead egg strain, and it is correspondingly more virulent for mice. Within macrophages, virus is protected from extracellular inhibitors such as antibody, and thus wandering macrophages can spread infection through the blood, lymph, and tissues, while fixed macrophages can provide an initial focus of infection in larger organs such as the liver (98). Some viruses are phagocytosed poorly or not at all by macrophages (95); this is a virulence mechanism for viruses which are destroyed by macrophages. On the other hand, for a virus which survives and replicates within macrophages, ability to be ingested is an essential virulence mechanism. The WE3 strain of lymphocytic choriomeningitis virus heavily infects the liver and spleen of mice because, in contrast to the Armstrong strain, it is ingested by the macrophages of these organs.

At present, we are ignorant of the viral products or mechanisms which determine virus ingestion, survival, or replication within macrophages. This is not surprising. Only recently have we learned a little regarding intracellular survival of bacteria, despite many years of work on their biology and chemistry (143). Perhaps the first point to note is that macrophages do not appear to provide a good environment for replication of any virus. Many macrophages in an inoculated population do not become infected, often viruses survive but do not multiply within macrophages, and, even when replication occurs, yields of infectious virus are small with much incomplete virus (98). Ingestion will probably be affected by the nature of the virus envelope, and serological and biochemical examination of the envelope proteins of strains differing in ease of ingestion might yield interesting results. Within the macrophage, the virus envelope may also play a role in virus survival by directly inhibiting viricidins, but it is equally possible that virus survival may be due to an overall inhibition of macrophage function by a cytotoxic action of the virus. Clearly some viruses such as myxoviruses, vaccinia virus, and measles virus exert cytotoxic effects on macrophages and inhibit phagocytic activity towards bacteria (57, 105, 130). These cytotoxic effects may be a result of virus replication and thus come under the heading of damage to the host which could aid or hinder, according to the function of the macrophage, further invasion by the same virus or another pathogen. But they would have little relevance to the survival and replication of the initial infecting virus particles. On the other hand, the constituents of these initial particles might themselves inhibit macrophage function, including viricidal activity sufficient to allow their limited replication. Until we know more about the biochemical basis of virus cytotoxicity, we cannot decide between these possibilities and recognize the basis of virus survival and replication within macrophages. Similarly, we are ignorant of the reasons for the inhibition of phagocytic activity towards bacteria of polymorphonuclear leukocytes after treatment with viruses such as mumps virus, influenza virus, and coxsackievirus (105, 130). If neutrophils contribute to defense against virus infection, such interference with their function by viruses may be a virulence mechanism.

Viruses could delay or reduce the protective effect of antibody by being present in such large amounts that any local antibody is swamped, by being "bad" antigens for inducing antibody, by antigenic variation, and by infecting and inhibiting the function of antibody-forming cells. Virus strains vary in their ability to evoke antibody (164), and "slow vi-

ruses," such as the scrapie agent, do not appear to induce antibody (169). The reasons why some antigens are "good" and others "bad" are unknown (144). The fact that viruses often have host-cell-membrane constituents in their envelope proteins provides the possibility of virus antigens being more "host-like" and therefore "bad" antigens, but this has not yet been proven. Furthermore, I am unaware of any comparison between virulent and attenuated strains of virus which has shown virulent strains to be the less immunogenic; it is usually the other way round (164). Antigenic variation occurs in influenza virus, rhinoviruses, foot-and-mouth disease virus, and other viruses infecting the respiratory and alimentary tracts, and this must contribute to the ability of these viruses to attack fresh hosts which have neutralizing antibodies only against previous variants. But we do not have evidence that antigenic variation during the course of infection contributes to virulence as for example in protozoal diseases (147). Some virus infections depress antibody synthesis, but it is not usually completely prevented. In some infections it is increased, for example, in Venezuelan encephalitis of mice and guinea pigs (105). Like depression of macrophage activity, the way in which antibody-forming cells are inhibited is unknown. Also, in all experiments so far, the methods for detecting change in antibody production have not involved the infecting virus (98). Hence we do not know whether response to the latter was suppressed, the crucial point as regards counteraction of host defenses in primary virus infection.

Cellular immunity as judged by graft rejection or delayed hypersensitivity reactions is depressed in most virus infections (105). Again, cellular immunity against the infecting virus has not been examined. Some viruses grow in lymphocytes and produce immunosuppression with or without cytotoxic damage (98). The mechanisms of this intracellular growth are obscure but, like macrophage infection, lymphocyte infection provides a ready vehicle for spread of virus infection in some diseases such as those caused by ectromelia virus, distemper virus, tick-borne encephalitis virus, and lymphochoriomeningitis virus (98).

HOST DAMAGE

In attempts to understand the mechanisms responsible for the pathological effects of virus disease, four broad questions arise. Which pathological effects are specific to virus attack rather than nonspecific responses to general injury (153)? Which cells are damaged by virus replication? Does this damage explain the specific pathological effects? And how is the damage produced?

In some cases, specific pathological effects are easy to detect, for example, paralysis in poliomyelitis resulting from damaged anterior horn tissue. On the other hand, the biochemical and pathological manifestations of shock that occur in poxvirus infections (149) are probably nonspecific responses to injury comparable to those found in anthrax (141) and malaria (51). When such blanketing nonspecific responses occur, identifying the trigger mechanisms in virus diseases will prove more difficult than for bacterial diseases (45). Unlike some bacterial toxins, the virus products responsible for the triggering effects have not been isolated (*see below*). Hence, infected animals or tissues must be used in all investigations. It is therefore difficult to distinguish the effects of virus replication (e.g., cellular amino acid changes due to virus-coded protein synthesis) from the results of damage to host tissue (e.g., cellular amino acid changes accompanying lysis), and there are also technical hardships in dealing with heavily infected animals. In bacteriology, the pathological syndrome has been studied successfully without interference from bacterial growth by removing bacteria with antibiotic treatment at a stage of the disease when the host was about to succumb (41). This method might be adapted for virus work if replication but not the disease syndrome in animals or organ cultures could be stopped by methods comparable to those used in tissue culture (6).

Cellular damage of animal tissues by virus attack has been recognized for many years by the classical methods of histopathology; for example, anterior horn cells are damaged by poliovirus, respiratory epithelium by influenza virus, and brain cells by Newcastle disease virus. Now that these methods have been supplemented by electron microscopy and immunofluorescent techniques, it is apparent that viral replication occurs in cells without significant damage. In seeking the important cell damage in virus disease, it would be unwise to assume that lack of morphological damage means absence of relevant biochemical damage, in view of the profound effect virus replication has on cell biochemistry and the experience from other fields, such as pharmacology, that small biochemical lesions can have striking pathological effects, especially if they occur in the nervous or vascular systems. Any cell type showing evidence of virus replication or presence should be considered a candidate

for the primary site of damage, although those overtly damaged should probably receive attention first.

A direct connection between cell damage and specific pathological effects is perhaps strongest for those viruses which damage cells of the nervous system, for example, poliovirus and the encephalitis-producing viruses. Respiratory tract viruses such as rhinoviruses and influenza virus damage the epithelial cells of the respiratory tract with resultant local symptoms, and the destruction of some respiratory tract cells by influenza virus will ease the way for its well-known secondary invaders, pneumococci and staphylococci. But it is difficult to believe that the unpleasant systemic and sometimes fatal effects of influenza are merely due to damage of respiratory epithelium. Either the virus grows in and damages other sites or virus components (or host cell breakdown products) are liberated from the damaged respiratory epithelium, and, like bacterial toxins, have a toxic action elsewhere. In this connection, it is interesting that large doses of influenza virus are toxic (95, 149, 154). Similarly, the systemic effects of viruses which produce rashes and skin pocks are possibly not directly connected with the skin cell damage but with damage elsewhere. If damage of host cells is widespread, then, as in malaria (112), death may follow from the nonspecific pathological effects of pharmacologically active materials liberated from the damaged host cells. This may be the explanation for the fatal shock syndrome seen in some poxvirus infections (149).

As suggested by Ginsberg (54), virus-induced cell damage may result from a passive role of the virus—a simple repercussion of the process of replication, such as the depletion of cellular components essential for cell life or mechanical harm due to excessive production of virus or its components. Nevertheless, there is increasing evidence that two more positive processes of cell damage occur, namely, virus cytotoxic activity (6, 54) and immunological reaction of the host against virus-infected cells (119, 129, 165).

There are two levels at which pathologically important cytotoxic activity can operate: biochemical damage without morphological damage and that occurring with morphological damage such as cell lysis, fusion, or death (6). The latter (called here morphological damage) is what is usually meant by cytotoxic (or cytopathic) effect. But both processes must be considered, since the former (called here biochemical damage) could cause the decisive pathological damage, for example in nerve cells, even when there is subsequent or accompanying morphological damage in the same or other tissues. In attempts to elucidate these cytotoxic effects, the first question is whether they can be divorced from the process of virus replication and be connected with virus-induced compounds which may or may not be components of the virion. Then we wish to know if the processes of morphological damage can be separated from aspects of biochemical damage. Finally, we need to know the nature and mode of action of the virus-induced compounds responsible for the cytotoxic effects. Some progress has been made in answering these questions for a few viruses, but only in tissue culture experiments. How far the findings can be extended to other viruses and to the pathology of animal infections remains to be seen.

Morphological damage can occur in tissue culture without production of infectious virus. Thus influenza virus, Newcastle disease virus, fowl plague virus, a murine picornavirus, and mengovirus damaged cells which were either incapable or poorly able to support virus replication (6). Cells were also damaged by poliovirus, vaccinia virus, and rabbit poxvirus in the presence of chemical inhibitors of virus replication, such as *p*-fluorophenylalanine, and also by ultraviolet light-inactivated vaccinia virus, rabbit poxvirus, and reovirus (6). In these experiments, high multiplicities of virus infection were used. Further support for the fact that virus replication and morphological damage need not be closely linked is provided by the observations that virulent strains of some viruses such as Newcastle disease virus (124, 125) have greater damaging effects in relation to replication rate than avirulent strains; and the damaging effects of the same virus, such as reovirus, in the same cell line can vary with different cultural conditions which provide similar yields of virus (45). With regard to biochemical damage, the cut-off phenomenon (90) or inhibition of host-cell macromolecular synthesis can occur in the absence of the production of infectious virus. Thus, RNA and protein synthesis were depressed in poliovirus-infected HeLa cells treated with guanidine, which prevented replication (64), and in cells treated with vesicular stomatitis virus after inactivation with ultraviolet light (163). Finally, some pathological damage occurs in animals in the absence of new infectious virus (131).

Some preformed virion components seem to exert cytotoxic effects. Sendai virus, New-

castle disease virus, measles virus, and simian virus 5 produced rapid polykaryocytosis in cell cultures, but only when high virus multiplicities were used. This indicated that preformed products were responsible for the fusion effects and, in the experiments with simian virus 5, puromycin and actinomycin D were added to stop de novo protein synthesis (26, 66, 83, 107). Components of herpesvirus also seem to produce syncytia (158). The penton of adenovirus causes cell rounding and cell detachment from glass (46, 55). A double-stranded RNA from bovine enterovirus caused rapid death, without the production of infectious virus, of cells susceptible and insusceptible to enterovirus infections (33). Whether the RNA itself was cytotoxic or incomplete replication occurred giving rise to cytotoxic proteins is a matter of speculation (6). Biochemical damage, more specifically interference with host-cell macromolecular synthesis, has been achieved with the fiber antigen of the adenovirus capsid which inhibited with RNA, deoxyribonucleic acid (DNA), and protein synthesis (88) and may have been achieved with a double-stranded RNA from poliovirus which interfered with protein synthesis in lysates of rabbit reticulocytes (44). Finally, it is interesting to mention here that large quantities of some viruses, such as influenza virus and poxviruses, cause rapid toxic effects in animals (97, 149, 154).

Bablanian, Tamm, and their colleagues have shown that morphological damage of cells infected with poliovirus or vaccinia virus is due to de novo synthesis and accumulation of virus-induced proteins; this was achieved by careful time-sequence examinations of the effects on morphological damage of adding and removing compounds which either interfered with the production of infectious virus such as guanidine or with protein synthesis such as streptovitacin A, cycloheximide, and puromycin (6–8). Similar conclusions that de novo protein synthesis is needed for morphological damage have been made for mengovirus (3, 21, 52, 59), influenza virus (133), *Molluscum contagiosum* virus (85), and Newcastle disease virus (124). Also, in some instances, inhibition of host-cell macromolecular synthesis appears to be due to virus-induced protein synthesis (6, 90).

Although virus-induced inhibition of host-cell macromolecular synthesis could produce decisive biochemical effects in animals (*see above*) and in time will kill cells, in several instances in tissue culture it appears that the rapid morphological damage of cells is not dependent on the "cut-off" phenomenon. First, noninfected cells with drug-inhibited macromolecular synthesis were not as damaged as infected cells. For example, L cells with RNA synthesis inhibited by actinomycin D to an extent comparable to that seen in mengovirus infection, and LLC-MK2 cells with protein synthesis inhibited with puromycin, cycloheximide, and streptovitacin A, comparable to that seen in vaccinia infection, did not suffer the rapid morphological damage seen in virus infection (6, 9). Second, sequential observations of virus-infected cells sometimes coupled with treatment with compounds inhibiting RNA and protein synthesis showed a lack of parallelism between the appearance of morphological damage and the occurrence of the "cut-off" phenomenon for poliovirus (6), mengovirus (3, 59), reovirus (45), influenza virus (133), simian virus (66), and herpesvirus (47). Third, in the case of adenovirus, different parts of the capsid have different activities, the penton affecting morphology and the fiber antigen macromolecular synthesis (47, 88).

In investigating the nature and mode of action of the virus-induced compounds that are responsible for cytotoxic effects, preformed virion components (*see above*) are the easier target. By fractionating split virions, it should be possible to identify cytotoxic components such as the penton and fiber antigens of adenovirus and possibly the double-stranded RNA from bovine enterovirus. But the majority of cytotoxic compounds are probably extravirion compounds found in infected cells. Hence the task of identification is more difficult. Stephen and Birkbeck (151) advocated a direct approach comparable to that used in bacteriology, namely, to isolate the virus-induced components from infected cells, to free them from intact virus, and to attempt to produce the toxic effects in uninfected cells. This approach required the design of techniques for the difficult process of introducing potentially cytotoxic viral products into fresh cells. Using magnesium sulfate solutions to increase membrane permeability, Stephen and his colleagues (19) have obtained evidence of cytotoxic factors induced by vaccinia virus in HeLa cells. Furthermore, by using appropriate immunosorbents, they (168) have provided evidence that the infected cells contain both virus-specific and host-specific cytotoxic factors. Thus, there is increasing evidence that viruses produce cytotoxins; but how do they act? Inhibition of host-cell macromolecular synthesis or other interference with the functions of the cell could be produced directly by the virus product, as diphtheria toxin interferes with protein

synthesis. On the other hand, the virus-induced product might release autolytic enzymes from the cell's own lysosomes (58), and the results of Stephen and his colleagues are interesting in this respect. Obviously we are beginning to know something of the mechanisms of virus cytotoxicity, but much remains to be done in tissue culture and even more in relating the results of such experiments to pathological effects in animals.

Immunopathology is responsible for host damage in bacterial and other microbial diseases (143, 147), but it is more likely to occur in virus diseases, because the obligate parasitism involved increases the chances of cell-bound antigens occurring and also the existence of autoimmune phenomena. It is now clearly established that some viruses incorporate host-cell constituents, especially membrane constituents, into their structure. Hence antibodies against these virus-host complexes could react with the membrane constituents of infected and *normal* cells. Also, virus infection may change the host-cell membrane constituents and form neoantigens, the antibodies against which could react with infected and normal cells.

Host damage could result from any of the four types of allergic reactions described by Coombs and Gell (32): type I, reaginic antibody-mediated anaphylactic reaction; type II, antibody and often complement-mediated cytotoxic reaction against cell-bound antigens; type III, antibody-antigen complex Arthus-type reaction; and type IV, reaction of actively allergized cells without antibody. In addition to cell destruction, cell proliferation might also occur as a result of type II reactions (165). It appears that one or more of these four types of allergic reactions may be involved, in some cases of damage, in a number of virus diseases such as encephalitis in measles, poxvirus rashes, pneumonia from respiratory syncytial virus, yellow fever, hemorrhagic dengue, mumps, coxsackie B virus infections, canine infective hepatitis, Kyasamur Forest disease, blue tongue of sheep, hog cholera, and Aleutian disease of mink (96, 165). In these diseases, the evidence for immunopathology is mostly suggestive. But for lymphochoriomeningitis in mice, we have the viral counterpart of tuberculosis and streptococcal nephritis where sufficient solid experimental evidence has accumulated for us to be reasonably sure that immunopathology (glomerulonephritis from immune complex, type III reaction in chronic disease, and cytotoxic type II reaction involving antibody and complement in acute disease) plays a major role in the observed damage. This evidence has been so well described and reviewed that it need not be repeated here (40, 99, 108–111, 165).

Immunopathology is such an attractive explanation for virus damage that it is receiving much current attention (111, 129, 165). Perhaps a few words of warning against too easy assumptions of its complicity in cases of virus damage may not be out of place. Firstly, mere demonstration that an infected host is immunologically sensitive to virus products by a diagnostic test such as a skin test is no proof of the implication of the sensitivity in the main pathological effects of disease. More extensive investigations are needed; the main systemic and local effects of the disease must be simulated by immunological reactions invoked in a sensitized host by products of the appropriate virus or in an infected host by antibody or immune cells from an immunized host. Such evidence is not easily obtained, particularly for human diseases lacking good animal models. Secondly, the lack of knowledge of the mechanisms of direct virus cytotoxicity adds to the difficulty of distinguishing such mechanisms from immunopathological ones. Thirdly, it should be remembered that, although interesting, the number of immunopathological cases is probably small compared with those due to direct virus cytotoxicity (165).

HOST AND TISSUE SPECIFICITY

The occurrence of host and tissue specificity in virus infections is so well documented (12, 65, 79, 149, 154) that descriptions of the many examples will not be repeated here. On the other hand, studies of the biochemical bases of these phenomena in virus infections are even more in their infancy than similar studies in bacteriology (12, 79, 115, 149). The two phenomena are considered together, because broadly they can be explained on similar principles, although most of the examples deal with tissue specificity. It should be emphasized that the two specificities are not all-or-none phenomena (limited virus replication may well occur in the nonsusceptible tissue or host, especially if the infecting dose is high), and they often vary with the age of the host. Thus, coxsackievirus infects muscle cells of young but not old mice (76), and the MHV (PRI) strain of mouse hepatitis virus infects liver macrophages of young but not old C_3H mice (12).

The real difficulty in studies of host and

tissue specificity is not lack of ideas of possible explanations for the phenomena but the design of experimental systems to investigate them in a manner relevant to natural infection. Clearly, the availability of "replication factors" in host cells and their surrounding fluids and their variation under different environmental conditions probably determine many cases of host and tissue specificities (146, 147). Similarly, other cases will depend on variation in levels of antiviral substances from host to host and tissue to tissue, or differential inductions of interferon and immune mechanisms either in level or in time (31, 65, 87). Also, the route of infection may play some role in tissue specificity. One susceptible tissue may be easily accessible to the incoming virus and become infected, whereas another equally susceptible tissue may be protected by a barrier of cells which either do not support virus replication or destroy virus. For example, in mice the Küpffer cells of the liver seem to protect the parenchyma cells from infection with blood-borne influenza and myxoma viruses, and infection via the bile duct circumvents the barrier (95). A blood-brain barrier appears to prevent infection of the brain by certain bloodborne viruses which attack neurons if inoculated directly into the central nervous system (14). This barrier may be insusceptible vascular endothelial cells, but general reduction of the viremia by the cells of the whole reticuloendothelial system may be a major factor in the "blood-brain barrier" (75). However, the latter does not seem to operate in some cases. Thus, in mice, an avirulent strain of Semliki Forest virus produced a viremia at least as high and probably higher than a virulent strain, yet its attack on the brain cells was abortive compared with the virulent strain whose lethal effect depended on rapid invasion and replication in the brain (121). Despite the clear implication of route of infection in some cases of tissue specificity, variations of "replication factors" and host defense mechanisms are almost certainly more important in both tissue and host specificity, and methods of investigating them outside the animal host are discussed below.

The first essential is that the specificities of animal infection should be retained in the experimental system. Since virus susceptibilities change when cells differentiate in normal tissue cultures (12, 68, 77, 142), the latter cannot be used directly to investigate host and tissue specificity in natural infection. Nevertheless, studies of differing cell susceptibilities in such cultures (48, 104), might serve as models for adaptation to the systems described below.

Infection in the chick embryo has been recommended as a system in which tissue specificities characteristic of human and fowl infections can be reproduced for a number of viruses such as fowl pox, vaccinia, herpes, pseudorabies, influenza, Rous sarcoma, and Newcastle disease viruses (12, 22, 23). However, this system does not appear to have been used extensively to investigate mechanisms of specificity, although experiments with whole embryos, primary chick cell cultures, and organ cultures could be conducted along the same lines as those described below. Thus, the difference in susceptibility between chicken lung cells and fibroblasts to Sendai virus appeared to be due to more efficient maturation of virus particles in the lung cells (38).

Short-term studies with primary cell cultures or suspensions of relevant tissues have yielded most of our available information on tissue and host specificity. Using such preparations and membrane fractions from them, Bang (12) and Holland (65) provided good evidence that the presence or absence of surface receptors for poliovirus determined susceptibility or resistance to infection of different primate tissues and of primate and nonprimate tissues; in particular, cell resistance to infection disappeared when the receptors were by-passed by using virion RNA as the infecting material. In other cases, experiments with primary cell cultures have shown that the cell receptors determining initial adsorption are probably not the important factors in susceptibility. The MHV (PRI) strain of mouse hepatitis virus infects PRI mice but not C_3H mice, and this difference in host specificity is reflected in the susceptibility of liver macrophages (12). Yet adsorption of MHV (PRI) virus to resistant mouse (C_3H) macrophages was similar to that occurring with susceptible mouse (PRI) macrophages; penetration of the resistant macrophages seems to have occurred, but there appeared to be no uncoating (136). Similarly, work with primary cell cultures showed initial adsorption, but absence of penetration occurred with insusceptible cells for Rous sarcoma virus (117) and feline herpesvirus (155). Also, experiments with primary cell cultures of human fetal tissue, placenta, and leukocytes with Sendai and rubella viruses showed that interferon production varied with cell type and virus (10).

In primary cell cultures, there have been attempts to confer susceptibility on resistant cells by extracts of or products from suscep-

tible cells. As for treatment of conventional tissue culture cells, susceptibilities of primary cell cultures have been affected by externally applied materials such as serum and tissue extracts. Bang and his colleagues reported that mouse (C_3H) liver macrophages resistant to mouse hepatitis virus were rendered susceptible by treatment with extracts of susceptible mouse (PRI) liver macrophages, but in these experiments there may have been a change in virus rather than a change in cell susceptibility (12). The source (horse, calf, or mouse) and concentration of the serum used to suspend mouse liver macrophages influenced their susceptibility to hepatitis virus, possibly by affecting intracellular events (86). Recently Mathews (91) reported that encephalomyocarditis virus promoted protein synthesis in cell-free systems of certain mammalian and avian cells, such as Krebs II ascites cells, but not in extracts of rabbit reticulocytes, until cell sap from ascites cells was added. The experiments indicated that a tissue-specific factor, possibly transfer RNA (5), was required for translation of the viral RNA by the rabbit reticulocyte extract. If cases are found where resistance or susceptibility of primary cell cultures can be changed by treatment with cell extracts of susceptible or resistant cells, then appropriate fractionation might provide the chemical basis for susceptibility or resistance. For example, as far as I am aware, no one has tried to extend the work of Holland and his colleagues on the importance of receptors for poliovirus infection by attempting to confer susceptibility on resistant cells by treatment with receptor extracts from susceptible cells. Such experiments would be comparable to the enhancement of infection of monkey kidney cultures by echovirus by treatment with receptor-like substances from red blood cells (139). In this connection, recent work (28, 30, 84) has shown the uncoating and release of nucleoprotein from polivirus by protein fractions from membranes of susceptible but not unsusceptible cell lines and similarly from influenza virus by chick embryo cell membranes.

In the future, experiments with organ cultures coupled with those in animals may prove of equal importance to work with primary cell cultures in studies of host and tissue specificity. Unlike tissue cultures, organ cultures usually retain their parent specificities of natural infection (12, 68) and, as suggested earlier, when this is not so, it indicates that the specific or nonspecific host defense mechanisms present in animals but absent in organ cultures may play an important role in the tissue or host specificity. As an indication of the course research might take when this situation occurs in studies of virus specificity, the reader is referred to descriptions of work on the host specificities of trachoma conjunctivitis agents (115) which, although not viruses, are handled technically as if they were. The following summary of recent attempts to identify the basis of tissue specificity of influenza virus (16, 17, 56; S. Rosztoczy, G. L. Toms, and H. Smith, *unpublished data*) illustrates the stages of research when specificities are reproduced in organ culture.

Influenza virus infection in the ferret seemed an appropriate system for study, since in ferrets influenza appeared to take the same course as in man, with a definite localization in the upper respiratory tract (49). First, a quantitative survey of ferret organs for virus following inoculation intranasally or into the bloodstream was conducted to identify not only highly susceptible tissues but, for comparison, insusceptible ones and possibly those of intermediate susceptibility. One to five days after intranasal inoculation, of 16 different tissues examined, only nasal mucosa, lungs, trachea, and esophagus contained detectable virus. The nasal mucosa appeared the most susceptible tissue: the average total amounts of infective virus in lungs, trachea, and esophagus were approximately 2%, 0.001%, and 0.1%, respectively, of that in nasal mucosa. When virus was inoculated into the bloodstream, only nasal mucosa became infected and to a considerable degree. The high susceptibility of nasal mucosa to influenza virus was thus established; other workers reached similar conclusions (116).

In organ cultures of ferret tissues, influenza virus infection followed the pattern in vivo for nasal mucosa, lung, trachea, esophagus, and aorta. After receiving the same inoculum, virus replicated significantly in nasal mucosa, lung, and trachea, and particularly well in nasal mucosa, but significant replication was not detected in the esophagus and aorta. Unexpectedly, organ cultures of bladder and oviduct (including the upper end of the uterus), which were not infected in the original experiments in vivo after intranasal or intracardial inoculation, supported virus growth. However, direct inoculation of ferrets into the bladder and upper end of the uterus resulted in local infection. The conclusions from the work thus far are as follows. First, influenza virus was more ubiquitous in infecting tissues than was first thought; the urogenital tract infection recalled reports of isolation of virus from urine

in some cases of human influenza (39). Second, route of infection plays a most important part in localization of influenza virus in ferrets and probably man, since the incoming virus meets a highly susceptible tissue first, and other susceptible tissues are protected by a blood-tissue barrier which may be a reduction of viremia by the whole reticuloendothelial system, as suggested for the blood-brain barrier by Johnson and Mims (75), or possibly an adsorption and inactivation by blood cells (S. Rosztoczy, G. L. Toms, and H. Smith, *unpublished data*). Third, some parallel had been established between infection in vivo and virus growth patterns in organ cultures, suggesting that "replication factors" rather than host defense mechanisms of the inflammatory and immune response play some role in tissue specificity. Fourth, highly susceptible, moderately susceptible, and poorly susceptible tissues had been indicated for future detailed comparison of their behavior when infected with influenza virus. Finally, factors which could vary in vivo and might be responsible for specificity differences, such as variations in temperature, *p*H, small molecular materials in the environment, and microbial flora, would not appear to play a decisive role in this example of tissue specificity. All these factors are standardized or controlled in the antibiotic-containing organ culture, the infection pattern of which paralleled the susceptibilities in vivo.

Deeper investigations on the differing susceptibilities of the organ cultures could be based on the methods and approaches which have had some success in studies of the susceptibility of tissue culture cells (48, 104) and primary cell cultures (*see above*). Single-cycle growth experiments with varying virus inocula and coupled with treatment with antiserum at different stages could be attempted to investigate more closely the stages of virus replication in poorly, moderately, and highly susceptible tissues. Investigations of receptors could be conducted by the methods used by Holland (65) for poliovirus, including infection experiments with viral nucleic acid and destruction of receptors by the *Vibrio cholerae* enzyme (neuraminidase). Attempts could be made to transfer susceptibility or resistance by isolated membranes or extracts from the various organ cultures or by feeder techniques using susceptible and poorly susceptible tissues in close proximity to one another. It is also possible that different strains of virus with a different envelope protein or neuraminidase may be blocked in infecting the normally highly susceptible nasal mucosa and thus provide some clue as to the important step in susceptibility. All these types of experiments are possible, but for their operation and interpretation the organ culture system has two serious disadvantages. First, in organ culture, cell types differ and only a relatively small proportion of them may be susceptible to infection; in fact, differences in susceptibility of organ cultures (and the parent tissue in vivo) may be merely a reflection of the relative number of susceptible cells rather than differences in inherent susceptibility of the cells. Second, organ cultures have cut edges and under-surfaces which will expose previously unexposed cells to virus attack, at least at the start of infection in vivo; virus attachment to or replication in these cells may confuse an issue. Some method, such as immunofluorescence or electron microscopy, of checking where virus attachment and replication is occurring in the different cell types of organ culture would seem an essential adjunct to the experimental approaches described above. Overall, it seems to me and my colleagues (S. Rosztoczy, G. L. Toms, and H. Smith, *unpublished data*) that only if susceptibility or resistance is determined by differential numbers of susceptible cells in the tissue or at early stages in replication (such as absorption, penetration, and possibly uncoating) is the organ culture system capable of providing the answers at its present stage of sophistication.

In a first attempt at such deeper investigations (56), infection of ferret nasal mucosa and esophagus were compared in experiments based on single-cycle growth studies. Virus adsorbed with equal efficiency to both tissues. Electron microscopy showed virus directly adsorbed to the cilia and cell surfaces of the respiratory tissue. However, in contrast to the nasal mucosa, virus adsorbed to esophagus appeared to be mainly associated with an amorphous substance covering the epithelial cells and rarely in direct contact with the cell membrane. Subsequently, virus penetrated the cells of the nasal mucosa, and large amounts of newly synthesized virus were recovered within 8 hr of infection. In contrast, with the small inoculum used in these experiments, virus failed to penetrate the esophageal surface in quantities sufficient to promote the production of significant amounts of new virus. It is possible that larger inocula or removal of the surface covering would allow some virus penetration and replication, but a strong adsorption to the thick surface covering, preventing close adhesion of most virus particles to the cells of the surface, appears to be the main

reason for the relative insusceptibility of esophagus to influenza infection. On the other hand, the reason for the high susceptibility of nasal mucosa is not clear. A strong adherence to the cilia and microvilli was observed in this and other work (41). Obviously the presence of cilia and microvilli is not essential for adsorption and penetration, since nonciliated cells of ferret bladder were susceptible to infection both in vivo and in vitro.

In conclusion, this review will have achieved its purpose if it encourages some virologists well versed in the elegant methods of tissue culture to turn their attention and adapt their methods to less well-defined organ culture and whole animal systems. In this way we may learn more about virus disease, the raison d'être for our subject.

LITERATURE CITED

1. Alexander, D. J., P. Reeve, and W. H. Allen. 1970. Characterisation and biological properties of the neuraminidase strains of Newcastle disease virus which differ in virulence. Microbios **6**:155–165.
2. Allison, A. C. 1967. Cell-mediated immune responses to virus infections and virus-induced tumors. Brit. Med. Bull. **23**:60–65.
3. Amako, K., and S. Dales. 1967. Cytopathology of meningovirus infection. I. Relationship between cellular disintegration and virulence. Virology **32**:184–200.
4. Asmundsson, T., and K. H. Kilburn. 1970. Mucociliary clearance rates at various levels in dog lungs. Amer. Rev. Resp. Dis. **102**:388–397.
5. Aviv, H., I. Boime, and P. Leder. 1971. Protein synthesis directed by encephalomyocarditis virus RNA: properties of a transfer RNA-dependent system. Proc. Nat. Acad. Sci. U.S.A. **68**:2303–2307.
6. Bablanian, R. 1972. Mechanisms of virus cytopathic effects. Symp. Soc. Gen. Microbiol. **22**:359–381.
7. Bablanian, R., H. J. Eggers, and I. Tamm. 1965. Studies on the mechanisms of poliovirus-induced cell-damage. I. The relation between poliovirus-induced metabolic and morphological alterations in cultured cells. Virology **26**:100–113.
8. Bablanian, R., H. J. Eggers, and I. Tamm. 1965. Studies on the mechanism of poliovirus-induced cell damage. II. The relation between poliovirus growth and virus-induced morphological changes in cells. Virology **26**:114–121.
9. Baltimore, D., and R. M. Franklin. 1962. The effect of mengovirus infection on the activity of DNA-dependent RNA polymerase of L-cells. Proc. Nat. Acad. Sci. U.S.A. **48**:1383–1398.
10. Banatvala, J. E., J. E. Potter, and J. M. Best. 1971. Interferon response to sendai and rubella viruses in human foetal cultures, leucocytes and placental cultures. J. Gen. Virol. **13**:193–201.
11. Bang, B. G., and F. B. Bang. 1969. Experimentally induced changes in nasal mucous secretory systems and their effect on virus infection in chickens. I. Effect on mucosal morphology and function. J. Exp. Med. **130**:105–119.
12. Bang, F. B. 1972. Specificity of viruses for tissues and hosts. Symp. Soc. Gen. Microbiol. **22**:415–435.
13. Bang, F. B., and M. A. Foard. 1969. Experimentally induced changes in nasal mucous secretory systems and their effect on virus infection in chickens. II. Effects on adsorption of Newcastle disease virus. J. Exp. Med. **130**:121–138.
14. Bang, F. B., and C. N. Luttrell. 1961. Factors in the pathogenesis of virus diseases. Advan. Virus Res. **8**:199–244.
15. Baron, S. 1970. The biological significance of the interferon system. Arch. Intern. Med. **126**:84–93.
16. Basarab, O., and H. Smith. 1969. Quantitative studies on the tissue localization of influenza virus in ferrets after intranasal and intravenous or intracardial inoculation. Brit. J. Exp. Pathol. **50**:612–618.
17. Basarab, O., and H. Smith. 1970. Growth patterns of influenza virus in cultures of ferret organs. Brit. J. Exp. Pathol. **51**:1–6.
18. Becker, Y., U. Olshevsky, and J. Levitt. 1967. The role of arginine in the replication of herpes simplex virus. J. Gen. Virol. **1**:471–478.
19. Birkbeck, T. H., C. G. Woodward, and J. Stephen. 1972. A test for vaccinia virus cytotoxicity. J. Gen. Microbiol., *in press*.
20. Bradish, C. J., K. Allner, and H. B. Maber. 1971. The virulence of original and derived strains of Semliki Forest virus for mice, guinea-pigs and rabbits. J. Gen. Virol. **12**:141–160.
21. Bubel, H. C. 1967. Protein leakage from mengovirus-infected cells. Proc. Soc. Exp. Biol. Med. **125**:783–786.
22. Buddingh, G. J. 1950. The culture and effects of viruses in chick embryo cells, p. 19–39. *In* J. G. Kidd (ed.), The pathogenesis and pathology of virus diseases. Columbia University Press, New York.
23. Buddingh, G. J. 1970. Editorial: The chick embryo for the study of infection and immunity. J. Infect. Dis. **121**:660–663.
24. Burrows, R. 1972. Early stages of virus infection: studies *in vivo* and *in vitro*. Symp. Soc. Gen. Microbiol. **22**:303–332.
25. Campbell, J. B., J. G. Buera, and F. M. Tobias. 1970. Influence of blood clearance rates on interferon production and virulence of Mengo virus plaque mutants in mice. Can. J. Microbiol. **16**:821–826.

26. Cascardo, M. R., and D. T. Karzon. 1965. Measles virus giant cell inducing factor (fusion factor). Virology **26**:311-325.
27. Chakrabortty, A. S., and T. S. L. Beswick. 1971. Morphological changes and resistance to vaccinia virus induced in human amnion cells by yeast extract. J. Med. Microbiol. **4**: 115-123.
28. Chan, V. F., and F. L. Black. 1970. Uncoating of poliovirus by isolated plasma membranes. J. Virol. **5**:309-312.
29. Chany, C., and C. Brailovsky. 1967. Stimulating interaction between viruses (stimulons). Proc. Nat. Acad. Sci. U.S.A. **57**:87-94.
30. Ciamphor, F., and O. Krizanova. 1971. Interaction of plasma membranes with influenza virus. III. Electron microscopic study of interactions between influenza virus and isolated plasma membranes. Acta Virol. **15**:361-366.
31. Colby, C., and M. J. Morgan. 1971. Interferon induction and action. Annu. Rev. Microbiol. **25**:333-360.
32. Coombs, R. R. A., and P. G. H. Gell. 1968. Classification of allergic reactions responsible for clinical hypersensitivity and disease, p. 575-596. *In* P. G. H. Gell and R. R. A. Coombs (ed.), Clinical aspects of immunology. Blackwell Scientific Publications, Ltd., Oxford.
33. Cordell-Stewart, B., and M. W. Taylor. 1971. Effect of double standard viral RNA on mammalian cells in culture. Proc. Nat. Acad. Sci. U.S.A. **68**:1326-1339.
34. Couch, R. B., T. R. Cate, R. G. Douglas, P. J. Gerone, and V. Knight. 1966. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bacteriol. Rev. **30**:517-529.
35. Couch, R. B., T. R. Cate, W. F. Fleet, P. J. Gerone, and V. Knight. 1966. Aerosol-induced adenoviral illness resembling the naturally occurring illness in military recruits. Amer. Rev. Resp. Dis. **93**:529-535.
36. Craighead, J. E. 1969. Immunological response to cytomegalovirus infection in renal allograft recipients. Amer. J. Epidemiol. **90**:506-513.
37. Dalhamn, T. 1956. Mucous flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases. Acta Physiol. Scand. **36**:Suppl. 123, p. 1-161.
38. Darlington, R. W., A. Portner, and D. W. Kingsbury. 1970. Sendai virus replication; an ultrastructural comparison of productive and abortive infections in avian cells. J. Gen. Virol. **9**:169-177.
39. Davenport, F. M. 1961. Pathogenesis of influenza. Bacteriol. Rev. **25**:294-300.
40. Dixon, F. J., M. B. A. Oldstone, and G. Tonietti. 1971. Pathogenesis of immune complex glomerulonephritis of New Zealand mice. J. Exp. Med. **134**:65s-71s.
41. Dourmashkin, R. R., and D. A. J. Tyrell. 1970. Attachment of two myxoviruses to ciliated epithelial cells. J. Gen. Virol. **9**:77-88.
42. Druett, H. A. 1967. The inhalation and retention of particles in the human respiratory system. Symp. Soc. Gen. Microbiol. **17**:165-202.
43. Ebisawa, I. T., O. Kitamoto, Y. Takeuchi, and M. Makino. 1969. Immunocytologic study of nasal epithelial cells in influenza. Amer. Rev. Resp. Dis. **99**:507-515.
44. Ehrenfeld, E. J., and T. Hunt. 1971. Double-stranded poliovirus RNA inhibits initiation of protein synthesis by reticulocyte lysates. Proc. Nat. Acad. Sci. U.S.A. **68**:1075-1089.
45. Ensminger, W., and I. Tamm. 1969. Cellular DNA and protein synthesis in reovirus-infected L cells. Virology **39**:357-360.
46. Everett, S. F., and H. S. Ginsberg. 1958. A toxin-like material separable from type 5 adenovirus particles. Virology **6**:770-771.
47. Falke, D., and W. Peterknecht. 1968. DNS-, RNS-, und Proteinsynthese und ihre Relation zur Riesenzellbildung *in vitro* nach Infektion mit *Herpesvirus hominis*. Arch. Ges. Virusforsch. **24**:267-287.
48. Fenner, F. 1970. The genetics of animal viruses. Annu. Rev. Microbiol. **24**:297-334.
49. Francis, T., Jr., and C. H. Stuart-Harris. 1938. Studies on the nasal histology of epidemic influenza virus infection in the ferret. I. The development and repair of the nasal lesion. J. Exp. Med. **68**:789-802.
50. Gabliks, J. 1969. Interaction of leucine with vaccinia virus infection in mice and cell cultures. J. Infect. Dis. **120**:679-686.
51. Garnham, P. C. C. 1967. Malaria in mammals excluding man, p. 139-204. *In* B. Dawes (ed.), Advances in parasitology, vol. 5. Academic Press Inc., New York.
52. Gauntt, C. J., and R. Z. Lockart, Jr. 1968. Destruction of L cells by mengovirus: use of interferon to study the mechanism. J. Virol. **2**: 567-575.
53. Ghendon, Y. Z. 1965. On the ability of certain viruses to block the effect of interferon. Acta Virol. **9**:186-187.
54. Ginsberg, H. S. 1961. Biological and biochemical basis for cell injury by animal viruses. Fed. Proc. Fed. Amer. Soc. Exp. Biol. **20**:656-660.
55. Ginsberg, H. S., H. G. Pereira, R. C. Valentine, and W. C. Wilcox. 1966. A proposed terminology for the adenovirus antigens and virion morphological sub-units. Virology **28**:782-783.
56. Gould, E. A., N. A. Ratcliffe, O. Basarab, and H. Smith. 1972. Studies of the basis of localization of influenza virus in ferret organ cultures. Brit. J. Exp. Pathol. **53**:31-36.
57. Gresser, I., and D. J. Lang. 1966. Relationships between viruses and leucocytes. Progr. Med. Virol. **8**:62-130.
58. Guskey, L. E., P. C. Smith, and D. A. Wolff. 1970. Patterns of cytopathology and lysosomal enzyme release in poliovirus-infected

HEp-2 cells treated with either 2-(α-hydroxybenzyl)-benzimidazole or guanidine HCl. J. Gen. Virol. 6:151-161.
59. Haase, A. T., S. Baron, H. Levy, and J. A. Kasel. 1969. Mengovirus-induced cytopathic effect in L-cells: protective effect of interferon. J. Virol. 4:490-495.
60. Hanson, R. J., J. E. Kempf, and A. V. Boand, Jr. 1957. Phagocytosis of influenza virus. II. Its occurrence in normal and immune mice. J. Immunol. 79:422-427.
61. Hermodsson, S. 1963. Inhibition of interferon by an infection with parainfluenza virus type 3 (PIV-3). Virology 20:333-343.
62. Hirsch, M. S., G. W. Gary, Jr., and F. A. Murphy. 1969. *In vitro* and *in vivo* properties of antimacrophage sera. J. Immunol. 102:656-661.
63. Hirsch, M. S., B. Zisman, and A. C. Allison. 1970. Macrophages and age-dependent resistance to herpes simplex virus in mice. J. Immunol. 104:1160-1165.
64. Holland, J. J. 1964. Inhibition of host cell macromolecular synthesis by high multiplicites of poliovirus under conditions preventing virus synthesis. J. Mol. Biol. 8:574-581.
65. Holland, J. J. 1964. Viruses in animals and in cell culture. Symp. Soc. Gen. Microbiol. 14:257-286.
66. Holmes, K. V., and P. W. Choppin. 1966. On the role of the response of the cell membrane in determining virus virulence. Contrasting effects of the para-influenza virus SV5 in two cell types. J. Exp. Med. 124:501-519.
67. Hoorn, B., and D. A. J. Tyrrell. 1966. A new virus cultivated only in organ cultures of human ciliated epithelium. Arch. Ges. Virusforsch. 18:210-225.
68. Hoorn, B., and D. A. J. Tyrrell. 1969. Organ cultures in virology. Prog. Med. Virol. 11:408-450.
69. Huang, A. S., and D. Baltimore. 1970. Defective viral particles and viral disease processes. Nature (London) 226:325-327.
70. Huang, C. H., and C. Wong. 1963. Relation of the peripheral multiplication of Japanese B encephalitis virus to the pathogenesis of the infection in mice. Acta Virol. 7:322-330.
71. Isaacs, A., Z. Rotem, and K. H. Fantes. 1966. An inhibitor of the production of interferon ("Blocker"). Virology 29:248-254.
72. Jandásek, L. 1970. Influence of anti-leucocyte serum on intraperitoneal vaccinia virus infection of rats. Acta Virol. 14:467-473.
73. Jandásek, L., R. Dvorak, and M. Votava. 1969. Vaccinia virus peritoneal infection in rats of different ages. Acta Virol. 13:88-95.
74. Johnson, J. S. 1970. The secretory immune system, a brief review. J. Infect. Dis. 121:115S-117S.
75. Johnson, R. T., and C. A. Mims. 1968. Pathogenesis of viral infections of the nervous system. N. Engl. J. Med. 278:23-30, 84-92.
76. Kantoch, M. and A. Sieminska. 1965. Studies on the susceptibility of mouse muscle cultures to Coxsackie A-4 viruses *in vitro*. Arch. Immunol. Ther. Exp. (Warszawa) 13:413-433.
77. Kaplan, A. S. 1955. The susceptibility of monkey kidney cells to poliovirus *in vivo* and *in vitro*. Virology 1:377-392.
78. Kato, N., F. Ohta, and A. Okada. 1966. Counteraction between interferon and enhancer. Virology 28:785-788.
79. Keppie, J. 1964. Host and tissue specificity. Symp. Soc. Gen. Microbiol. 14:44-63.
80. Kilburn, K. H. 1968. A hypothesis for pulmonary clearance and its implications. Amer. Rev. Resp. Dis. 98:449-463.
81. Knight, V. 1970. The importance of particle size in airborne viral respiratory infections, p. 273-288. *In* I. H. Silver (ed.) Aerobiology, Proceedings of the third international symposium. Academic Press Inc., London.
82. Koch, A., Cs. Dren, and E. György. 1968. Saturated fatty acids in poliovirus host cell interaction. I. Stimulation and inhibition of virion uptake. Acta Microbiol. 15:77-85.
83. Kohn, A. 1965. Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. Virology 26:228-245.
84. Krizanova, G., D. Kociskova, V. Rathova, and B. Styk. 1971. Interaction of plasma membranes with influenza virus. II. Release of viral nucleoprotein. Acta Virol. 15:352-360.
85. La Placa, M. 1966. On the mechanism of the cytopathic changes produced in human amnion cell cultures by the molluscum contagiosum virus. Arch. Ges. Virusforsch. 18:374-378.
86. Lavelle, G. C., and F. B. Bang. 1971. Influence of type and concentration of sera *in vitro* on susceptibility of genetically resistant cells to mouse hepatitis virus. J. Gen. Virol. 12:233-238.
87. Leader, R. W. 1970. Infection, genetics and immunological disease, p. 25-29. *In* R. H. Dunlop and H. W. Moon (ed.), Resistance to infectious disease. Modern Press, Saskatoon, Canada.
88. Levine, A. J., and H. S. Ginsberg. 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. 1:747-757.
89. Lockart, R. Z., Jr. 1967. Recent progress in research on interferons. Prog. Med. Virol. 9:451-475.
90. Martin, E. M., and I. M. Kerr. 1968. Virus-induced changes in host-cell macromolecular synthesis. Symp. Soc. Gen. Microbiol. 18:15-46.
91. Mathews, M. B. 1970. Tissue-specific factor required for the translation of a mammalian viral RNA. Nature (London) 228:661-663.
92. Maw, J., and G. G. Meynell. 1968. The true division and death rates of *Salmonella typhimurium* in the mouse spleen determined with superinfecting phage P22. Brit. J. Exp. Pathol. 49:597-613.

93. Miles, A. A. 1955. The meaning of pathogenicity. Symp. Soc. Gen. Microbiol. **5**:1-16.
94. Miller, W. S. 1966. Infection of pigeons by airborne Venezuelan equine encephalitis virus. Bacteriol. Rev. **30**:589-595.
95. Mims, C. A. 1964. Aspects of the pathogenesis of virus diseases. Bacteriol. Rev. **28**:30-71.
96. Mims, C. A. 1966. Pathogenesis of rashes in virus diseases. Bacteriol. Rev. **30**:739-760.
97. Mims, C. A. 1968. The response of mice to the intravenous injection of cowpox virus. Brit. J. Exp. Pathol. **49**:24-32.
98. Mims, C. A. 1972. Host defences against viruses and the latter's ability to counteract them. Symp. Soc. Gen. Microbiol. **22**:333-358.
99. Mims, C. A., and F. A. Tosolini. 1969. Pathogenesis of lesions in lymphoid tissue of mice infected with lymphocytic choriomeningitis (LCM) virus. Brit. J. Exp. Pathol. **50**:584-592.
100. Mirchamsy, H., and F. Rapp. 1969. Role of interferon in replication of virulent and attenuated strains of measles virus. J. Gen. Virol. **4**:513-522.
101. Montgomerie, J. Z., D. M. O. Becroft, M. C. Croxson, P. B. Doak, and J. D. K. North. 1969. Herpes-simplex-virus infection after renal transplantation. Lancet **2**:867-871.
102. Morahan, P. S., and S. E. Grossberg. 1970. Age-related cellular resistance of the chick embryo to viral infections. I. Interferon and natural resistance to myxovirus and vesicular stomatitis virus. J. Infect. Dis. **121**:615-623.
103. Nash, D. R., S. B. Halstead, A. C. Stenhouse, and C. McCue. 1971. Nonspecific factors in monkey tissues and serum causing inhibition of plaque formation and hemagglutination by dengue viruses. Infect. Immunity **3**:193-199.
104. Newton, A. A. 1970. The requirements of a virus. Symp. Soc. Gen. Microbiol. **20**:323-358.
105. Notkins, A. L., S. E. Mergenhagen, and R. J. Howard. 1970. Effect of virus infections on the function of the immune system. Annu. Rev. Microbiol. **24**:525-538.
106. Oh, J. O. 1970. Enhancement of virus multiplication and interferon production by cortisone in ocular herpesvirus infection. J. Immunol. **104**:1359-1363.
107. Okada, Y., and J. Tadokoro. 1963. The distribution of cell fusion capacity among several cell strains or cells caused by HV. J. Exp. Cell Res. **32**:417-430.
108. Oldstone, M. B. A., and F. J. Dixon. 1970. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. II. Relationship of the anti-lymphocytic choriomeningitis immune response to tissue injury in chronic lymphocytic choriomeningitis disease. J. Exp. Med. **131**:1-20.
109. Oldstone, M. B. A., and F. J. Dixon. 1970. Tissue injury in lymphocytic choriomeningitis viral infection; virus induced immunologically specific release of a cytotoxic factor from immune lymphoid cells. Virology **42**:805-813.
110. Oldstone, M. B. A., and F. J. Dixon. 1971. Acute viral infection; tissue injury mediated by anti-viral antibody through a complement effector system. J. Immunol. **107**:1274-1280.
111. Oldstone, M. B. A., and F. J. Dixon. 1971. Immune complex disease in chronic viral infections. J. Exp. Med. **134**:32s-40s.
112. Onabanjo, A. O., A. R. Bhabani, and B. G. Maegraith. 1970. The significance of kinin-destroying enzymes activity in *Plasmodium Knowlesi* malarial infection. Brit. J. Exp. Pathol. **51**:534-540.
113. Padawer, J. 1971. Poxvirus phagocytosis *in vivo*: electron microscopy of macrophages, mast cells and leukocytes. J. Reticuloendothel. Soc. **9**:23-41.
114. Panijel, J., and P. Cayeux. 1968. Immunosuppressive effects of macrophage antiserum. Immunology **14**:769-780.
115. Pearce, J. H., and D. B. Lowrie. 1972. Tissue and host specificity in bacterial infection. Symp. Soc. Gen. Microbiol. **22**:193-216.
116. Pinto, C. A., R. F. Haff, and R. C. Stewart. 1969. Pathogenesis of and recovery from respiratory syncytial and influenza infections in ferrets. Arch. Ges. Virusforsch. **26**:225-237.
117. Piraino, F. 1967. The mechanism of genetic resistance of chick embryo cells to infection by Rous sarcoma virus-Bryan strain (BS-RSV). Virology **32**:700-707.
118. Platt, H. 1970. Factors contributing to resistance and susceptibility of stratified squamous and respiratory epithelia, p. 173-183. *In* R. H. Dunlop and H. W. Moon (ed.), Resistance to infectious diseases. Modern Press, Saskatoon, Canada.
119. Porter, D. D. 1971. Destruction of virus infected cells by immunological mechanisms. Annu. Rev. Microbiol. **25**:283-290.
120. Postic, B., C. J. Schleupner, J. A. Armstrong, and M. Ho. 1969. Two variants of Sindbis virus which differ in interferon induction and serum clearance. I. The phenomenon. J. Infect. Dis. **120**:339-347.
121. Pusztai, R., E. A. Gould, and H. Smith. 1971. Infection patterns in mice of an avirulent and a virulent strain of Semliki Forest virus. Brit. J. Exp. Pathol. **52**:669-677.
122. Quinlan, M. F., S. D. Salman, D. L. Swift, H. N. Wagner, Jr., and D. F. Proctor. 1969. Measurement of mucociliary function in man. Amer. Rev. Resp. Dis. **99**:13-23.
123. Reed, S. E. 1969. Persistent respiratory virus infection in tracheal organ cultures. Brit. J. Exp. Pathol. **50**:378-388.
124. Reeve, P., D. J. Alexander, G. Pope, and G. Poste. 1971. Studies on the cytopathic effects of Newcastle disease virus: metabolic requirements. J. Gen. Virol. **11**:25-34.

125. Reeve, P., and G. Poste. 1971. Studies on the cytopathogenicity of Newcastle disease virus: relation between virulence, polykaryocytosis and plaque size. J. Gen. Virol. **11**:17–24.
126. Reeve, P., M. Rosenblum, and D. J. Alexander. 1970. Growth in chick chorioallantoic membranes of strains of Newcastle disease virus of differing virulence. J. Hyg. **68**:61–69.
127. Reeve, P., and A. P. Waterson. 1970. The growth cycle of avirulent strains of Newcastle disease virus. Microbios **5**:5–9.
128. Reinarz, A. B. G., M. G. Broome, and B. P. Sagik. 1971. Age-dependent resistance of mice to Sindbis virus infection: viral replication as a function of host age. Infect. Immunity **3**:268–273.
129. Russell, W. O., and others. 1971. Fifth annual A.S.C.P. research symposium. Viruses and auto-immune disease. Amer. J. Clin. Pathol. **56**:259–353.
130. Sawyer, W. D. 1969. Interaction of influenza virus with leukocytes and its effect on phagocytosis. J. Infect. Dis. **119**:541–556.
131. Schlesinger, R. W. 1950. Incomplete growth cycle of influenza virus in mouse brain. Proc. Soc. Exp. Biol. Med. **74**:541–548.
132. Schloer, G. M., and R. P. Hanson. 1971. Virulence and *in vitro* characteristics of four mutants of Newcastle disease virus. J. Infect. Dis. **124**:289–296.
133. Scholtissek, C., H. Becht, and R. Drzeniek. 1967. Biochemical studies on the cytopathic effect of influenza viruses. J. Gen. Virol. **1**: 219–225.
134. Sellers, R. F., A. I. Donaldson, and K. A. J. Herniman. 1970. Inhalation, persistence and dispersal of foot-and-mouth disease virus by man. J. Hyg. **68**:565–573.
135. Sheaff, E. T., and R. B. Stewart. 1968. A substance enhancing virus growth and antigenistic to interferon action. Can. J. Microbiol. **14**:965–969.
136. Shif, I., and F. B. Bang. 1970. *In vitro* interaction of mouse hepatitis virus and macrophages from genetically resistant mice. I. Adsorption of virus and growth curves. J. Exp. Med. **131**:843–850.
137. Shroyer, E. L., and B. C. Easterday. 1968. Growth of infectious bovine rhino-tracheitis virus in organ cultures. Amer. J. Vet. Res. **29**: 1355–1362.
138. Simizu, B., and N. Takayama. 1969. Isolation of two plaque mutants of Western equine encephalitis virus differing in virulence for mice. J. Virol. **4**:799–800.
139. Simon, M., and I. Dömök. 1966. Enhancing effect of human erythrocyte extracts on the susceptibility of monkey kidney cells to certain entroviruses. Acta Microbiol. **13**:229–234.
140. Smith, H. 1958. The use of bacteria grown *in vivo* for studies on the basis of their pathogenicity. Annu. Rev. Microbiol. **12**:77–102.
141. Smith, H. 1960. Biochemical response to bacterial injury, p. 341–359. *In* H. B. Stoner and C. J. Threlfall (ed.), The biochemical response to injury. Blackwell Scientific Publications, Oxford.
142. Smith, H. 1964. Microbial behaviour in natural and artificial environments. Symp. Soc. Gen. Microbiol. **14**:1–29.
143. Smith, H. 1968. Biochemical challenge of microbial pathogenicity. Bacteriol. Rev. **32**:164–184.
144. Smith, H. 1969. The search for protective antigens. Brit. Med. Bull. **25**:126–130.
145. Smith, H. 1969. Toxic activities of microbes. Brit. Med. Bull. **25**:288–292.
146. Smith, H. 1971. Host factors influencing microbial proliferation *in vivo*, p. 141–151. *In* R. H. Dunlop and H. W. Moon (ed.), Resistance to infectious disease. Modern Press, Saskatoon, Canada.
147. Smith, H. 1972. The little-known determinants of microbial pathogenicity. Symp. Soc. Gen. Microbiol. **22**:1–24.
148. Smith, H., and J. H. Pearce. 1972. Microbial pathogenicity in man and animals. Symp. Soc. Gen. Microbiol. **22**.
149. Smith, W. 1963. Mechanisms of virus infection. Academic Press Inc., New York.
150. Smorodintsev, A. A. 1960. Basic mechanisms of non-specific resistance to viruses in animals and man. Advan. Virus Res. **7**:327–376.
151. Stephen, J., and T. H. Birkbeck. 1969. The biochemistry of virus cytotoxicity. J. Gen. Microbiol. **59**: p xvi–xvii.
152. Stevens, J. G., and M. L. Cooke. 1971. Restriction of herpes simplex virus by macrophages. An analysis of the cell-virus interaction. J. Exp. Med. **133**:19–38.
153. Stoner, H. B. 1972. Specific and non-specific effects of bacterial infection on the host. Symp. Soc. Gen. Microbiol. **22**:113–128.
154. Tamm, I., and F. L. Horsfall. 1965. Viral and rickettsial infections of man, 4th ed. J. B. Lippincott Co., Philadelphia.
155. Tegtmeyer, P., and J. F. Enders. 1969. Feline herpesvirus infection in fused cultures of naturally resistant human cells. J. Virol. **3**:469–476.
156. Thind, I. S., and W. H. Price. 1970. The role of serum protective factor and neutralizing antibody on pathogenesis of experimental infection with Langat virus in mice. J. Infect. Dis. **121**:378–382.
157. Thomas, L. H. 1970. Mycoplasmas as infectious agents. Annu. Rev. Med. **21**:179–184.
158. Tokumaru, T. 1968. The nature of toxins of herpes virus. I. Syncytial giant cell producing components in tissue culture. Arch. Gesamte Virusforsch. **24**:104–122.
159. Tomasi, T. B. 1970. Structure and function of mucosal antibodies. Annu. Rev. Med. **21**:281–298.
160. Truden, J. L., M. M. Sigel, and L. S. Dietrich. 1967. An interferon antagonist; its effect on

interferon action in Mengo-infected Ehrlich ascites tumour cells. Virology **33**:95-103.

161. Tyrrell, D. A. J. 1967. The spread of viruses of the respiratory tract by the airborne route. Symp. Soc. Gen. Microbiol. **17**:286-306.
162. Veltri, R. W., and B. E. Kirk. 1971. An antiviral substance in the tissues of mice acutely infected with lymphocytic choriomeningitis virus. J. Gen. Virol. **10**:17-27.
163. Wagner, R. R., and A. S. Huang. 1966. Inhibition of RNA and interferon synthesis in Krebs-2 cells infected with vesicular stomatitis virus. Virology **28**:1-10.
164. Waterson, A. P., T. H. Pennington, and W. H. Allan. 1967. Virulence in Newcastle disease virus. A preliminary study. Brit. Med. Bull. **23**:138-143.
165. Webb, H. E., and J. G. Hall. 1972. An assessment of the role of the allergic response in the pathogenesis of viral disease. Symp. Soc. Gen. Microbiol. **22**:383-414.
166. Webster, A. J. F. 1970. Environmental and physiological interactions influencing resistance to infectious disease, p. 61-80. *In* R. H. Dunlop and H. W. Moon (ed.), Resistance to infectious disease. Modern Press, Saskatoon, Canada.
167. Wheelock, E. F., and R. Edelman. 1969. Specific role of each human leukocyte type in viral infections. III. 17D yellow fever virus replication and interferon production in homogenous leukocyte cultures treated with phytohaemagglutinin. J. Immunol. **103**:429-436.
168. Woodward, C. G., T. H. Birkbeck, and J. Stephen. 1972. Possible vaccinia virus cytotoxic factor(s). J. Gen. Microbiol., *in press*.
169. Worthington, M., and R. Clark. 1971. Lack of effect of immunosuppression on Scrapie infection in mice. J. Gen. Virol. **13**:349-351.
170. Zisman, B., M. S. Hirsch, and A. C. Allison. 1970. Selective effect of anti-macrophage serum, silica and anti-lymphocyte serum on pathogenesis of herpes virus infection of young adult mice. J. Immunol. **104**:1155-1165.