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Pathogenicity of Influenza Virus

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INTRODUCTION

Influenza and its causative virus have been the subjects of extensive research and publication. Overwhelming attention has been given to the structure and biochemistry of the virus (for reviews, see 31, 41, 174, 207, 212, 223), its mechanism of replication in cell culture (for reviews, see 189, 200, 223), clinical and pathological descriptions of the disease (for reviews, see 56, 161, 223), epidemiology, antigenic variation and animal reservoirs (for reviews, see 59, 114, 131, 171, 222, 255), immunology (for reviews, see 176, 204, 223), and vaccines (for reviews, see 223, 245). In contrast, the pathogenicity of influenza virus, i.e. the mechanisms whereby it produces illness, receives little attention (155, 244). The aim of this review is to describe what is known about pathogenicity and to highlight the gaps in the hope of stimulating research. Reference to the subjects listed above will be minimal and confined to their relevance to pathogenicity.

The main aspects of microbial pathogenicity (or the synonymous term, virulence) are (i) mucous surface interaction, (ii) entry to host tissues, (iii) replication in vivo, (iv) interference with or avoidance of host defenses, (v) spread from the initial site of replication, (vi) damage to the host, and (vii) tissue and host specificity. The microbial and host components responsible for these processes are the determinants of pathogenicity (216). There are three essential steps in identifying these determinants. First, methods for comparing virulence must be established to identify strains of high and low virulence. Second, these strains must be compared in tests relevant to the above aspects of pathogenicity to recognize properties associated with virulence such as rapid growth, serum resistance, phagocyte inhibition, or cytotoxicity. Finally, the biochemical bases for these properties of the virulent strain must be identified (216). The first two observational steps are relatively easy to accomplish, but identifying determinants is difficult and has only been achieved substantially for bacteria (216). Not only are most investigations of pathogenicity of viruses at the observational rather than the determinant stage, but only replication has received much attention (214, 217). However, viral pathogenicity is not determined solely by ability to replicate in host cells, since virulent and attenuated strains often replicate similarly in vitro yet differ fundamentally in infection of animals. This is due to different capacities to accomplish one or more of the other aspects of pathogenicity listed above. All

of these general statements regarding viral pathogenicity apply to influenza viruses.

The plan is to deal solely with the pathogenicity of influenza virus A as seen in humans and the two animal models, ferret and mouse infection, most used in the study of human influenza. Experiments with ferrets will receive major attention because of the recent appearance of much relevant work. Reference to influenza in other animals will be made only to illustrate particularly important points. The following facets of pathogenicity will be considered: (i) criteria of pathogenicity for selecting strains of differing virulence; (ii) survival on and penetration of the respiratory mucous membranes; (iii) replication of virus in and release from host cells: tissue specificity; (iv) counteracting nonspecific defense mechanisms; (v) counteracting immunospecific defense mechanisms; (vi) spread of virus: barriers and tissue specificity; (vii) damage to the host—adult, fetus, and neonate; (viii) exacerbation of bacterial infection; and (ix) virulence in relation to genetic analysis.

CRITERIA OF PATHOGENICITY FOR SELECTING STRAINS OF DIFFERING VIRULENCE

Human influenza is predominantly an upper respiratory tract infection with some lung involvement but rare viremia (56, 221). Typically, symptoms include nasal discharge, cough, fever, headache, myalgia, anorexia, malaise, and depression (56, 221) but occasionally neurological and gastrointestinal effects are also seen (166, 221). It is rarely lethal when uncomplicated by bacterial infection (56, 221). Information on the relative virulence of strains has come less from observations on epidemics than from volunteer studies.

Interpretation of morbidity and mortality data from epidemics is complicated by population immunity and confusion between virulence (ability to cause disease) and communicability (ability to spread). The second wave of an epidemic appears to be more devastating than the first or later waves, but this is probably due to greater communicability rather than increased virulence (58). The changes in severity of disease between pandemics may indicate changes in virus virulence. The mortality rate in 1918 was about 16 times higher than that in 1957 (161), suggesting that the former pandemic was due to a more virulent virus. Although population resistance may have been low and the effect of bacterial pneumonia may have been high in the absence of antibacterial drugs (19), some of the greater number of deaths were due to true viral pneumonia (161).

Trials with volunteers have shown significant differences in virulence between recombinants produced as prospective vaccines (19, 20, 162). Assessment is based on the frequency and amount of virus shedding, the levels of serum antibody before and after inoculation, and clinical surveillance of subjective symptoms (e.g, headache, myalgia, malaise, chill, hoarseness, and sore throat) and more objective criteria (handkerchief count, oral temperature, cough, mucopurulent nasal discharge, and sneezing). These reactions are scored numerically and then collectively graded as very mild (trivial effects), mild (local symptoms), moderate (local and constitutional symptoms), or severe (febrile or other unpleasant reactions necessitating bed rest) (21). Inevitably, most trials must be conducted with relatively few heathly adult volunteers of variable antibody status. They can be misleading (36, 61), especially about the virulence of strains for the old and young. Hence, such trials should not form the immutable basis against which all other assessments of virulence are judged. Rather, they should be viewed together with animal experiments as indications of what may happen in more extensive human trials (36).

In ferrets, the pattern of infection after nasal instillation of human influenza viruses is similar to that in humans, mainly an infection of the upper respiratory tract with fever, some lung involvement, and rare viremia (15, 148, 218, 221, 238). As for humans, the infection is not usually fatal, and assessment of virulence must be based on less definite criteria than the 50% lethal dose used for lethal pathogens. Sneezing, nasal discharge, listlessness, and anorexia may be observed (221) but are difficult to quantify. Criteria used include the 50% infectious dose, the level and persistance of upper respiratory tract infection, the height and duration of fever, the level of lung infection (36, 148, 238), the numbers of inflammatory cells (84, 85, 135, 148, 239), the amount of protein in nasal washes (177), and the degree of nasal obstruction (83). Differences in virulence between strains are easily detected and, with a few exceptions, agree well with those observed in human volunteers (36, 48, 64, 148, 238). Ferrets appear slightly more susceptible to influenza virus infection than adult humans with previous experience of influenza (36), but they might accurately reflect human infection in infants or when a new serotype appears.

Intranasal instillation of influenza virus freshly isolated from humans into mice does not produce overt disease, although virus may multiply in the lungs, bronchioles, and trachea as well as in nasal tissue (94, 103, 161). Passage of virus through mice produces strains which cause

severe disease in which, unlike in human and ferret influenza, the lungs rather than the upper respiratory tract are predominantly affected (3, 139) and death often follows, probably from pneumonia (179, 266). Parameters used for comparing the virulence of mouse-adapted strains are death rate or time to death or both, lung consolidation scored on a five-point scale, lung weight (which increases with consolidation), hemagglutinin in macerated lung, food and water intake, and body weight (175). Sometimes virulence comparisons in mice do not correlate well with those in humans (150), which is not surprising in view of the difference in disease syndrome. The primary advantage of using mice is that the pulmonary pathology is similar to that seen in the rare cases of viral pneumonia in humans (139, 161).

In this section of the review, efforts to identify virulence markers should be noted. These markers are properties associated with virulent strains; they need not necessarily contribute to the disease process (216). The aim is merely to have convenient tests in vitro for recognizing virulent and attenuated strains and serological and cultural properties can suffice (216). Some success has been achieved for influenza virus, for example, in relating the virulence of strains to ability to inhibit cilial activity of tracheal organ cultures, but usually not all strains comply with any one criterion (89, 95, 160). This is not surprising because of the multifactorial nature of virulence. Only those markers that have relevance to the disease process in vivo are of interest here. Examples are the replication in organ cultures of respiratory tissues with damage to cilia (89, 160) and temperature sensitivity which may relate to abilities to replicate in the upper and lower respiratory tract (40, 148, 159, 162).

Virulent and attenuated strains of influenza virus have been identified for humans, ferrets, and mice. This allows the search for virulence determinants by comparing them in various aspects of pathogenicity. Investigations in humans are necessarily limited, but those in ferrets should have relevance to human influenza because both the disease syndrome and virulence differences between strains are similar in the two hosts. Studies with mice might prove useful in delineating the determinants of lung infection.

SURVIVAL ON AND PENETRATION OF THE RESPIRATORY MUCOUS MEMBRANES

Influenza virus invades across the epithelial surfaces of the respiratory tract. Factors operating against virus attack on this tract, such as the mucociliary "blanket," competition from commensal microorgainisms, and humoral and cellular defenses, have been described before (34, 214). The objective here is to highlight the lack of specific information on how influenza virus counteracts these factors and to suggest future experiments.

Observations made late in the infectious process show that influenza virus can infect many different cell types in the upper and lower respiratory tracts of humans, ferrets, and mice. Immunofluorescence studies have shown influenza virus antigens in ciliated, intermediate, basal, and goblet cells of human nasal epithelium (60, 136, 236) but not in bronchial or bronchiolar cells (146); also, in the rare cases of pneumonia, antigen has been found in the alveolar lining (161) and macrophages (146). In ferrets, antigen has been detected in nasal epithelial and inflammatory cells, bronchial epithelium, and macrophages but not in the trachea (135, 227), and for certain strains of virus it has been detected in alveolar cells (161, 221). In mice, antigen has been found in epithelial cells of the nasal turbinates, trachea, bronchi, bronchioles, and alveoli (67, 163). These studies give no clue to the initial site of attack, but demonstrate that virulent strains can breach mucosal defenses and, when they do, many types of cells are available for infection.

With respect to the initial lodgement site in natural infection of humans, we must note that the infecting aerosol contains a range of particle sizes as was present in early aerosol experiments with volunteers (56). Since larger particles lodge in the upper respiratory tract and smaller particles are carried to the lung (34), the infecting virus probably lodges throughout the respiratory tract (56). However, the initial lodgement site appears not to influence subsequent events. Thus, studies with human volunteers inoculated either by intranasal instillation or with homogeneous aerosols of small particle size, indicated that uncomplicated influenza can follow from lodgement of virus in either the upper or lower respiratory tract (126, 214). For ferrets, we are not aware of studies with aerosol inoculation; intranasal instillation is the usual procedure (135, 238), and here virus lodges predominantly on the nasal epithelium. Lung infection occurs, but whether it is derived from the original inoculum is unknown. Virus might be carried to the lung in fine particles resulting from breathing during nasal instillation, or it may follow later by a similar mechanism from the upper respiratory tract infection. Aerosol inoculation of mice leads to uniform infection throughout the respiratory tract (67, 140) with primary

lodgement on the epithelial cells lining the airways (268). Even with nasal instillation, virus is deposited in the lung as well as in the nasal tract (67).

Thus, in human and animal influenza, all areas of the respiratory tract probably receive virus, and most of them contain cells that are susceptible to attack. The questions to be answered include the following. (i) What promotes contact between the virus and the walls of the respiratory tract despite the mucus layer? (ii) What prevents removal by mucociliary action? (iii) How does the virus resist, if necessary, any inhibitory action of commensals? (iv) How does it interfere with humoral and cellular defense mechanisms in the mucus? (v) What determines the predominantly upper respiratory tract infection that characterizes influenza despite the seeding of the lung and the susceptibility of some of its cell types? Before discussing these points, two suggestions that might help research should be mentioned. Ferrets, rather than mice, provide a disease syndrome similar to that of humans, but the presently used method of inoculation—nasal instillation—does not mimic natural infection. If the aim of investigations is to study mucous surface interactions, infection of ferrets should be with aerosols of heterogeneous particle size so that virus reaches the lower as well as the upper respiratory tract. Also, virulent and attenuated strains should be compared. Such comparisons in bacteriology have revealed virulence determinants acting at mucous surfaces (215), but we are not aware that they have been made with influenza virus.

Contact between virus and respiratory tract cells will be a chance event because virus is trapped in the mucus layer and swept upwards to the pharynx or backwards from the nose to be swallowed (34). Host and environmental factors that may affect the chance of contact include variations in thickness, flow rate, and viscosity of mucus and gaps in the blanket (214). Also, mucus secretion and ciliary action may be inhibited by changes in temperature, ion concentration, and humidity of the air (34, 214). Although experiments to assess the influence of these factors on virus infection are difficult to perform, this has been accomplished with Newcastle disease virus in chickens, where temperature changes and drugs were used to vary mucociliary action (9, 10). Similar investigations could be done with influenza virus in ferrets, if not humans, by using aerosol inoculation of strains of differing virulence. Undoubtedly, host and environmental factors play a part in infection, but during epidemics influenza occurs in too many individuals for simultaneous defects or impairment of the mucociliary blanket to be the sole cause of the increased rate of disease. Viral properties must also be involved in infection. Virus concentration in the aerosol would be important, and viral neuraminidase might facilitate access to the surface by liquefying mucus (77). The effect of the neuraminidases of virulent and attenuated strains of influenza virus on respiratory mucus of humans, ferrets, and mice should be examined.

Adherence to the cilia themselves may be a mechanism whereby influenza virus resists removal by mucociliary action. In organ cultures of ciliated epithelium from various animal species including ferrets and mice, the cilia continue to beat, and after infection virus adheres strongly to them (23, 57, 78, 258). The adherence to cilia may not mean that virus enters the cell via them; electron microscopy of infection of guinea pig trachea showed adhesion but produced no evidence of penetration through cilia (57). However, in vivo, the adherence could promote infection by immobilizing the virus, thus avoiding its elimination in the mucus blanket, and by inhibiting ciliary action, sometimes by clumping (258). The apparent predilection for cilia may occur because cilia have receptors for influenza virus of higher affinity or in greater number than the ubiquitous receptors found on most host cells. Also, the accumulation of virus on cilia may be helped mechanically by their combing action in the mucus flow and the large surface area offered for virus adsorption. Although in ciliated tissues themselves virus appears to adsorb preferentially to cilia, it should be noted that differences in adsorption to organ cultures of ciliated (nasal turbinate and trachea) and nonciliated (alveoli, bladder, and uterus) ferret tissues could not be detected (120, 240). It is possible, however, that adsorption to the ciliated tissues may have been affected by the fact that mucus is not produced in organ cultures of ferret tissue (93, 120). A decrease in susceptibility of hamsters with age to influenza virus correlated with adsorption of virus to tracheal organ cultures (197), but the reasons were not investigated. The adherence of virulent and attenuated strains to appropriate mucus-producing ciliated tissues should be compared not only in organ culture but also in vivo (for example, by electron microscopy immediately after inoculation of large amounts of virus). Mucus production in organ culture might be induced by vitamin A (8).

Commensals may influence mucosal attack by influenza virus. Fungi, bacteria, and their products induce interferon (34); mycoplasma can damage ciliated epithelium (237) and prevent interferon induction (42); and *Haemophilus influenzae*, a persistent colonizer of chronic bronchitics (243), produces a substance which destroys the ciliated epithelium of human fetal

trachea (52).To determine whether commensals influence the primary stages of influenza, experiments should be conducted as for bacterial infections and infection of pigeons with Venezuelan equine encephalitis virus (34, 216). Volunteers, ferrets, or mice could be treated systemically or locally with antibiotics to remove commensals from the upper respiratory tract before aerosol inoculation to observe the effect on infection by virulent and attenuated strains of virus. Comparisons with animals having normal flora would reveal any dramatic influence of these microbes. Also, gnotobiotic ferrets or mice could be inoculated similarly; in the one instance known to us, germfree mice were more susceptible to influenza virus than ordinary mice (55).

The mucus lining the respiratory tract contains nonspecific inhibitors, polymorphonuclear phagocytes, and macrophages, and these may increase during infection (34, 154, 228, 239). In humans, previous experience of influenza means that these defenses may be to some extent immunospecific even at the beginning of infection. Ferrets and mice are usually shown to lack detectable antibody before use. Interference with nonspecific and specific humoral and cellular defenses will be considered in later sections.

How far can factors affecting the initial stages of mucosal attack explain the fact that influenza in humans and ferrets is predominantly an upper and middle respiratory tract infection, despite the seeding of the lower regions of the lung and the presence of susceptible cells (see above; 119, 120)? In the lung the mucus layer is thinner and moves more slowly (34) and, together with the relative absence of commensals, should enhance the chances of infection compared with the upper respiratory tract. If adherence of virus to cilia in vivo was an important aspect of infection (see above), this might explain the lack of alveolar cell infection and the bronchial and bronchiolar involvement sometimes seen in addition to upper respiratory tract infection. The effectiveness of alveolar macrophages may be another important factor. Such mechanisms could be tested, at least for ferrets, with appropriate organ cultures or alveolar macrophages inoculated with virulent and attenuated strains. Similar studies with mice, which are more prone to lung infection, might also be useful. The next section deals with other mechanisms which influence the susceptibilities of different parts of the respiratory tract.

REPLICATION OF VIRUS IN AND RELEASE FROM HOST CELLS: TISSUE SPECIFICITY

Ability to replicate in vivo is an essential but not the only determinant of virulence (214). All stages of replication, namely attachment and penetration, uncoating, synthesis of viral nucleic acid and proteins, and assembly and release, require complementation between virion components and host cell factors that have been termed replication factors (214). The degree to which complementation occurs determines whether replication ensues, its rate, and the infective quality of the progeny. It may involve an "all or nothing" response, but in many cases a lack of full complementarity reduces the yield of infectious virus per unit time but does not eliminate virus production completely.

Replication in Tissue Culture Cells

Some determinants of influenza virus replication in cell culture are known, and they probably apply to infection in vivo. Studies on events in permissive and nonpermissive cells, on abortive infection, and on defective interfering particles are of most interest in relation to pathogenicity. The subject has been reviewed (188, 189, 200, 223) and is summarized here. Attachment of virus to cells is mediated by the hemagglutinin (200) and *N*-acetyl neuraminic acid residues of glycoprotein or glycolipid cell receptors (207) which are present in most, if not all, cell membranes (169). Thus, influenza virus can attach to many cell types. However, penetration does not necessarily follow adsorption, for example, with erythrocytes. It occurs only in certain cell types and appears to depend on factors supplied by these cells as well as on a fully glycosylated and cleaved hemagglutinin on the virus (125, 187, 200, 207). Penetration probably occurs by energy-independent viropexis (170), but a role for membrane fusion cannot be excluded (200). The subsequent events of replication occur successfully only if virus and host factors complement one another completely; abortive infections with influenza virus have resulted from errors in uncoating, macromolecular synthesis (6, 26, 66, 72, 133), maturation, budding, and release (35, 69, 137). In some cases, defects in viral matrix protein (32, 306) and misintegration of neuraminidase into host cell membranes (106) have been implicated in abortive infection.

The most investigated event of replication in relation to infectivity and virulence is the posttranslational cleavage of the surface hemagglutinin, without which progeny virus is noninfectious (132, 188). Glycosylation and proteolytic cleavage of this hemagglutinin takes place during migration from the rough endoplasmic reticulum via the smooth endoplasmic reticulum to the plasma membrane before incorporation into the virus envelope at maturation and budding (200). The virulence of strains of avian influenza viruses is associated with the facility for cleavage of the hemagglutinin in a broad spectrum of cells, namely in MDCK cells as well as chicken,

duck, turkey, and quail fibroblasts (27, 188). Less pathogenic strains were produced in a noninfectious form in MDCK cells and chicken and turkey fibroblasts; in chicken embryos their replication was restricted to endodermal cells, unlike that of the pathogenic strains which could also infect mesodermal and ectodermal tissue (27, 188). Thus, the extent to which cleavage of hemagglutinin occurs depends not only on the particular structure of that hemagglutinin (124) but on the proteases of the host cell and on its environment (125); as for infection of the chicken embryo, these factors could determine tissue specificity in vivo (125, 188). Although cleavage of the hemagglutinin is important in the virulence of influenza virus, it is not the only factor involved, and some strains with easily cleaved hemagglutinins are not virulent (191). It has been suggested that viral neuraminidase may promote cleavage of the hemagglutinin by exposing relevant sites (205) as well as possibly being involved in release of budding particles (31).

Lack of a full set of replication factors may not only block or reduce the production of infectious virus but also result in formation of incomplete virus particles. As already mentioned, some cannot replicate. Others can, but only in the presence of standard infectious virus whose replication is interfered with specifically. Such defective interfering particles (DIPs) were first recognized for influenza virus (248, 249). If produced in vivo, DIPs could influence pathogenicity by reducing the replication of infectious virus (101).

Replication In Vivo

Experiments of the detail described above cannot be conducted with infected animals. Nevertheless, some information has come from observations of the behavior in vivo of temperature-sensitive (*ts*) mutants produced in vitro. More importantly, attempts have been made recently to investigate replication in relevant tissues at the cellular level with organ cultures which retain many in vivo characteristics (98). Results from organ cultures should be interpreted with care for three reasons, only the first two of which reflect the situation in vivo: (i) more than one cell type is present; (ii) only a small proportion of the total cells may be susceptible; and (iii) cut ends are exposed to infection as well as surfaces of tissues unexposed in vivo (215). Nevertheless, differences in the replication of virulent and attenuated strains of influenza virus have been detected in cultures held at normal and pyrexial temperatures, differences which related to their behavior in vivo. Also, organ culture experiments have indicated some mechanisms which could explain the tissue specificity of influenza virus observed in humans and ferrets, namely, high levels of infection in the nasal tract, little in the lung, and almost none elsewhere. This specificity could result either from a greater inherent susceptibility (infectious virus-producing capacity) of nasal mucosa compared with lung and other tissues, or from limitation of virus spread from the nasal tract by barriers and host defenses. Experiments with organ cultures, mainly of ferret tissue, have shown: (i) influenza virus adsorbs to most tissues but replicates in few; (ii) the virus can replicate well in tissues where it is not found in vivo, thus invoking barrier phenomena in tissue specificity; and (iii) more virus is produced in and released from nasal turbinates than from lung tissue, showing that differences in inherent susceptibility contribute to the infection pattern found in the respiratory tract. The experiments are summarized below.

Replication in human tissue. *ts* mutants of influenza virus have been produced in vitro and tested in volunteers as potential vaccine strains. Strains which replicate at 32 to 34°C, the temperature of the upper respiratory tract, but not at that of the lower tract (37°C), may immunize without pneumonic complications (162). Mutants were produced by growth of wild-type virus in the presence of 5-fluorouracil, and relevant recombinants were made. Two sets of *ts* mutants, *ts*-1 [E] and *ts* IA2, have been evaluated. The two *ts*-1 [E] mutations were located on the ribonucleic acid (RNA) genes coding for polymerase protein P3 and nucleoprotein, and those of *ts* IA2 were on the genes coding for polymerase proteins P1 and P2; therefore, all were defective in complementary RNA and viral RNA synthesis (40, 162). Their replication was markedly restricted in tissue culture at 38 and 37°C, respectively, and they replicated poorly in hamster lungs. In human volunteers, they replicated in the upper respiratory tract, but did not induce systemic or lower respiratory tract symptoms (40, 162). There is no evidence to suggest that genes other than those with the *ts* lesion played a role in attenuation (145). Thus, it appears that a defect in polymerase function at the temperatures of internal tissues leads to an inability to replicate in them in vivo and therefore loss of virulence.

In organ culture, human adult tissues (nasal mucosal scrapings, uterus, bladder, and conjunctiva) and fetal tissues (nasal mucosa, trachea, esophagus, intestine, bladder, lung, conjunctiva, and umbilical cord) supported replication of influenza virus; adult and fetal nasal mucosa were

more susceptible than fetal lung (98, 186). Fetal brain, spleen, liver, thymus, and amnion did not support replication, although they adsorbed virus as well as susceptible tissues (186). Virus replication in human fetal trachea correlated with the virulence of strains in humans in some but not all cases (89, 160). The mechanisms of replication in human tissues have not been investigated in depth.

Replication in ferret tissue. In early work the virulence of virus strains for humans could not always be correlated with their ability to replicate in organ cultures of ferret trachea at 33°C (89, 158). Recently, for members of one recombinant system, correlations were obtained between ability to replicate in organ cultures of nasal turbinate tissue at normal and pyrexial temperatures and infection patterns in the upper respiratory tract; and, in one case, similar correlations applied to infection of lung tissue in organ culture and in vivo (148, 229, 230, 239). The background is as follows. The normal rectal temperature of ferrets is about 38.5°C. This temperature probably occurs in the lung, but in the nasal tract it is normally a few degrees lower (229). Fever occurs in infected ferrets during day 2 after intranasal inoculation, and temperatures of both the upper and lower respiratory tracts probably rise to 40 to 41°C (148, 229, 238, 239).

Experiments began with two clones of a recombinant influenza virus A/PR/8/34-A/England/939/69 (H_3N_2) which exhibited in ferrets differences in virulence (clone 7a, virulent; clone 64d, attenuated) similar to those found in humans (238). After intranasal inoculation, both clones produced similar upper respiratory tract infections during day 1, then their behavior diverged. The titers of clone 64d in nasal washings leveled off, then declined at the beginning of day 2, whereas those of clone 7a continued to rise; they leveled off and declined 6 to 12 h later than those of clone 64d. Clone 7a also produced a more consistent and greater lung infection than did clone 64d (238). The two clones were adsorbed and inactivated equally by the phagocytes of the nasal inflammatory response (228), but in organ culture of nasal turbinates at normal and pyrexial temperatures, they showed differences. First, at the normal temperature of the nasal tract (about 34°C), although initial production of virus by the two clones was similar for about 20 h, the maximal yields of clone 7a at 24 to 27 h were greater (230). Second, clone 64d was more thermolabile and its replication was more restricted at elevated temperature than that of clone 7a (228, 229). These differences in ability to replicate could explain the behavior of the clones in the upper respiratory tract of infected ferrets (229, 230). Furthermore, clone 64d was less able than clone 7a to replicate in ferret lung tissue at normal and pyrexial temperatures, and this could explain its behavior in vivo (229). Extension of this work to the two parent strains (see above) and two further recombinants (clones 6 and 64c) confirmed the relation between ability to replicate at pyrexial temperatures and the pattern of infection in the upper respiratory tract (148). But it failed to do so for lung infection where, for some strains, other factors must play a role in vivo (148). A similar lack of complete correlation between temperature sensitivity and lung infection has been noted for other strains of virus in hamsters (C. W. Potter, personal communication).

The information in the previous paragraph overlaps with the next section, because fever in some cases appears to be a host defense mechanism against virus attack (142). It should be emphasized that the experiments just described with ferrets differ fundamentally from those with *ts* mutants in humans and hamsters. The latter experiments implicate in attenuation restriction of virus replication at the normal temperatures of the lower respiratory tract, whereas the former correlate restriction of replication at pyrexial temperatures with loss of virulence. The molecular basis for the ability to replicate at pyrexial temperatures is unknown. Lwoff (142) suggested that raised temperature inhibited the replicases of attenuated strains of poliovirus and also, with other factors, promoted the release of lysosomal nucleases which destroyed virus nucleic acid. Virulent viruses had replicases that worked rapidly at high temperatures, so virus development took place before the nucleases were released. This may be an oversimplification, and we do not know whether such mechanisms operate for influenza virus. Clearly, however, resistance of replication to pyrexial temperatures may be an important aspect of influenza, and the molecular determinants could be identified by appropriate experiments.

Tissue specificity in ferrets is similar to that in humans, with the nasal mucosa being the most susceptible tissue (15, 84, 85, 135). The degree of lung infection varies with the virus strain (148, 239, 241). Of 16 tissues taken from infected ferrets, only nasal turbinates, lung, trachea, and esophagus contained significant quantities of virus, and nasal turbinates contained 95% of the total (15). In organ culture, similar to the situation with human tissues, bladder, uterus, oviduct, conjuctiva, esophagus, and pharynx could be infected in vitro in addition to respiratory tissues. Many other tissues were insusceptible, although, like insusceptible human

tissue, they adsorbed virus (16, 186, 240). In vivo, barriers must stop spread of infection to susceptible tissues like the urogenital tract, and this is discussed later. In contrast, the inherent susceptibility of the respiratory tissue to virus replication could determine the natural infection pattern, since replication in organ culture paralleled that in vivo (15, 16, 240). The minimal infectious dose for organ cultures of nasal mucosa was lower, and virus was produced in the medium more rapidly and to a higher titer than in corresponding cultures of trachea and lung alveolar tissue (16, 119, 120, 240).

Research was concentrated on the differences between nasal turbinate and lung tissue (39, 119, 120). Virus yields in the tissue pieces and media of organ cultures of both tissues were compared. Over a 24-h period, when the first and possibly second cycles of infection were occurring, nasal turbinate organ cultures produced approximately 10-fold more virus in total than did lung cultures (39). These differences were not caused by differences in: (i) the amounts of virus adsorbed (120, 240), (ii) the total numbers of susceptible cells and the actual numbers becoming infected (39, 119, 120), (iii) the occurrence of powerful inhibitors (120), or (iv) the kinetics of virus replication (39, 119, 120, 240). Thus, individual susceptible cells of nasal turbinates produced about 10-fold more virus than the alveolar type I and II cells which became infected in lung tissue (39, 119, 120), and the difference seemed to be determined by factors that influenced either the amount and quality of virus components synthesized or their assembly. Lung alveolar cells have characteristics which might either limit influenza virus production or result in the formation of incomplete virus. In kidney and other cell types, the hemagglutinin and possibly other envelope proteins are synthesized on the rough endoplasmic reticulum and translocated along the smooth endoplasmic reticulum to the plasma membrane (200). Alveolar type II cells have little smooth endoplasmic reticulum, and translocation may be further hindered by surfactant produced in the endoplasmic reticulum (see 39). This surfactant contains dipalmitoylphosphatidylcholine and phosphatidylglycerol, compounds that are rare in other mammalian cells (185). Alveolar type I cells are relatively devoid of ribosomes and mitochondria, and this may limit virus (39).

Lung tissue infected immediately after its removal from the animal (fresh tissue) released only about 6% of the total virus produced in 24 h whereas fresh nasal turbinates released approximately 30% (39). The poor release of virus by lung cells may be as important as the smaller total production of virus in restricting infection in the lower respiratory tract. The following observation provided a basis for investigating the poor release. When lung tissue was maintained for 24 h before inoculation, the total virus produced was not significantly greater than that in fresh tissue, but a greater proportion (about 40%) was released (39, 119, 120). This increased release was not due to obvious artefacts such as cell necrosis, fibroblast outgrowth, inhibitors, or killing of commensals by the antibiotic-containing medium (119). Furthermore, fresh adult nasal turbinate tissue and fresh lung tissue of 2-day-old ferrets, which released a substantial proportion of their total virus production, showed no marked increase in release when inoculated after maintenance (38, 39, 118, 119, 120). Hence, the change of adult lung on maintenance may be in the mechanism which prevents virus release from fresh tissue in vitro and in vivo. Three possibilities have already been discounted. Overlying mucus, which may have prevented release, appeared absent from both fresh and maintained lung (119). Alveolar surfactant may have either prevented release or inactivated virus, but similar amounts were present in fresh and maintained lung (120). Virus released from fresh lung may have been noninfectious in the assay system because the hemagglutinin was uncleaved, but trypsin treatment showed that this was not so (39). The mechanism preventing release in adult lung tissue is at present unknown. It must operate outside the host cell membrane, because influenza virus is not infectious until it has budded through the membrane, and the total infectious virus produced by fresh and maintained tissue was similar. One possibility is an inhibitor of viral neuraminidase which has been implicated in virus release (31).

In the organ culture experiments with ferret tissues, autointerference was noted, i.e., a depression in virus yield at high inocula (120, 240) which may have been due to DIPs (101, 248, 249). Whether these particles are produced in vivo and affect pathogenicity is unknown.

Replication in mouse tissue. We are not aware of any organ culture studies with mouse tissue either for comparing virulent and attenuated strains or for studying the basis of tissue specificity. In vivo, with unadapted strains, virus titers may be higher in either the nasal or lung mucosa (67, 103). Although the amount of virus in the lung may be similar for unadapted and adapted strains, the latter seem better able to infect alveolar cells (179, 266). This alveolar cell susceptibility could be investigated in organ cultures along the lines described for ferret tissue. In mice, DIPs interfere with the replication of

infectious virus in the lung (249), with increased survival and decreased disease (96, 178). However, the protection appeared not to be related to autointerference with replication of wild-type virus but to augmented humoral immune responses (178). Whether DIPs are produced during mouse infection with wild-type virus is unknown. Their role in the pathogenesis of infection is unclear both in mice and in humans.

COUNTERACTING NONSPECIFIC DEFENSE MECHANISMS

As background to this and the next section, the reader is referred to articles (154, 214, 217) in which the general nature of host defense against virus attack was summarized. Also, the lack of knowledge of the mechanisms whereby viruses counteract this host defense was emphasized, and some speculations were made about these mechanisms.

During the first few days of influenza in nonimmune hosts, the virus must inhibit or avoid nonspecific defenses on, within, and below the surface epithelium of the respiratory tract. These defenses include the mucociliary blanket, possible inhibitory action of surface commensals, and nonspecific virus inhibitors in mucus, serum, and tissue fluids (11, 29, 94, 128, 179, 240; Husseini, Sweet, and Smith, unpublished data). Then, there is the cellular inflammatory response which, for influenza in humans, ferrets, and mice, consists predominantly of polymorphonuclear (PMN) phagocytes in contrast to the mononuclear (MN) phagocytes seen in other virus infections (88, 135, 136, 146, 154, 161, 179, 239). The response occurs in the epithelium, but the inflammatory cells are extruded onto the mucosal surface. The acidity of inflammatory exudates (214) may also be a defense, because influenza viruses are normally labile to acidic pH (100). Lung macrophages also increase during the inflammatory process in humans, ferrets, and mice (161, 179, 238). Fever produced by interaction of virus with PMN and MN phagocytes is another defense mechanism in humans and ferrets (184). Finally, interferon is formed in influenza of humans, ferrets, and mice and is found in respiratory tract washings and serum (80, 84, 86, 172, 183, 213, 225). As for host cell nucleases which might destroy viral nucleic acid (214), we are not aware of any being implicated in the destruction of influenza virus in vivo.

We have already discussed the ability of influenza virus to counteract the moving mucus blanket, any inhibitory action of commensals, and fever. We will now take the remainder of nonspecific defenses and examine how they may be overcome by influenza virus in our three hosts.

Nonspecific inhibitors of influenza virus comprise the α and γ mucoprotein inhibitors, which inhibit hemagglutination, and the β inhibitors, which neutralize infectivity (128, 207). These could prevent virus attaching to receptors on susceptible cells. In humans, resistance to β inhibitors may have allowed Asian strains of influenza virus to infect the lung more readily (161). In ferrets, although low titers of virus were rapidly inactivated by ferret blood in vitro (240), inhibitors present in nasal washings from ferrets infected with either a virulent or an attenuated clone of influenza virus had a similar action on both clones (Husseini, Sweet, and Smith, unpublished data). Adaptation to mice was accompanied by viral resistance to β inhibitors in one study (29) but not in others (11, 94, 179). Hence, for influenza of humans, ferrets, and mice, it seems unlikely that resistance to nonspecific inhibitors is important in virulence. If in fact it is involved, viral neuraminidase could be the virulence determinant, since it digests some nonspecific inhibitors.

The influence of inflammatory cells on infections with virulent and attenuated strains of influenza virus has been studied only with ferrets. After intranasal inoculation with a virulent (clone 7a) or an attenuated (clone 64d) clone, a nasal inflammatory response consisting of 90% PMN and 10% MN phagocytes occurred at about the same time (25 to 29 h after inoculation) and to a similar extent for both clones (239). This response coincided with the occurrence of fever and correlated with decline of virus titers in nasal washes (239). Extension of this work to the parent viruses (A/PR/8/34; A/England/939/69) and two further recombinants confirmed the relation between the appearance of the inflammatory response and the decline of virus in nasal washes, but revealed a striking difference for A/PR/8/34 (148). Although this virus produced a similar and possibly greater upper respiratory tract infection, the inflammatory response was delayed and less marked than with the other strains. This relative lack of stimulation or depression of the inflammatory response has been noted in influenza of rats (194) and is a possible virulence mechanism. It may result from a decreased chemotaxis of PMN phagocytes (17, 195, 198) and could possibly be related to the surface antigens of A/PR/8/34 (H_0N_1), which are different from those of the parent and recombinant strains (H_3N_2) (148). Experiments with more strains or recombinants possessing the same surface antigens as A/PR/8/34 (H_0N_1) might clarify any role of these antigens in this respect.

When influenza virus interacts with PMN

phagocytes, experiments in vitro with cells of humans, ferrets, and mice show that: (i) virus is adsorbed and inactivated, but productive virus replication does not occur (82, 88, 130, 196, 228); and (ii) the phagocytes are damaged, as indicated by loss of chemotactic activity and ability to phagocytose bacteria (17, 82, 130, 195, 198). As yet, there is no evidence that virulent strains are more able either to resist adsorption and phagocytosis or to damage phagocytes compared with attenuated strains. On the contrary, ferret peritoneal and nasal inflammatory cells adsorbed and inactivated clones 7a (virulent) and 64d (attenuated) equally (228). Further work along these lines with different virus strains and cells from different animal species is needed. The decreased chemotactic and ingestive activity of PMN phagocytes caused by influenza virus almost certainly contributes to bacterial superinfection (see below), and similar mechanisms probably operate in promoting infection with influenza virus itself. The determinants of this detrimental effect of virus infection on PMN phagocytes are unknown.

What has been said for interaction with PMN phagocytes also applies to MN phagocytes of the respiratory tract, lymph nodes, spleen, and liver. Adsorption and inactivation of virus with subsequent reduction of chemotactic, ingestive, and killing ability of the phagocytes are the pattern of numerous experiments with MN phagocytes from humans, ferrets, mice, and other animals (82, 121, 122, 123, 153, 196, 209, 228, 256). Productive infection of macrophages by influenza virus has not been demonstrated as it has for other viruses (51, 156, 214, 217) except for an avian influenza virus adapted to certain types of mice (134, 247). Influenza virus antigen has been detected in MN phagocytes in vivo (135, 146, 161, 227), but experiments with MN phagocytes in vitro indicate that only abortive infection occurs (51, 209, 256). Experiments with attenuated and virulent strains do not appear to have been conducted. Thus, as for PMN phagocytes, infection of and damage to MN phagocytes are probably a virulence mechanism for influenza virus, but we have no proof of this nor any idea of the mechanisms involved.

Although influenza virus is acid labile (100) and inflammatory exudates can be acidic (214), we are not aware of comparisons of virulent and attenuated strains for resistance to pH equivalent to that in inflammatory exudates.

The role of interferon in ameliorating virus disease is not clear, and attempts to relate the virulence of virus strains with a lower capacity to induce interferon or a greater resistance to it have not been successful (154, 214, 217). This situation applies to influenza and influenza viruses. The absence of interferon in lung tissues of fatal cases of human influenza was noted (14). A temporal relation between appearance of interferon and recovery is apparent in humans, but usually the quantities are directly related to the degree of virus replication and severity of illness (56). In one study attenuated influenza viruses induced more interferon in human leukocytes than a virulent strain (173). However, in chick cells a negative correlation occurred between interfering ability and virulence (50). The role of interferon in infection of ferrets is unclear (84, 172, 213), and preliminary experiments on two strains of differing virulence (clones 7a and 64d) indicate no marked differences in ability to induce interferon in the nasal tract (Husseini, Sweet, and Smith, unpublished data). Although interferon appeared to be an important defense mechanism in some studies with mice (86, 225, 269), anti-interferon serum had no effect on infection in another study (81). No correlation was found between the virulence of strains and interferon production in the lung (152, 179). A material which inhibited interferon production in murine cells in vitro was detected in the sera of infected mice and may have impaired interferon production in vivo (220). Its relevance to virus infection is unclear, since similar inhibitors have been found in uninfected tissue (154). Overall, it appears that both virulent and attenuated strains of influenza virus induce interferon and show no marked differences in resistance to it.

The lack of knowledge as to how influenza viruses counteract nonspecific defense mechanisms at the beginning of infection is regrettable. The most fruitful avenues for research appear to be interactions with PMN and MN phagocytes and the reasons for the resistance of viral replication at pyrexial temperatures.

COUNTERACTING IMMUNOSPECIFIC DEFENSE MECHANISMS

Immunity to influenza in humans, ferrets, and mice has been well studied and reviewed (176, 204, 224, 247). On balance, humoral immunity—local and systemic—appears to play the predominant role. In summary, the main mechanisms are as follows. Antibody to the hemagglutinin combines with virus and prevents its attachment to cells; circulating anti-hemagglutinin antibodies correlate with resistance to disease in humans and animals. Some workers believe anti-neuraminidase antibody prevents release of virus from cells. Destruction of virus by increased phagocytosis or lysis may follow from activation of complement by reactions of antibodies with the viral envelope. Infected cells may be destroyed by two antibody-mediated mechanisms: K-cell killing and complement lysis, after interaction of

antibody and viral antigens incorporated in the host cell membrane. These antigens include not only the viral surface antigens but also the nucleoprotein and matrix protein. Cellular immunity is also induced, and in addition to the usual events that follow T-lymphocyte activation, e.g., lymphokine production, cytotoxic T-lymphocyte destruction of infected target cells of the same histocompatibility type may also contribute to protection.

It appears that influenza virus lacks a strong ability to counteract the specific host defenses because in humans, ferrets, and mice (inoculated with unadapted strains) influenza is usually an acute disease with a complete and rapid recovery. Nevertheless, persistence of influenza virus possibly occurs in some humans during interepidemic periods (99) and has been detected in the lungs of some human neonates (129) and mice (70). Do any of the possible mechanisms for counteracting immune defenses outlined elsewhere (154, 156, 214, 217) operate for influenza virus?

Persistent infections with influenza virus have been established in cell lines (73, 242, 260) and in organ cultures of human and other animal tissue (181; Sweet, unpublished data). The mechanisms are not known, although in one case persistence may have been mediated by interferon (242). In human influenza, the virus in goblet cells (60) may be in an immunologically privileged site (156); in bovine tracheal organ cultures, parainfluenza virus persisted in the submucous glands despite antibody in the medium (182). DIPs may be involved, since inoculation of defective virions into mice produced a persistent lung infection (70).

Antigenic shift and drift (255) occur between influenza epidemics and undoubtedly promote initial infection of incompletely immune hosts. Does it occur during infection of individuals, as for equine infectious anemia (217)? It has been suggested that virus persistence and antigenic variation may occur in interepidemic periods, but the evidence is not yet strong (99). Even if antigenic variation occurs, it would probably result from, and not be a mechanism of, persistence if, for example, influenza virus behaves like vesicular stomatitis virus. Persistent infection with DIPs of vesicular stomatitis virus resulted in mutation and consequent antigenic variation (97). As for other possibilities for persistence (154, 156, 214), there is no evidence that the envelopes of virulent strains are more "host-like" (and therefore less antigenic) than those of attenuated strains or that nonneutralizing antibodies block the activities of other antibodies or K cells in vivo.

Considering the possibility of direct inactivation of B and T lymphocytes, temporary suppression of cellular immunity with lymphocytopenia has been reported for influenza in humans (46, 110, 193) and mice (147) but not ferrets (112). Suppression of human T but not B cells by virulent and attenuated strains has been reported (110), as well as a decrease in both types, with the greatest suppression occurring in T cells (46). In humans (111) and mice (147) influenza pneumonitis is associated with immunosuppression. The lack of an effect on cell-mediated immunity in ferrets was attributed to the absence of pneumonia (112). Some lymphocytes pass through the lung en route from the central lymph system to the arterial blood, and if the lung is infected, virus-lymphocyte interaction can take place (263). Virus antigen has been found on blood lymphocytes after infection of humans (262), and virus adhered to human T and B lymphocytes in vitro (82). However, virus replication does not occur in lymphocytes, and infection, if it happens, is abortive (30, 51, 82). Nevertheless, the lymphocytes appear to be harmed. Chemotaxis of T and B cells is depressed in influenza of humans (54). Attachment of influenza virus to rat thoracic duct lymphocytes temporarily affected their ability to migrate into lymphoid tissue (263). The cause of the interference in activity is unknown, although the effect on rat lymphocytes was attributed to neuraminidase-induced changes in their membranes (264). All the tests for B- and T-cell function were made with antigens different from influenza virus. Hence, we cannot be certain whether any immunosuppression induced by influenza virus would actually potentiate the persistence of influenza virus itself.

To sum up, interference with the specific defense mechanisms may not be an important facet of the pathogenicity of influenza virus because recovery from disease is rapid. Although viral persistence may occur in vivo, it has not been convincingly demonstrated. Immunosuppression occurs to some extent. In either case, the mechanisms and determinants are unknown.

SPREAD OF VIRUS: BARRIERS AND TISSUE SPECIFICITY

We have already discussed the factors which determine the pattern of respiratory tract involvement after infection of humans by natural aerosols and of ferrets and mice by intranasal or aerosol inoculation. In summary, virus can be delivered to all parts of the tract either by the initial inoculum or by aerosols set up by passage of air over previously infected tissues. Also, virus can spread locally from cell to cell and generally to all parts. The general spread can be either upward following mucociliary action or down-

ward by drainage if mucosal secretion swamps this action (155). There are no strong barriers between the different parts, and tissue specificity is determined by the inherent abilities of the various tissues to produce and release virus coupled, in some cases, with the influence of host defense mechanisms.

In this section attention is focused on infection of tissues outside the respiratory tract. Organ culture experiments show that many of these tissues, particularly urogenital and fetal tissues, can support replication of influenza virus. In some cases they do so as well as if not better than respiratory tissues (186, 233, 240). Do these extrarespiratory tissues become infected in vivo and, if not, why not?

Although enteric effects have been noted in human influenza (56), we are not aware of virus isolations from adult alimentary tract tissues lower than the esophagus of humans, ferrets, or mice. This is not surprising since adult tissues are not susceptible in organ culture (186, 240), and even if virus is swallowed (34), infection would probably be prevented by the acid lability of influenza virus (100, 232), i.e., stomach acid provides a barrier. Avian influenza viruses which are more stable than human viruses appear to replicate in the alimentary tract of birds (254).

In human influenza, cardiac and neurological complications have been noted occasionally (221), and virus has been isolated infrequently from urine (48) and, in fatal cases, from cerebrospinal fluid, pericardial fluid, and liver and fetal tissues (56, 143). Viremia has been reported, but only rarely (56), and virus may be carried on blood lymphocytes as well as in plasma (262). In ferrets inoculated intranasally, infectious virus was detected in cervical lymph nodes (238) and spasmodically in liver, spleen, and kidney but not in uterus, bladder of nonpregnant animals, or the placentas and fetuses of pregnant ferrets (15, 44, 233, 238). Virus was found spasmodically in plasma (238), but consistent viremia or antigenemia was not detected (12, 84, 227). If a viremia occurs, experiments in vivo and in vitro (15, 238, 240) suggest that virus is transported in the plasma rather than on blood cells. Thus, in humans and in ferrets, infection of extrarespiratory tissues occurs only rarely and, despite their high inherent susceptibility, urogenital and fetal tissues do not usually become infected. In mice with adapted strains, the picture is different. Virus has been isolated from liver, spleen (68, 87), pancreas, kidneys, salivary glands, heart, cervical and mediastinal lymph nodes (68), and fetuses and fetal membranes (211, 235; Collie, Sweet, and Smith, unpublished data). Viremia has been demonstrated consistently with virus in the plasma and on blood cells (68, 87, 105, 219).

In humans and ferrets, barriers must prevent the spread of virus from the respiratory tract to susceptible tissues in all but a few individuals on rare occasions. The barriers occur at three stages: between the respiratory tract and the lymph/blood system; in the blood and its associated reticuloendothelial system; and at blood-tissue junctions. Experiments to test the nature and strength of these barriers are difficult in humans. Hence, as a model, experiments in ferrets were conducted particularly in relation to spread of infection to the urogenital tract of nonpregnant animals and the placenta and fetal tissues of pregnant animals.

The first barrier between the respiratory tract and the lymph/blood system can be breached because, after intranasal inoculation, virus or virus antigen was found consistently in the cervical (238) or mediastinal (135) lymph nodes. Also, nasal infection was established by bloodstream inoculation of virus into nonpregnant and pregnant animals (15, 233). However, only moderate levels of virus appear to escape through this barrier. After intranasal inoculation and at the height of infection, antigen was not detected in the spleens of animals by fluorescent antibody, whereas it was detected after bloodstream inoculation of 10^8 50% egg-bit infectious doses ($EBID_{50}$) of virus ($\simeq 10^6$ $EBID_{50}$/ml). In addition, infection of fetal membranes of pregnant ferrets occurred after bloodstream inoculation of $10^{7.4}$ $EBID_{50}$ of virus ($\simeq 10^{5.4}$ $EBID_{50}$/ml) but not after intranasal infection. Hence, the amount of virus escaping from the nasal tract was such that the blood titer at any one time was less than 10^5 to 10^6 $EBID_{50}$/ml. Also, nasal infection after bloodstream inoculation could only be initiated with very large quantities of virus (15, 233).

It appears that, at least in ferrets, the second barrier to virus spread, namely the blood and reticuloendothelial system, is formidable. First, inhibitors are present in the blood of ferrets (and humans), and they can inactivate in 1 h at 37°C an amount of virus greater than the maximum that might be found in the blood after liberation from the respiratory tract (240). Second, it appears that phagocytosis by spleen and liver cells can quickly remove blood-borne virus. After bloodstream inoculation with $10^{9.4}$ $EBID_{50}$ of influenza virus ($\simeq 10^7$ $EBID_{50}$/ml), the viremia was 10^4 $EBID_{50}$ in 5 min and undetectable within 30 min (240). Two hours later, infectious virus was only detected significantly in the spleen (240). Furthermore, fluorescent antibody detected antigen in the spleen 0.5 h after blood-

stream inoculation of 10^8 $EBID_{50}$ of virus (227). Thus, the second barrier will reduce quickly and effectively any viremia that develops from the respiratory infection. In individual ferrets (and probably humans), where the inhibitors may be low and phagocytosis impaired, a viremia might occur, but only rarely.

The third barrier is between the blood and susceptible extrarespiratory tissues. It seems inpenetrable in the case of urogenital tissues in nonpregnant ferrets. Although urogenital tissues could be infected in vivo by local inoculation (16), bloodstream inoculation of large quantities of virus failed to infect them (15). Also, virus was not detected either in the blood or in the nasal turbinates of ferrets infected urogenitally (16) or intra-amniotically (Collie, Sweet, and Smith, unpublished data). The nature of this barrier is unknown, but one factor may be insusceptibility of the endothelial cells lining blood vessels (240).

The third barrier is modified in pregnancy and can be breached if the viremia is sufficiently high. After intracardial inoculation ($10^{7.4}$ $EBID_{50}$), virus could be isolated not only from the uterus and bladder of pregnant ferrets but also from the fetal membranes (placenta, hematoma, umbilical cord, amnion, and chorion) (44, 233). Only in ferrets at late gestation was fetal infection established with certainty (233). The route to the fetus is unclear. In organ culture all the fetal membranes and uterus were susceptible to infection (233, 240). After blood-borne infection of placenta or uterus, virus could spread to the fetus from the placenta along the blood vessels of the umbilical cord or from the uterine lining through the chorion and amnion, as with reovirus infection in rats (117). Within the fetus, most tissues were infected, indicating viremic spread (233). Even if maternal viremia is high, fetal infection in humans may not occur so easily as in ferrets for two reasons. First, although in organ culture human endometrium and decidua were susceptible to infection, human placenta, amnion, and chorion were relatively insusceptible (186; Collie, Sweet, and Smith, unpublished data), and they might therefore act as a barrier to fetal infection. Second, the structure of the human placenta (hemomonochorial) is different from that of ferrets (endotheliochorial) (233). Guinea pigs have hemomonochorial placentas (231). In organ culture all their fetal membranes except chorion were insusceptible, and after bloodstream inoculation of large quantities of virus, the fetal membranes contained only low titers and there was no evidence of replication in fetal tissues (231).

Thus, in ferrets and probably humans, the reasons for the lack of virus infection of extrarespiratory tissues include the small escape from the respiratory tract and the rapid removal of blood-borne virus by nonspecific inhibitors and the reticuloendothelial tissues. In the absence of a substantial viremia, relatively weak blood-tissue barriers (such as in ferret fetal membranes) cannot be breached. However, all experiments were conducted with one Asian and one Hong Kong strain of virus, and the barriers may be breached with more virulent strains. In human influenza significant extrarespiratory infection has not been detected as new virus strains have appeared. Thus, its rare occurrence probably results from host variation, particularly with respect to inadequate host defenses in the blood and reticuloendothelial tissues.

Why the barriers to spread of infection are weaker in mice is not clear, although this may well follow from the adaptation of strains to this animal. Viremia (around 10^5 to 10^7 50% egg infections doses per ml) occurs with virus present on blood cells and in the plasma for up to 48 h after aerosol inoculation (68). The occurrence and level of viremia correlate with high titers of virus in the lung (68), and it may represent "spillover" from this site (87). As for the reasons, either mouse-adapted strains may be better able to counteract inhibitors in the blood and the phagocytes of the reticuloendothelial tissues, or these defenses are less effective than those in ferrets. However, mouse phagocytes appear effective against influenza virus; liver parenchyma cells, which could be infected via the bile duct, were not infected by blood-borne virus due to phagocytosis by the Kupffer cells (153). Virus association with erythrocytes (87) may be a mechanism for avoiding phagocytosis, but mouse-adapted strains are no more resistant to serum inhibitors than are nonadapted strains (11, 94, 179). Hence, we have no valid explanation for the more easily demonstrable viremia which occurs in mice compared with that in humans and ferrets. Almost certainly it largely determines the frequency of extrarespiratory infection in mice.

DAMAGE TO THE ADULT, FETUS, AND NEONATE

Overall harm to the host follows from viral damage to individual cells. This damage may result from virus replication depleting cells of essential components, from direct virus cytotoxicity, or from immunopathology. These mechanisms have been discussed in detail elsewhere (7, 214, 217, 253) using as examples viruses other than influenza virus, for there is little knowledge of how this virus damages cells. Here we describe

the damage influenza virus causes in the adult, fetus, and neonate (human, ferret, and mouse) with recent information on the mechanism involved at the animal level. Then, the little knowledge available at the cellular level will be summarized.

Damage to Adults

Uncomplicated influenza of humans and ferrets is rarely fatal, lasting 1 to 2 weeks, and is accompanied by respiratory and constitutional effects. Mice, however, are killed by adapted strains.

Respiratory effects. In humans, respiratory symptoms include sneezing, nasal obstruction and discharge, sore throat, hoarseness, and cough (56, 221) and probably result from damage produced by viral replication in the upper and lower respiratory tract (63). The upper respiratory tract pathology shows an inflammatory response and desquamation of ciliated, goblet, intermediate, and basal cells in which virus replication has occurred (60, 250). Although damage is predominantly confined to the upper respiratory tract, tracheitis, bronchitis (250), and impaired lower respiratory function may also occur (56). Pneumonia is rare and occurs usually in patients predisposed by heart disease and other conditions (141, 146); alveolar and capillary damage leads to edema and hemorrhage and often death from respiratory failure and shock (141, 161, 221).

In ferrets, respiratory damage is similar to that in humans. Sneezing and nasal discharge are seen (148, 221, 238), and lesions are mainly confined to the upper respiratory tract (135, 221). There is inflammation, hypersecretion of mucus, and necrosis of respiratory epithelium leading to desquamation of epithelial cells but not the basal layer (221). Some virus strains replicate in lung tissue and produce bronchiolitis and bronchitis but little, if any, pneumonitis; damage to ciliated epithelium is the primary feature, and its extent probably depends on the virulence of the strain (Macartney, Sweet, and Smith, unpublished data). Pneumonia can be produced in ferrets by passaged strains, and the pathology is similar to that in humans (161, 221).

In mice, unadapted strains of virus produce bronchitis and bronchiolitis, and adapted strains produce lung lesions similar to those in human influenzal pneumonia (161, 179).

Constitutional effects. In humans, constitutional effects commonly include headache, myalgia, shivering, listlessness, nausea, vomiting, anorexia, and fever, and the severity of the symptoms is related to the height of fever (56). These effects have been attributed either to the toxic effects of products (viral or host) from cells destroyed by viral replication or to complement activation by antigen-antibody complexes of viral components (63). However, recent evidence suggests that fever and the other constitutional effects may follow from release of leukocyte pyrogen from phagocytes (PMN and MN) after reaction with influenza virus, predominantly in the respiratory tract. The evidence comes from experiments with rabbits, ferrets, and humans.

Leukocyte (endogenous) pyrogen acts on the hypothalamus, via synthesis of prostaglandin E, to produce fever (22). Rabbit PMN and MN phagocytes released leukocyte pyrogen on interaction with influenza virus in vitro (22, 109, 113). Also, fever, with detectable leukocyte pyrogen in the bloodstream, followed intravenous inoculation of rabbits with large quantities of semipurified influenza virus (4, 127). The pyrogen was probably produced by phagocytes in the spleen after interaction with influenza virus, since cultures of spleen cells from inoculated rabbits released pyrogen in vitro (71).

We are not aware of experiments showing release of leukocyte pyrogen by human phagocytes after interaction with influenza virus. However, this would probably occur as for rabbit phagocytes since, like them, human phagocytes release pyrogen after interaction with other inducers such as endotoxin (22, 180). If this is so, then release of leukocyte pyrogen may well be the cause of fever in influenza. The problem is to determine where the main virus-phagocyte interaction occurs: locally in the upper respiratory tract with release of pyrogen (or possibly the phagocytes themselves) into the bloodstream, or systemically after escape of virus into the bloodstream and reaction with the reticuloendothelial phagocytes (45). Both mechanisms are possible, since pyrogen is produced at inflammatory sites and released into the blood (24), and bloodstream inoculation of rabbits with influenza virus showed that pyrogen could come from spleen cells (see above). However, recent experiments with ferrets support the local origin of fever (227).

Intranasal inoculation of ferrets with the virulent clone 7a produced a fever of approximately 24 h in duration. Its onset coincided with the inflammatory response in the infected upper respiratory tract (239). There were strong positive correlations between the rise of inflammatory cells in nasal washings and the rise of pyrexia (239). Also, insufficient virus escaped into the bloodstream from the respiratory tract to produce a 24-h fever by interacting with reticuloendothelial phagocytes. Ten bloodstream inoculations of 10^8 $EBID_{50}$ of virus were needed over

a 5-h period to produce a 3- to 8-h fever (227). Thus, a viremia corresponding to this quantity of virus ($\approx 10^6$ $EBID_{50}$/ml) over about 24 h would be needed to induce the 24-h fever observed in intranasally infected ferrets. However, the total virus in the nasal mucosa was below 10^8 $EBID_{50}$ before the onset of fever and only exceeded this level for a 4-h period during the 24-h fever. Furthermore, influenza virus antigen could not be detected by fluorescent antibody in the spleens of intranasally infected animals immediately before and during the fever, but could be detected after a single intravenous inoculation of 10^8 $EBID_{50}$ of virus, suggesting that escape of such amounts does not occur from the upper respiratory tract (227). Finally, with regard to the local origin of fever: (i) the majority of thePMN and MN phagocytes of the nasal inflammatory response had influenza virus antigen associated with them; (ii) these infected cells, on incubation in vitro, released a pyrogen that was active in ferrets and that had the characteristics of leukocyte pyrogen; and (iii) a similar pyrogen was released from ferret peripheral blood leukocytes after interaction with influenza virus in vitro. Thus, fever probably results from interaction of virus with phagocytes in the upper respiratory tract and release of leukocyte pyrogen into the bloodstream. Phagocytes primed by virus interaction in the respiratory tract might themselves enter the bloodstream to release their pyrogen there (45), and virus-phagocyte interactions in the cervical lymph nodes, where virus is found 2 days after inoculation, may contribute some pyrogen. But essentially all these interactions are local, not systemic.

We cannot be certain that human phagocytes release pyrogen after interaction with influenza virus as do rabbit and ferret phagocytes. Nor can we be sure that the findings on ferret infection regarding the local origin of fever apply to human influenza. Nevertheless, it is reasonable to suggest that similar processes occur because of the similar disease syndromes. If this is so, then the local release of human leukocyte pyrogen by virus-phagocyte interaction in the respiratory tract has wider implications. Most constitutional effects of influenza such as headache, malaise, myalgia, shivering, and nausea have followed the administration of autologous leukocyte pyrogen to humans (180). Thus, the constitutional effects of uncomplicated influenza in adults could follow from local interactions in the respiratory tract.

Damage to the Fetus

Controversial evidence links influenza during human pregnancy with fetal death, congenital malformations, and neoplasms in lymphatic and hemopoietic tissues (143). The controversy is not surprising since fetal infection probably occurs in only a few individuals on rare occasions. We can only speculate on how damage might follow from fetal infection, should this occur. Many human fetal tissues support virus replication in organ culture (186), and thus damage in vivo could follow directly from virus replication. However, those tissues—neural and lymphopoietic—in which the major developmental or postnatal deformities have been reported, appeared insusceptible in organ cultures (186). This damage might result from abortive replication. Alternatively, multiplication in susceptible tissues could release toxic or teratogenic materials (186). Influenza of chicken embryos produced neural abnormalities, but replication was limited to extraneural sites (108). Fetal damage could also follow from virus-induced endothelial lesions in fetal blood vessels. This might cause vasculitis and intravascular coagulation in the placenta and umbilical cord and hence hypoxia in fetal organs (37); intravascular coagulation has been noted in influenza (49, 259).

Experiments with ferrets and mice show that, if virus reaches the fetus, gross damage may occur, although the mechanisms are unknown. Intranasal inoculation of pregnant ferrets failed to infect the fetuses, which remained unaffected despite the maternal illness and fever (44). When virus reached the fetuses either from bloodstream inoculation of pregnant animals at late gestation or by intra-amniotic inoculation at early gestation, death and resorption of fetuses occurred (44; Collie, Sweet, and Smith, unpublished data). The effect of fetal infection appeared to be "all or none." Deaths and resorptions readily occurred, but abnormalities in the survivors after subsequent birth and growth have not been detected (Collie, Sweet, and Smith, unpublished data). However, intranasal inoculation of pregnant mice with adapted strains of virus not only produced fetal resorptions and stillbirths, but also poor neonatal survival and malformations in the progeny (157, 235, 261).

Clearly in pregnant women and ferrets suffering from respiratory influenza, the fetuses are protected by barriers to spread of infection rather than by resistance to virus damage.

Damage to Neonates

Respiratory disease in human babies is hazardous because of their narrow air passages, their shallow and irregular breathing, and their inability to take breath orally (234); death can occur quickly by occlusion of the airways. Also,

pneumonia may have more severe effects because of the low proportion of alveolar cells in neonates (53). Influenza is a common respiratory infection of young children for whom its effects are generally mild (56). However, convulsions and croup are common (223), and bronchiolitis, bronchiectasis, and pneumonia can occur, sometimes with fatal consequences (129, 166). Influenza has also been implicated in the sudden infant death syndrome, so called "cot-death" (25, 164), although precise diagnosis is difficult.

Infection of neonatal ferrets might be a useful model for providing information on cot-death and infant pneumonia (43). After intranasal inoculation of small doses of influenza virus, all neonates died within 10 days. Some showed destruction of the epithelium with mucopurulent exudates in both the upper and middle respiratory tract. They showed nonspecific changes in the lung reminiscent of those occurring in cot-death and appeared to have suffocated from occlusion of nasal passages. In other neonates there was a frank pneumonia which probably caused death. The predominant pathology was attack and destruction of ciliated epithelium. Ferret neonates could be used to define the role of the inflammatory response in death due to influenza by examining infections with strains such as A/PR/8/34 which do not stimulate an intense response (148).

Some possible reasons for the increased susceptibility of the middle and lower respiratory tract of neonates compared with adults are as follows. First, the primary susceptible cells—ciliated epithelial cells—may, as in human infants (53), be in greater proportion in the neonatal lungs compared with adults because of incomplete development of alveolar tissue. Second, virus release from infected neonatal lung is greater than that from adult lung (38). Finally, the lung macrophages of neonatal animals may be less effective in destroying virus.

Possible Mechanisms of Cell Damage

The pathological effects of influenza described above follow largely from virus-induced damage to epithelial cells in the respiratory tract as well as to the phagocytes of the inflammatory response which, in itself, can be mechanically harmful. Unfortunately, little is known about mechanisms of influenza virus-induced cell damage even in tissue culture.

The virus appears to exert its toxic effect on cells directly, rather than by the replication process depleting cells of essential components (7), because cellular damage can follow from abortive replication in tissue culture cells (199). Also, both PMN and MN phagocytes and T and B lymphocytes may be damaged after virus interaction, even though they fail to support the full replicative cycle of influenza virus in vitro and in vivo (see above). Thus, phagocytes are less chemotactic and less able to ingest and kill bacteria, and the lymphocytes also seem to have impaired functions.

In tissue culture cells, influenza virus depressed both RNA and protein synthesis (138, 199), and this effect appeared to be dependent upon the synthesis of virus-specified protein (138). However, whether such a viral protein shuts off host protein and RNA synthesis specifically or whether it interferes with some control mechanism essential for cellular macromolecular synthesis is unknown (138, 199). With fowl plague virus (FPV), which produced an abortive infection in HeLa cells, the cytopathic effect was tentatively attributed to the viral neuraminidase (199) which might have acted on lysosomes to release their enzymes (144, 199).

In vivo, influenza viruses demonstrate toxic activity, suggesting the presence of toxic components. Large doses are toxic and pyrogenic when inoculated into animals (13, 63, 115). The virus is toxic to mouse lung tissue, and this has been attributed to viral RNA which may code for cytotoxic proteins (13). The lipid component has been implicated in its pyrogenicity (210). A suggestion has been made (130) that the adverse effects of virus on phagocytes may involve two separate processes: a surface effect, which impairs phagocytosis, and an intracellular event, which inhibits chemotaxis. The relevance of these studies with large doses of virus and on abortive replication to normal infection of the respiratory tract is unclear. In the respiratory tract, productive replication occurs and epithelial cells are rapidly damaged. More research is needed not only with tissue culture cells but also with epithelial cells and phagocytes.

The role of immunopathology in influenza appears to be minimal since the main clinical and pathological events happen quickly after infection, and recovery occurs usually within 2 weeks. However, macrophages may be involved in the lung lesions of humans (221). In pneumonia of mice there is some evidence for cell-mediated immunopathology. T lymphocytes have been incriminated in the severity of pneumonia (257, 265), with the degree of lymphocyte infiltration depending upon the virulence of the strain (266). Nude mice infected with virus died later than infected normal mice (224), and normal mice treated with antilymphocyte serum showed decreased lung consolidation and mortality (226). Antibody appears to be less important. Passive administration of antibody or treatment with cyclophosphamide did not affect the severity of the disease (62, 102).

In summary, damage caused by influenza virus appears to follow mainly from a direct toxic effect on cells, the nature of which is little understood.

EXACERBATION OF BACTERIAL INFECTION

Influenza alone is rarely lethal, but it promotes secondary bacterial infections that are often fatal (161, 221). In humans, the secondary invaders are staphylococci, pneumococci, and to a lesser extent *Haemophilus influenzae* (155, 221). β-Hemolytic streptococci as secondary invaders have virtually disappeared, probably because they are now less prevalent (130, 161). Pneumococci, natural respiratory pathogens, having gained a foothold in areas of virus-damaged epithelium in the upper respiratory tract, probably spread to the lung without further viral assistance (161). Staphylococci, though, are not normally respiratory pathogens and attack mainly those areas of the tract that have been damaged by virus (161).

Several possible mechanisms for promoting bacterial infection are suggested in previous sections: destruction of ciliated epithelium would inhibit mucociliary clearance of bacteria; it would also promote entry of bacteria to the tissues; release of substances from damaged cells might stimulate bacterial growth; and inhibition of phagocytes might prevent bacterial destruction. Some of these processes appear to be involved, but the viral determinants are unknown.

There is no direct evidence for virus attack inhibiting mucociliary clearance and promoting entry of bacteria to human respiratory tissues, although adherence of staphylococci via protein A to antibody-coated virus-infected cells may be a factor (5). Human PMN phagocytes after interaction with influenza virus showed decreased chemotaxis and phagocytic activity for staphylococci in vitro (130).

In ferrets, a mixed infection of influenza virus with group C streptococci was more severe than the individual infections (28, 76). However, exacerbation of bacterial infection in ferrets has not been intensively studied. Staphylococci are normal commensals of the nasopharynx of ferrets (28), and attempts to exacerbate these or inoculated staphylococci by infection with virus strains of differing virulence may shed light on similar human infection.

Most experiments on bacterial superinfection have been done with mice. Coliform infection of lungs was promoted by influenzal pneumonia (267). Similarly, simultaneous inoculation of virus and pneumococci produced higher mortality than either alone (74). If influenza virus behaves as parainfluenza virus, inhibition of mucociliary clearance seems to play little role in promoting the bacterial pneumonia (79). However, the pneumonia may in part have been due to the promotion of bacterial growth by virus-induced lung edema (90); inoculation of the virus-free fluid enhanced the ability of pneumococci to produce pneumonia in mice (90). Influenza of mice depressed macrophage accumulation at inflammatory sites (121) and impaired their capacity to remove staphylococci from the lungs (107, 208) but did not affect bactericidal defenses in the liver and spleen (107). Inhaled staphylococci were internalized with equal facility by alveolar macrophages in virus-infected and uninfected mice (79). In the former, however, possibly because of virus-induced decrease of lysosomal enzyme activity (251), the bacteria proliferated internally, whereas they were killed in the latter (79, 252).

VIRULENCE IN RELATION TO GENETIC ANALYSIS

Production of disease by any microbe is so complex that more than one factor must be involved (216). This multifactorial nature of virulence means that: (i) a strain is only fully virulent when it carries a whole armory of virulence determinants; (ii) strains attenuated because of loss of one or more determinants often possess the remaining members of the armory; and (iii) an avirulent strain can be transformed into a virulent strain by transfer of the appropriate virulence determinants from another avirulent strain (216). We have dealt here with the many facets of virulence of influenza virus; its polygenic nature has been noted before (33, 149, 190). Recent genetic analyses of parent viruses and recombinants derived from them, when viewed in relation to virulence, have confirmed the principles outlined above. No one gene segment determines virulence; the latter is dependent upon an optimal constellation of genes, some of which may be derived from attenuated parent strains.

Analysis of the RNA genome of influenza virus shows eight RNA segments (151) coding for nine gene products (104), and RNA-polypeptide assignments have been made for several influenza A virus strains (2, 91, 168). Gene segments 1, 2, and 3 code for polymerase-associated proteins (P1, P2, and P3); segment 4 codes for hemagglutinin (HA); segment 5 codes for nucleoprotein (NP); segment 6 codes for neuraminidase (NA); segment 7 codes for matrix protein (M); and segment 8 codes for one, possibly two, nonstructural proteins (NS1 and NS2). Under suitable conditions the RNA segments of different strains migrate at different rates in polyacrylamide slab gels, allowing the identity and parental origin of genes in recombinants to be determined (91, 165,

167, 168). Alternatively, differential migration of double-stranded RNA molecules produced by hybridization of recombinant RNA with parental RNA has been used for analysis of recombinants (75, 92).

Although each of the eight designated gene segments in any particular strain of virus has features in common with the corresponding gene segments of other strains, their different physicochemical behavior indicates that there are subtle differences between them; some of these differences in the same gene type may well determine the properties related to high or low virulence (214). Thus, in exchange of gene segments, the parental source as well as the specific gene (1-8) could influence pathogenicity. Although it is probable, with such a small genome, that each segment is involved in pathogenicity, some may have a greater influence than others. Furthermore, in any particular strain, cooperation between gene products may be required for a virulence attribute. In recombination all gene segments can probably be exchanged, and thus a recombinant may derive from both pathogenic parents gene segments which are not particularly involved in pathogenicity, or a segment may be transferred without its cooperating segment. Hence, in addition to pathogenic recombinants being formed from less pathogenic parents, nonpathogenic recombinants may be formed from pathogenic parents.

Most studies have related genetic composition with overall virulence rather than to particular aspects of it. Studies on recombinants of avian influenza viruses (particularly FPV) made between themselves or with human and swine strains have provided the main information. Exchange of any RNA segment can modify pathogenicity (191). The virulence of a recombinant is determined by the parent from which the gene segment was derived as well as the particular gene segment exchanged (18, 188, 202). Thus, FPV recombinants in which segment 1 alone was derived from strain A/PR/8/34 were nonpathogenic for chickens, whereas recombinants with this segment replaced by that from strain A/Swine/1976/31 were fully pathogenic (202). However, if RNA segment 2 alone originated from A/Swine/1976/31, then the recombinant was nonpathogenic, but if segment 2 was from a human A_2 or avian influenza virus, a pathogenic recombinant resulted (202). In recombination between a pathogenic and an attenuated strain, recombinants with larger numbers of genes from the attenuated parent tended towards attenuation (65, 165, 192), but, as expected from the principles outlined above, virulent recombinants were obtained from two attenuated parents and attenuated recombinants were obtained from two virulent parents (191, 203, 246). An optimal constellation of genes was required for a highly pathogenic virus (188), and indications of this constellation have been obtained. FPV recombinants which derived some genes for the RNA polymerase complex (P1, P2, P3, and NP genes) from one pathogenic parent and some from another were nonpathogenic, whereas with one exception, recombinants which derived all these genes from one or other parent were pathogenic (191). In addition, all of the pathogenic recombinants derived both their HA and NA genes from the same parent, whereas all but two of the nonpathogenic recombinants did not (191). The genes coding for P1, P2, HA, and M polypeptides have been implicated in neurovirulence of FPV recombinants for mice but not with mouse pneumovirulence or pathogenicity for chickens (203, 246).

More recently two recombinant series have been analyzed genetically in relation to their virulence for humans and for ferrets (36, 148). The few conclusions that can be drawn from the small numbers of recombinants that have been tested (36, 148, 165) support the principles outlined above and the findings with avian influenza virus recombinants.

With clones of the recombinant virus A/PR/8/34-A/England/939/69 (H_3N_2), no gene or combination of genes was recognized that related to overall virulence for humans or ferrets (148, 165). The attenuated parent A/PR/8/34 had some virulence attributes, such as the ability to replicate in the upper respiratory tract. Although substitution of genes from the virulent parent A/England/939/69 produced recombinants more virulent than A/PR/8/34, clone 64d, which contained four gene segments from each parent, was more attenuated than A/PR/8/34, at least for the ferret (148). In addition, recombinants containing A/PR/8/34 genes were more virulent than A/England/939/69 (148).

A second series of recombinants was derived from A/Okuda/57 (attenuated) and A/Finland/4/74 (virulent). Only four were tested in humans, and it was impossible to draw conclusions regarding the relation of virulence to genetic constitution (36). Preliminary results from studies with ferrets involving 10 recombinants of the A/Okuda/57-A/Finland/4/74 series (36; Campbell, Sweet, and Smith, unpublished data) have suggested the following tentative correlations between gene constitution and virulence. The only recombinants (two) that were as attenuated as the A/Okuda/57 parent possessed gene segments 4 (HA) and 6 (NA) from that parent. The recombinants possessing gene segments 4 and 6 from A/Finland/4/74 were as virulent as A/Finland or of intermediate virulence, and there was

a tendency for strains to become less virulent as the number of gene pieces from A/Okuda/57 increased. Unlike A/PR/8/34 in the previous recombinant series, there was no evidence of A/Okuda/57 providing gene segments that markedly increased pathogenicity. The optimal constellation of genes for virulence in this series may follow from analysis and testing of more recombinants.

Although less attention has been paid to the relation of genetic analysis to particular facets of virulence, some information has emerged. Regarding the ability to replicate, gene segments 4 (HA) and 6 (NA) of the correct parental origin seem to be important in infectivity for tissue culture cells (75, 205). These genes have also been implicated in virus infectivity for humans (20) and animals (18, 27, 246) including ferrets (Campbell, Sweet, and Smith, unpublished data), although they are not the sole determinants of virulence (149, 150, 190). RNA segments from the appropriate parent coding for P3, NP, and M proteins appear important for high yields of virus from eggs (165, 206), and the genes coding for P1 and P3 polypeptides apparently control the specificity of FPV recombinants for tissue culture cells (1, 201). How far these latter results apply to infection of animals is not clear.

When the genetic analysis of recombinants of the A/PR/8/34-A/England/939/69 series was viewed in relation to facets of virulence in ferrets, other than replication (the persistence of nasal infection, the presence of lung infection, the height and duration of fever), no correlations with gene segments could be seen except the possibility that the lower stimulation of the inflammatory response of A/PR/8/34 might be related to its surface antigens (148).

Clearly, the relation of the genetic composition of a virus to its virulence is neither simple nor fully understood yet. Even what has already been learned should be regarded with caution since isolates of wild-type virus may contain more than one variant having significantly different pathogenic properties for the host (116). Recombinants may, therefore, have their gene segments derived from different parental variants. Hence, the virulence of each recombinant may reflect chance mutations as well as particular combinations of genes from the two parents. In future studies we should use cloned parental viruses, test recombinants with identical genetic compositions, and devise methods for detecting mutations.

CONCLUSIONS

Apart from replication, little is known about the determinants of the pathogenicity of influenza virus. Nevertheless, this review has identified important areas other than replication that are amenable to deeper investigation by making comparisons of virulent and attenuated strains. We need to know the mechanisms responsible for the following: (i) the initial attack on mucous surfaces; (ii) the lower stimulation of the inflammatory response by some strains; (iii) the inhibition of phagocyte function; (iv) the replication of virulent strains in respiratory tract tissues at pyrexial temperatures; (v) the greater attack of some strains on the lower respiratory tract; (vi) the spread of virus from the respiratory tract in some animals on rare occasions; (vii) the greater susceptibility of the middle and lower respiratory tract of neonates; (viii) the liberation of endogenous pyrogen from phagocytes; (ix) cell damage which may relate to iii and viii; (x) bacterial superinfection.

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LITERATURE CITED

1. **Almond, J. W.** 1977. A single gene determines the host range of influenza virus. Nature (London) **270**:617–618.
2. **Almond, J. W., D. McGeoch, and R. D. Barry.** 1977. Method of assigning temperature-sensitive mutations of influenza viruses to individual segments of the genome. Virology **81**:62–73.
3. **Andrewes, C. H., P. P. Laidlaw, and W. Smith.** 1934. The susceptibility of mice to the viruses of human and swine influenza. Lancet **ii**:859–862.
4. **Atkins, E., and W. C. Huang.** 1958. Studies on the pathogenesis of fever with influenzal viruses. I. The appearance of an endogenous pyrogen in the blood following intravenous injections of virus. J. Exp. Med. **107**:383–401.
5. **Austin, R. M., and C. A. Daniels.** 1978. The role of protein A in attachment of staphylococci to influenza-infected cells. Lab. Invest. **39**:128–132.
6. **Avery, R. J.** 1975. Abortive infection of L cells by influenza virus: absence of virion RNA synthesis. J. Virol. **16**:311–314.
7. **Bablanian, R.** 1972. Mechanisms of virus cytopathic effects. Symp. Soc. Gen. Microbiol. **22**:359–381.
8. **Bang, F. B.** 1972. Specificity of viruses for tissues and hosts. Symp. Soc. Gen. Microbiol. **22**:415–435.
9. **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.
10. **Bang, F. B., and M. A. Foard.** 1969. Experimentally induced changes in nasal mucous se-

cretory systems and their effect on virus infection in chickens. II. Effects on adsorption of Newcastle disease virus. J. Exp. Med. **130**:121-140.

11. **Barb, K., and G. Takatsy.** 1972. Inhibitor sensitive and inhibitor resistant strains of influenza A_2 virus populations. Acta Microbiol. Acad. Sci. Hung. **19**:195-205.
12. **Barber, W. H., and P. A. Small.** 1977. Construction of an improved tracheal pouch in the ferret. Am. Rev. Respir. Dis. **115**:165-169.
13. **Barker, S. M., and L. Hoyle.** 1972. The nature of the toxic reaction of influenza virus towards lung tissue. J. Hyg. **70**:425-437.
14. **Baron, S., and A. Isaacs.** 1962. Absence of interferon in lungs from fatal cases of influenza. Br. Med. J. **1**:18-20.
15. **Basarab, O., and H. Smith.** 1969. Quantitative studies on the tissue localization of influenza virus in ferrets after intranasal and intravenous or intracardial inoculation. Br. J. Exp. Pathol. **50**:612-618.
16. **Basarab, O., and H. Smith.** 1970. Growth patterns of influenza virus in cultures of ferret organs. Br. J. Exp. Pathol. **51**:1-6.
17. **Baum, J., and R. G. Douglas.** 1979. The effect of immunization on polymorphonuclear leukocyte chemotaxis in influenza. Acta Virol. **23**: 433-436.
18. **Bean, W. J., and R. G. Webster.** 1978. Phenotypic properties associated with influenza genome segments, p. 685-692. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, Inc., London.
19. **Beare, A. S.** 1975. Live viruses for immunization against influenza. Prog. Med. Virol. **20**:49-83.
20. **Beare, A. S.** 1978. Recombinant influenza viruses from parents of known virulence for man, p. 745-755. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, Inc., London.
21. **Beare, A. S., and S. E. Reed.** 1977. The study of antiviral compounds in volunteers, p. 27-55. *In* J. S. Oxford (ed.), Chemoprophylaxis and virus infections of the respiratory tract, vol. 2. CRC Press Inc., Cleveland.
22. **Bernheim, H. A., L. H. Block, and E. Atkins.** 1979. Fever: pathogenesis, pathophysiology, and purpose. Ann. Intern. Med. **91**:261-270.
23. **Blaskovic, P., A. J. Rhodes, F. W. Doane, and N. A. Labzoffsky.** 1972. Infection of chick embryo tracheal organ cultures with influenza A_2 (Hong Kong) virus. II. Electron microscopy. Arch. Gesamte Virusforsch. **38**:250-266.
24. **Bodel, P. T., and J. W. Hollingsworth.** 1968. Pyrogen release from human synovial exudate cells. Br. J. Exp. Pathol. **49**:11-19.
25. **Bonser, R. S. A., B. H. Knight, and R. R. West.** 1978. Sudden infant death syndrome in Cardiff, association with epidemic influenza and with temperature, 1955-1974. Int. J. Epidemiol. **7**:335-340.
26. **Bosch, F. X., A. J. Hay, and J. J. Skehel.** 1978. RNA and protein synthesis in a permissive and an abortive influenza virus infection, p. 465-474. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, Inc., London.
27. **Bosch, F. X., M. Orlich, H.-D. Klenk, and R. Rott.** 1979. The structure of the hemagglutinin, a determinant for the pathogenicity of influenza viruses. Virology **95**:197-207.
28. **Brightman, I. J.** 1935. Streptococcus infection occuring in ferrets inoculated with human influenza virus. Yale J. Biol. Med. **8**:127-135.
29. **Briody, B. A., W. A. Cassel, and M. A. Medill.** 1955. Adaptation of influenza virus to mice. 3. Development of resistance to β inhibitor. J. Immunol. **74**:41-45.
30. **Brownson, J. M., B. W. J. Mahy, and B. L. Hazleman.** 1979. Interaction of influenza A virus with human peripheral blood lymphocytes. Infect. Immun. **25**:749-756.
31. **Bucher, D., and P. Palese.** 1975. The biologically active proteins of influenza virus: neuraminidase, p. 83-123. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
32. **Bukrinskaya, A. G., A. K. Gitelman, and V. B. Martynenko.** 1978. Abortive infection of influenza virus in Ehrlich Ascites tumor cells. Unusual fragility of virus particles. Arch. Virol. **56**:279-290.
33. **Burnet, F. M.** 1959. Genetic interactions between animal viruses, p. 275-306. *In* F. M. Burnet and W. M. Stanley (ed.), The viruses, vol. 3. Academic Press, Inc., London.
34. **Burrows, R.** 1972. Early stages of virus infection: studies *in vivo* and *in vitro*. Symp. Soc. Gen. Microbiol. **22**:303-332.
35. **Caliguiri, L. A., and K. V. Holmes.** 1979. Host-dependent restriction of influenza virus maturation. Virology **92**:15-30.
36. **Campbell, D., C. Sweet, and H. Smith.** 1979. Comparisons of virulence of influenza virus recombinants in ferrets in relation to their behaviour in man and their genetic constitution. J. Gen. Virol. **44**:37-44.
37. **Catalano, L. W., and J. L. Sever.** 1971. The role of viruses as causes of congenital defects. Annu. Rev. Microbiol. **25**:255-282.
38. **Cavanagh, D., M. H. Collie, C. Sweet, and H. Smith.** 1979. Production and release of influenza virus from fresh and maintained organ cultures of ferret neonatal lung. FEMS Microbiol. Lett. **5**:431-433.
39. **Cavanagh, D., F. Mitkis, C. Sweet, M. H. Collie, and H. Smith.** 1979. The localization of influenza virus in the respiratory tract of ferrets: susceptible nasal mucosa cells produce and release more virus than susceptible lung cells. J. Gen. Virol. **44**:505-514.
40. **Chanock, R. M., and B. R. Murphy.** 1979. Genetic approaches to control of influenza. Perspect. Biol. Med. **22**:S37-S48.
41. **Choppin, P. W., and R. W. Compans.** 1975. The structure of influenza virus, p. 15-51. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.

42. **Cole, B. C., P. S. Lombardi, J. C. Overall, and L. A. Glasgow.** 1978. Inhibition of interferon induction in mice by mycoplasmas and mycoplasmal fractions. Proc. Soc. Exp. Biol. Med. **157:**83-88.
43. **Collie, M. H., D. I. Rushton, C. Sweet, and H. Smith.** 1980. Studies of influenza virus infection in newborn ferrets. J. Med. Microbiol., in press.
44. **Collie, M. H., C. Sweet, D. Cavanagh, and H. Smith.** 1978. Association of foetal wastage with influenza infection during ferret pregnancy. Br. J. Exp. Pathol. **59:**190-195.
45. **Cranston, W. I., M. D. Rawlins, R. H. Luff, and G. W. Duff.** 1971. Relevance of experimental observations to pyrexia in clinical situations, p. 155-164. *In* G. E. W. Wolstenholme and J. Birch (ed.), Pyrogen and fever. A Ciba Foundation Symposium. Churchill Livingstone, London.
46. **Criswell, B. S., R. B. Couch, S. B. Greenberg, and S. L. Kimzey.** 1979. The lymphocyte response to influenza in humans. Am. Rev. Respir. Dis. **120:**700-704.
47. **Davenport, F. M.** 1961. Pathogenesis of influenza. Bacteriol. Rev. **25:**294-300.
48. **Davenport, F. M., A. V. Hennessy, H. F. Maassab, E. Minuse, L. C. Clark, G. D. Abrams, and J. R. Mitchell.** 1977. Pilot studies on recombinant cold-adapted live type A and B influenza virus vaccines. J. Infect. Dis. **136:**17-25.
49. **Davison, A. M., D. Thomson, and J. S. Robson.** 1973. Intravascular coagulation complicating influenza A virus infection. Br. Med. J. **1:**654-655.
50. **Dekhtyareva, N. I., T. E. Medvedeva, and G. I. Aleksandrova.** 1976. Interfering activity of virulent and attenuated influenza virus strains. Acta Virol. **20:**189-195.
51. **Denman, A. M., B. Rager-Zisman, T. C. Merigan, and D. A. J. Tyrrell.** 1974. Replication or inactivation of different viruses by human lymphocyte preparations. Infect. Immun. **9:** 373-376.
52. **Denny, F. W.** 1973. The damage of cilia by bacteria, p. 186-189. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. Sixth International Symposium on Aerobiology. Oosthoek Publishing Co., Utrecht.
53. **Doershuk, C. F., B. J. Fisher, and K. W. Matthews.** 1975. Pulmonary physiology of the young child, p. 166-182. *In* E. M. Scarpelli (ed.), Pulmonary physiology of the fetus, newborn and child. Lea and Febiger, Philadelphia.
54. **Dolin, R., D. D. Richman, B. R. Murphy, and A. S. Fauci.** 1977. Cell-mediated immune response in humans after induced infection with influenza A virus. J. Infect. Dis. **135:**714-719.
55. **Dolowy, W. C., and R. L. Muldoon.** 1964. Studies of germfree animals. I. Response of mice to infection with influenza A virus. Proc. Soc. Exp. Biol. Med. **116:**365-371.
56. **Douglas, R. G.** 1975. Influenza in man, p. 395-481. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
57. **Dourmashkin, R. R., and D. A. J. Tyrrell.** 1970. Attachment of two myxoviruses to ciliated epithelial cells. J. Gen. Virol. **9:**77-88.
58. **Dowdle, W. R.** 1976. Influenza: epidemic patterns and antigenic variation, p. 17-21. *In* P. Selby (ed.), Influenza: virus, vaccines and strategy. Proceedings of a working group on pandemic influenza, Rougemont, 26-28 January, 1976. Academic Press, Inc., London.
59. **Easterday, B. C.** 1975. Animal influenza, p. 449-481. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
60. **Ebisawa, I. T., O. Kitamoto, Y. Takeuchi, and M. Makino.** 1969. Immunocytologic study of nasal epithelial cells in influenza. Am. Rev. Respir. Dis. **99:**507-515.
61. **Evans, A. E. J., R. L. L. Simmons, R. D. Ferris, S. K. Hasell, E. Letley, and D. S. Freestone.** 1979. Antibody responses to vaccination with WRL 105 strain live influenza vaccine in previously vaccinated and unvaccinated volunteers. J. Hyg. **82:**489-495.
62. **Fazekas de St. Groth, S., and D. M. Graham.** 1954. Studies on experimental immunology of influenza. X. Passive immunity and its enhancement. Aust. J. Exp. Biol. Med. Sci. **32:** 369-386.
63. **Fenner, F., B. R. McAuslan, C. A. Mims, J. Sambrook, and D. O. White.** 1974. The biology of animal viruses, 2nd. ed. Academic Press, Inc., London.
64. **Fenton, R. J., R. Jennings, and C. W. Potter.** 1977. Differential response of ferrets to infection with virulent and avirulent influenza viruses: a possible marker of attenuation. Arch. Virol. **55:**55-66.
65. **Florent, G., M. Lobmann, A. S. Beare, and N. Zygraich.** 1977. RNA's of influenza virus recombinants derived from parents of known virulence for man. Arch. Virol. **54:**19-28.
66. **Franklin, R. M., and P. M. Breitenfeld.** 1959. The abortive infection of Earle's L cells by fowl plague virus. Virology **8:**293-307.
67. **Frankova, V.** 1975. Inhalatory infection of mice with influenza A_0/PR8 virus. I. The site of primary virus replication and its spread in the respiratory tract. Acta Virol. **19:**29-34.
68. **Frankova, V., and V. Rychterova.** 1975. Inhalatory infection of mice with influenza A_0/PR8 virus. II. Detection of virus in the blood and extrapulmonary organs. Acta Virol. **19:**35-40.
69. **Fraser, K. B.** 1967. Immunofluorescence of abortive and complete infections by influenza A virus in hamster BHK21 cells and mouse L cells. J. Gen. Virol. **1:**1-12.
70. **Frolov, A. F., A. M. Shcherbinskaya, N. A. Maksimovich, and L. V. Kuz'menkova.** 1978. Experimental influenza caused by an incomplete form of the virus. Bull. Exp. Biol. Med. **85:**475-476.

71. **Gander, G. W.** 1973. The source of pyrogen in viral fever, p. 472–473. *In* E. Schonbaum and P. Lomax (ed.), The pharmacology of thermoregulation. Proceedings of a Satellite Symposium Held in Conjunction with the Fifth International Congress on Pharmacology, San Francisco, July 23–28, 1972. S. Karger, Basel.
72. **Gandhi, S. S., H. B. Bell, and D. C. Burke.** 1971. Abortive infection of L cells by fowl plague virus: comparison of RNA and protein synthesis in infected chick and L cells. J. Gen. Virol. **13**:423–432.
73. **Gavrilov, V. I., D. M. Asher, S. D. Vyalushkina, L. S. Ratushkina, R. G. Zmieva, and B. G. Tumyan.** 1972. Persistent infection of continuous line of pig kidney cells with a variant of WSN strain of influenza A_0 virus. Proc. Soc. Exp. Biol. Med. **140**:109–117.
74. **Gerone, P. J., T. G. Ward, and W. A. Chappel.** 1957. Combined infections in mice with influenza virus and *Diplococcus pneumoniae*. Am. J. Hyg. **66**:331–341.
75. **Ghendon, Y., A. Klimov, O. Blagoveshenskaya, and D. Genkina.** 1979. Investigation of recombinants of human influenza and fowl plague viruses. J. Gen. Virol. **43**:183–191.
76. **Glover, R. E.** 1941. Spread of infection from the respiratory tract of the ferret. II. Association of influenza A virus and streptococcus group C. Br. J. Exp. Pathol. **22**:98–107.
77. **Gottschalk, A.** 1957. Neuraminidase: the specific enzyme of influenza virus and *Vibrio cholerae*. Biochim. Biophys. Acta **23**:645–646.
78. **Gould, E. A., N. A. Ratcliffe, O. Basarab, and H. Smith.** 1972. Studies on the basis of localization of influenza virus in ferret organ cultures. Br. J. Exp. Pathol. **53**:31–36.
79. **Green, G. M., G. J. Jakab, R. B. Low, and G. S. Davis.** 1977. Defence mechanisms of the respiratory membrane. Am. Rev. Respir. Dis. **115**:479–514.
80. **Gresser, I. G., and H. B. Dull.** 1964. A virus inhibitor in pharyngeal washings from patients with influenza. Proc. Soc. Exp. Biol. Med. **115**: 192–196.
81. **Gresser, I. G., M. G. Tovey, C. Maury and M. T. Bandu.** 1976. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with *Herpes simplex*, Moloney sarcoma, vesicular stomatitis, Newcastle disease and influenza viruses. J. Exp. Med. **144**:1316–1323.
82. **Hackemann, M. M. A., A. M. Denman, and D. A. J. Tyrrell.** 1974. Inactivation of influenza virus by human lymphocytes. Clin. Exp. Immunol. **16**:583–591.
83. **Haff, R. F., and C. A. Pinto.** 1973. The nasal decongestant action of aspirin in influenza infected ferrets. Life Sci. **12**:9–14.
84. **Haff, R. F., P. W. Schriver, C. G. Engle, and R. C. Stewart.** 1966. Pathogenesis of influenza in ferrets. I. Tissue and blood manifestations of disease. J. Immunol. **96**:659–667.
85. **Haff, R. F., P. W. Schriver, and R. C. Stewart.** 1966. Pathogenesis of influenza in ferrets: nasal manifestations of disease. Br. J. Exp. Pathol. **47**:435–444.
86. **Haller, O., H. Arnheiter, I. Gresser, and J. Lindenmann.** 1979. Genetically determined, interferon-dependent resistance to influenza in mice. J. Exp. Med. **149**:601–612.
87. **Hamre, D., J. Appel, and C. G. Loosli.** 1956. Viremia in mice with pulmonary influenza A virus infections. J. Lab. Clin. Med. **47**:182–193.
88. **Hanson, R. J., J. E. Kempf, and A. V. Boand.** 1957. Phagocytosis of influenza virus. II. Its occurrence in normal and immune mice. J. Immunol. **79**:422–427.
89. **Hara, K., A. S. Beare, and D. A. J. Tyrrell.** 1974. Growth and pathogenicity of influenza viruses in organ cultures of ciliated epithelium. I. Experiments in ferret and human. Arch. Gesamte Virusforsch. **44**:227–236.
90. **Harford, C. G., and M. Hara.** 1950. Pulmonary edema in influenza pneumonia of the mouse and the relation of fluid in the lung to the inception of pneumococcal pneumonia. J. Exp. Med. **91**:245–259.
91. **Harms, E., W. Rohde, F. Bosch, and C. Scholtissek.** 1978. Biochemical studies on influenza viruses. II. Assignment of gene functions to RNA segments 5, 7 and 8 of fowl plague virus and virus N. Virology **86**:413–422.
92. **Hay, A. J., A. R. Bellamy, G. Abraham, J. J. Skehel, C. M. Brand, and R. G. Webster.** 1977. Procedures for characterization of the genetic material of candidate vaccine strains. *In* International Symposium on Influenza Immunization, vol. II, Geneva 1977. Developments in Biological Standardization **39**:15–24. S. Karger, Basel.
93. **Herbst-Laier, R.** 1970. Organ cultures in evaluation of antiviral drugs. I. Virus infection in trachea explants as potential model systems for evaluation of antiviral drugs against respiratory viruses. Arch. Gesamte Virusforsch. **30**: 379–396.
94. **Hirst, G. K.** 1947. Studies on the mechanism of adaption of influenza virus to mice. J. Exp. Med. **86**:357–366.
95. **Hobson D., and E. A. Gould.** 1969. Laboratory markers of virulence in influenza viruses. Prog. Immunobiol. Stand. **3**:70–72.
96. **Holland, J. J., and M. Doyle.** 1973. Attempts to detect homologous autointerference in vivo with influenza virus and vesicular stomatitis virus. Infect. Immun. **7**:526–531.
97. **Holland, J. J., E. A. Grabau, C. L. Jones, and B. L. Semler.** 1979. Evolution of multiple genome mutations during long-term persistent infection by vesicular stomatitis virus. Cell **16**: 495–504.
98. **Hoorn, B., and D. A. J. Tyrrell.** 1969. Organ cultures in virology. Prog. Med. Virol. **11**:408–450.
99. **Hope-Simpson, R. E.** 1979. Epidemic mechanisms of type A influenza. J. Hyg. **83**:11–26.
100. **Hoyle, L.** 1968. The influenza viruses. Springer-Verlag, New York.

101. **Huang, A. S.** 1973. Defective interfering viruses. Annu. Rev. Microbiol. **27**:101-117.
102. **Hurd, J., and R. B. Heath.** 1975. Effect of cyclophosphamide on infections in mice caused by virulent and avirulent strains of influenza virus. Infect. Immun. **11**:886-889.
103. **Iida, T., and F. B. Bang.** 1963. Infection of the upper respiratory tract of mice with influenza A. Am. J. Hyg. **77**:169-176.
104. **Inglis, S. C., T. Barrett, C. M. Brown, and J. W. Almond.** 1979. The smallest genome RNA segment of influenza virus contains 2 genes that may overlap. Proc. Natl. Acad. Sci. U.S.A. **76**:3790-3794.
105. **Ishida, N., T. Morizuka, and Y. Hinuma.** 1964. Virus antigens in kidneys of mice infected with influenza A. Nature (London) **201**:421-422.
106. **Israel, A., A. Niveleau, G. Quash, and M.-H. Richard.** 1979. Latex fetuin spheres as probes for influenza virus neuraminidase in productively and abortively infected cells. Arch. Virol. **61**:183-199.
107. **Jakab, G. J., G. A. Warr, and M. E. Knight.** 1979. Pulmonary and systemic defences against challenge with *Staphylococcus aureus* in mice with pneumonia due to influenza A virus. J. Infect. Dis. **140**:105-108.
108. **Johnson, K. P., R. Klasnja, and R. T. Johnson.** 1971. Neural tube defects of chick embryos: an indirect result of influenza A virus infection. J. Neuropathol. Exp. Neurol. **30**:68-74.
109. **Kanoh, S., and H. Kawasaki.** 1966. Studies on myxovirus pryogen. I. Interaction of myxovirus and rabbit polymorphonuclear leucocytes. Biken J. **9**:177-184.
110. **Kantzler, G. B., S. F. Lauteria, C. L. Cusumano, J. D. Lee, R. Ganguly, and R. H. Waldman.** 1974. Immunosuppression during influenza virus infection. Infect. Immun. **10**: 996-1002.
111. **Kauffman, C. A., C. C. Linnemann, G. M. Schiff, and J. P. Phair.** 1976. Effect of viral and bacterial pneumonia on cell-mediated immunity in humans. Infect. Immun. **13**:78-83.
112. **Kauffman, C. A., G. M. Schiff, and J. P. Phair.** 1978. Influenza in ferrets and guinea-pigs: effect on cell-mediated immunity. Infect. Immun. **19**: 547-552.
113. **Kawasaki, H., and S. Kanoh.** 1974. Studies on the myxovirus pyrogen. III. Comparative studies on the leucocytic pyrogens induced by influenza virus and bacterial pyrogen *in vitro*. Virus (Tokyo) **24**:51-56.
114. **Kilbourne, E. D.** 1975. Epidemiology of influenza, p. 483-538. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
115. **Kilbourne, E. D., R. M. Chanock, P. W. Choppin, F. M. Davenport, J. P. Fox, M. B. Gregg, G. G. Jackson, and P. D. Parkman.** 1974. Influenza vaccines—summary of influenza workshop V. J. Infect. Dis. **129**:750-771.
116. **Kilbourne, E. D., S. McGregor, and B. C. Easterday.** 1979. Hemagglutinin mutants of swine influenza virus differing in replication characteristics in their natural host. Infect. Immun. **26**:197-201.
117. **Kilham, L., and G. Margolis.** 1973. Pathogenesis of intrauterine infection in rats due to reovirus type 3. I. Virologic studies. Lab. Invest. **28**:597-604.
118. **Kingsman, S. M., C. Sweet, G. L. Toms, and H. Smith.** 1977. The differential susceptibilities to infection with influenza virus of fresh and maintained organ cultures of ferret urogenital, foetal and neonatal tissues. FEMS Microbiol. Lett. **2**:251-253.
119. **Kingsman, S. M., G. L. Toms, and H. Smith.** 1977. The localization of influenza virus in the respiratory tract of ferrets: the differential susceptibilities of fresh and maintained organ cultures. J. Gen. Virol. **37**:249-258.
120. **Kingsman, S. M., G. L. Toms, and H. Smith.** 1977. The localization of influenza virus in the respiratory tract of ferrets: the importance of early replication events in determining tissue specificity. J. Gen. Virol. **37**:259-270.
121. **Kleinerman, E. S., C. A. Daniels, R. P. Polisson, and R. Snyderman.** 1976. Effect of virus infection on the inflammatory response. Depression of macrophage accumulation in influenza infected mice. Am. J. Pathol. **85**:373-382.
122. **Kleinerman, E. S., R. Snyderman, and C. A. Daniels.** 1974. Depression of human monocyte chemotaxis by *Herpes simplex* and influenza viruses. J. Immunol. **113**:1562-1567.
123. **Kleinerman, E. S., R. Snyderman, and C. A. Daniels.** 1975. Depressed monocyte chemotaxis during acute influenza infection. Lancet **ii**:1063-1066.
124. **Klenk, H.-D., R. Rott, and M. Orlich.** 1977. Further studies on the activation of influenza virus by proteolytic cleavage of the haemagglutinin. J. Gen. Virol. **36**:151-161.
125. **Klenk, H.-D., R. Rott, M. Orlich, and J. Blodorn.** 1975. Activation of influenza A viruses by trypsin treatment. Virology **68**:426-439.
126. **Knight, V.** 1973. Airborne transmission and pulmonary deposition of respiratory viruses, p. 175-182. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. Sixth International Symposium on Aerobiology. Oostehoek Publishing Co., Utrecht.
127. **Kosel, B., and H. Kohlhage.** 1970. Effects of actinomycin on *in vivo* and *in vitro* formation of endogenous rabbit pyrogen induced with myxoviruses or stimulated with endotoxin. Arch. Gesamte Virusforsch. **32**:209-220.
128. **Krizanova, O., and V. Rathova.** 1969. Serum inhibitors of myxoviruses. Curr. Top. Microbiol. Immunol. **47**:125-151.
129. **Laraya-Cuasay, L. R., A. Deforest, D. Huff, H. Lischner, and N. N. Huang.** 1977. Chronic pulmonary complications of early influenza virus infection in children. Am. Rev. Respir. Dis. **116**:617-625.
130. **Larson, H. E., R. P. Parry, C. Gilchrist, A.**

Luquetti, and D. A. J. Tyrrell. 1977. Influenza viruses and staphylococci *in vitro*: some interactions with polymorphonuclear leucocytes and epithelial cells. Br. J. Exp. Pathol. **58**:281-288.

131. **Laver, W. G., and R. G. Webster.** 1979. Ecology of influenza viruses in lower mammals and birds. Br. Med. Bull. **35**:29-33.
132. **Lazarowitz, S. G., R. W. Compans, and P. W. Choppin.** 1973. Proteolytic cleavage of the hemagglutinin polypeptide of influenza virus. Function of the uncleaved polypeptide HA. Virology **52**:199-212.
133. **Lerner, R. A., and L. D. Hodge.** 1969. Nonpermissive infections of mammalian cells: synthesis of influenza virus genome in HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **64**:544-551.
134. **Lindenmann, J., E. Deuel, S. Fanconi, and O. Haller.** 1978. Inborn resistence of mice to myxoviruses: macrophages express phenotype *in vitro*. J. Exp. Med. **147**:531-540.
135. **Liu, C.** 1955. Studies on influenza infection in ferrets by means of fluorescein-labelled antibody. I. The pathogenesis and diagnosis of the disease. J. Exp. Med. **101**:665-676.
136. **Liu, C.** 1961. Diagnosis of influenza infection by means of fluorescent antibody staining. Am. Rev. Respir. Dis. **83**:130-133.
137. **Loffler, H., G. Henle, and W. Henle.** 1962. Attempts to influence the incomplete cycle of influenza virus in HeLa cells by antibodies. J. Immunol. **88**:763-776.
138. **Long, W. F., and D. C. Burke.** 1970. The effect of infection with fowl plague virus on protein synthesis in chick embryo cells. J. Gen. Virol. **6**:1-14.
139. **Loosli, C. G.** 1949. The pathogenesis and pathology of experimental air-borne influenza virus A infections in mice. J. Infect. Dis. **84**:153-168.
140. **Loosli, C. G., M. S. Hertweck, and R. S. Hockwald.** 1970. Airborne influenza PR8-A virus infections in actively immunized mice. Arch. Environ. Health **21**:332-346.
141. **Louria, D. B., H. L. Blumenfeld, J. T. Ellis, E. D. Kilbourne, and D. E. Rogers.** 1959. Studies on influenza in the pandemic of 1957-58. II. Pulmonary complications of influenza. J. Clin. Invest. **38**:213-265.
142. **Lwoff, A.** 1969. Death and transfiguration of a problem. Bacteriol. Rev. **33**:390-403.
143. **Mackenzie, J. S., and M. Houghton.** 1974. Influenza infections during pregnancy: association with congenital malformations and with subsequent neoplasms in children, and potential hazards of live virus vaccines. Bacteriol. Rev. **38**:356-370.
144. **Malluci, L., and A. C. Allison.** 1965. Lysosomal enzymes in cells infected with cytopathic and non-cytopathic viruses. J. Exp. Med. **121**:477-485.
145. **Markoff, L. J., F. Thierry, B. R. Murphy, and R. M. Chanock.** 1979. Relationship of genotype of recombinants of influenza A/Hong Kong/68-*ts*-1[E] virus used as live virus vaccines to virulence in humans. Infect. Immun. **26**:280-286.
146. **Martin, C. M., C. M. Cunin, L. S. Gottlieb, M. W. Barnes, C. Liu, and M. Finland.** 1959. Asian influenza A in Boston 1957-58. I. Observations in thirty-two influenza-associated fatal cases. Arch. Intern. Med. **103**:515-531.
147. **Massanari, R. M.** 1979. Suppression of tuberculin hypersensitivity during influenza infection in mice. Infect. Immun. **24**:501-507.
148. **Matsuyama, T., C. Sweet, M. H. Collie, and H. Smith.** 1980. Different aspects of virulence in ferrets exhibited by influenza virus recombinants of known genetic constitution. J. Infect. Dis. **141**:351-361.
149. **Mayer, V., J. L. Schulman, and E. D. Kilbourne.** 1973. Nonlinkage of neurovirulence exclusively to viral hemagglutinin or neuraminidase in genetic recombinants of A/NWS(HON1) influenza virus. J. Virol. **11**:272-278.
150. **McCahon, D., and G. C. Schild.** 1972. Segregation of antigenic and biological characteristics during influenza virus recombination. J. Gen. Virol. **15**:73-77.
151. **McGeoch, D., P. Fellner, and C. Newton.** 1976. Influenza virus genome consists of eight distinct RNA species. Proc. Natl. Acad. Sci. U.S.A. **73**:3045-3049.
152. **McLaren, C., and C. W. Potter.** 1973. The relationship between interferon and virus virulence in influenza virus infections of the mouse. J. Med. Microbiol. **6**:21-32.
153. **Mims, C. A.** 1964. Aspects of the pathogenesis of virus diseases. Bacteriol. Rev. **28**:30-71.
154. **Mims, C. A.** 1972. Host defences against viruses and the latter's ability to counteract them. Symp. Soc. Gen. Microbiol. **22**:333-358.
155. **Mims, C. A.** 1976. The pathogenesis of influenza, p. 95-105. *In* P. Selby (ed.), Influenza: virus, vaccines, and strategy. Proceedings of a Working Group on Pandemic Influenza, Rougement, 26-28 January 1976. Academic Press, Inc., London.
156. **Mims, C. A.** 1977. The pathogenesis of infectious disease. Academic Press, Inc., London.
157. **Molnarova, A., and D. Blaskovic.** 1975. The influence of influenza virus infection of pregnant mice on the development of their foetuses. Acta Virol. **19**:259.
158. **Mostow, S. R., S. Flatauer, M. Paler, and B. R. Murphy.** 1977. Temperature-sensitive mutants of influenza virus. XIII. Evaluation of influenza A/Hong Kong/68 and A/Udorn/72 *ts* and wild-type viruses in tracheal organ culture at permissive and restrictive temperatures. J. Infect. Dis. **136**:1-6.
159. **Mostow, S. R., J. A. Hopkins, and P. F. Wright.** 1979. Behaviour of vaccine revertants of temperature-sensitive mutants of influenza virus in ferret tracheal organ cultures. Infect. Immun. **26**:193-196.
160. **Mostow, S. R., and D. A. J. Tyrrell.** 1973. The behaviour *in vitro* of attenuated recombinant influenza viruses. Arch. Gesamte Virusforsch.

43:385-392.

161. **Mulder, J., and J. F. Ph. Hers.** 1972. Influenza. Wolters-Noordhoff, Groningen.

162. **Murphy, B. R., S. B. Spring, and R. M. Chanock.** 1976. Live-vaccine: production and use, p. 179-197. *In* P. Selby (ed.), Influenza: virus, vaccines, and strategy. Proceedings of a Working Group on Pandemic Influenza, Rougemont, 26-28 January 1976. Academic Press, Inc., London.

163. **Nayak, D. P., G. W. Kelley, G. A. Young, and N. R. Underdahl.** 1964. Progressive descending influenza infection in mice determined by immunofluorescence. Proc. Soc. Exp. Biol. Med. **116**:200-206.

164. **Nelson, K. E., M. A. Greenberg, M. A. Mufson, and V. K. Moses.** 1975. The sudden infant death syndrome and epidemic viral disease. Am. J. Epidemiol. **101**:423-430.

165. **Oxford, J. S., D. J. McGeoch, G. C. Schild, and A. S. Beare.** 1978. Analysis of virion RNA segments and polypeptides of influenza A virus recombinants of defined virulence. Nature (London) **273**:778-779.

166. **Paisley, J. W., F. W. Bruhn, B. A. Laver, and K. McIntosh.** 1978. Type A_2 influenza viral infections in children. Am. J. Dis. Child. **132**: 34-37.

167. **Palese, P., M. B. Ritchey, and J. L. Schulman.** 1977. Mapping of the influenza virus genome. II. Identification of the P1, P2 and P3 genes. Virology **76**:114-121.

168. **Palese, P., and J. L. Schulman.** 1976. Differences in RNA patterns of influenza A viruses. J. Virol. **17**:876-884.

169. **Papkoff, H.** 1966. Glycoproteins with biological activity, p. 532. *In* A. Gottschalk (ed.), Glycoproteins. Elsevier, Amsterdam.

170. **Patterson, S., J. S. Oxford, and R. R. Dourmashkin.** 1979. Studies on the mechanisms of influenza virus entry into cells. J. Gen. Virol. **43**:223-229.

171. **Pereira, M. S.** 1979. Global surveillance of influenza. Br. Med. Bull. **35**:9-14.

172. **Pinto, C. A., R. F. Haff, and R. C. Stewart.** 1969. Pathogenesis of and recovery from Respiratory Syncytial and influenza infections in ferrets. Arch. Gesamte Virusforsch. **26**:225-237.

173. **Polezhaev, F. I., G. S. Makariev, and G. I. Aleksandrova.** 1974. Interferon-inducing ability in human leukocyte cultures of influenza viruses of different virulence. Acta Virol. **18**: 362.

174. **Pons, M. W.** 1975. Influenza virus RNA(s), p. 145-170. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.

175. **Potter, C. W., and J. S. Oxford.** 1977. Animal models of influenza virus infection as applied to the investigation of antiviral compounds, p. 1-26. *In* J. S. Oxford (ed.), Chemoprophylaxis and virus infections of the respiratory tract, vol. II. CRC Press, Inc., Cleveland.

176. **Potter, C. W., and J. S. Oxford.** 1979. Determinants of immunity to influenza in man. Br. Med. Bull. **35**:69-75.

177. **Potter, C. W., S. L. Shore, and C. McLaren.** 1972. Immunity to influenza in ferrets. III. Proteins in nasal secretions. J. Infect. Dis. **126**: 387-393.

178. **Rabinowitz, S. G., and J. Hupriker.** 1979. The influence of defective-interfering particles of the PR-8 strain of influenza A virus on the pathogenesis of pulmonary infection in mice. J. Infect. Dis. **140**:305-315.

179. **Raut, S., J. Hurd, R.-J. Cureton, G. Blandford, and R. B. Heath.** 1975. The pathogenesis of infections of the mouse caused by virulent and avirulent variants of an influenza virus. J. Med. Microbiol. **8**:127-136.

180. **Rawlins, M. D., and W. I. Cranston.** 1973. Clinical studies on the pathogenesis of fever, p. 264-277. *In* E. Schonbaum and P. Lomax (ed.), The pharmacology of thermoregulation. Proceedings of a Satellite Symposium Held in Conjunction with the Fifth International Congress on Pharmacology, San Francisco, July 23-28, 1972. S. Karger, Basel.

181. **Reed, S. E.** 1969. Persistent respiratory virus infection in tracheal organ culture. Br. J. Exp. Pathol. **50**:378-388.

182. **Reed, S. E., and P. S. Nolan.** 1973. The cytopathology of infection with parainfluenza 3 virus in organ cultures of ciliated epithelium, and the effect of treatment with antiserum, p. 193-196. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. Sixth International Symposium on Aerobiology. Oosthoek Publishing Co., Utrecht.

183. **Richman, D. D., B. R. Murphy, S. Baron, and C. Uhlendorf.** 1976. Three strains of influenza A virus (H_3N_2): interferon sensitivity in vitro and interferon production in volunteers. J. Clin. Microbiol. **3**:223-226.

184. **Roberts, N. J.** 1979. Temperature and host defense. Microbiol. Rev. **43**:241-259.

185. **Rooney, A. S.** 1976. Function of type II cell lamellar inclusion in surfactant production, p. 147-152. *In* A. Bouhuys (ed.), Lung cells in disease. North-Holland, Amsterdam.

186. **Rosztoczy, I., C. Sweet, G. L. Toms, and H. Smith.** 1975. Replication of influenza virus in organ cultures of human and simian urogenital tissues and human foetal tissues. Br. J. Exp. Pathol. **56**:322-328.

187. **Rott, R.** 1977. The structural basis of the function of influenza virus glycoproteins. Med. Microbiol. Immunol. **164**:23-33.

188. **Rott, R.** 1979. Molecular basis of infectivity and pathogenicity of myxovirus. Arch. Virol. **59**: 285-298.

189. **Rott, R., and H.-D. Klenk.** 1977. Structure and assembly of viral envelopes, p. 47-81. *In* G. Poste and G. L. Nicolson (ed.), Virus infections and the cell surface. Cell surface reviews, vol. 2. Elsevier, Amsterdam.

190. **Rott, R., M. Orlich, and C. Scholtissek.** 1976. Attenuation of pathogenicity of fowl plague virus by recombination with other influenza A

viruses nonpathogenic for fowl: nonexclusive dependence of pathogenicity on hemagglutinin and neuraminidase of the virus. J. Virol. **19:** 54–60.

191. **Rott, R., M. Orlich, and C. Scholtissek.** 1979. Correlation of pathogenicity and gene constellation of influenza A viruses. III. Non-pathogenic recombinants derived from highly pathogenic parent strains. J. Gen. Virol. **44:**471–477.
192. **Rott, R., C. Scholtissek, H.-D. Klenk, and M. Orlich.** 1978. Structure and pathogenicity of influenza viruses, p. 653–662. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, Inc., London.
193. **Rudenko, L. G., N. Ya. Zoshchenkova, T. I. Tunkun, M. G. Shamanova, M. P. Zykov, and P. D. Starshov.** 1978. T lymphocytes as an indicator in influenza vaccination, influenza and acute respiratory diseases. Acta Virol. **22:** 167–169.
194. **Ruutu, P.** 1977. Depression of rat neutrophil exudation and motility by influenza virus. Scand. J. Immunol. **6:**1113–1120.
195. **Ruutu, P., A. Vaheri, and T. U. Kosunen.** 1977. Depression of human neutrophil motility by influenza virus *in vitro*. Scand. J. Immunol. **6:**897–906.
196. **Sawyer, W. D.** 1969. Interaction of influenza virus with leukocytes and its effect on phagocytosis. J. Infect. Dis. **119:**541–556.
197. **Schiff, L. J.** 1974. Studies on the mechanisms of influenza virus infection in hamster trachea organ culture. Correlation of reduced viral adsorption with age-related resistance to infection. Arch. Gesamte Virusforsch. **44:**195–204.
198. **Schlesinger, J. J., C. Ernst, and L. Weinstein.** 1976. Inhibition of human neutrophil chemotaxis by influenza virus. Lancet **i:**650–651.
199. **Scholtissek, C., H. Becht, and R. Drzeniek.** 1967. Biochemical studies on the cytopathic effect of influenza viruses. J. Gen. Virol. **1:**219–225.
200. **Scholtissek, C., and H.-D. Klenk.** 1975. Influenza virus replication, p. 215–242. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
201. **Scholtissek, C., and B. R. Murphy.** 1978. Host range mutants of an influenza A virus. Arch. Virol. **58:**323–333.
202. **Scholtissek, C., R. Rott, M. Orlich, E. Harms, and W. Rohde.** 1977. Correlation of pathogenicity and gene constellation of an influenza virus (fowl plague). I. Exchange of a single gene. Virology **81:**74–80.
203. **Scholtissek, C., A. Vallbracht, B. Flehmig, and R. Rott.** 1979. Correlation of pathogenicity and gene constellation of influenza A viruses. II. Highly neurovirulent recombinants derived from non-neurovirulent or weakly neurovirulent parent virus strains. Virology **95:** 492–500.
204. **Schulman, J. L.** 1975. Immunology of influenza, p. 373–393. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
205. **Schulman, J. L., and P. Palese.** 1977. Virulence factors of influenza A viruses: WSN virus neuraminidase required for plaque production in MDBK cells. J. Virol. **24:**170–176.
206. **Schulman, J. L., and P. Palese.** 1978. Biological properties of recombinants of influenza A/Hong Kong and A/PR8 viruses: effects of genes for matrix protein and nucleoprotein on virus yield in embryonated eggs, p. 663–674. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, Inc., London.
207. **Schulze, I. T.** 1975. The biologically active proteins of influenza virus: the hemagglutinin, p. 53–82. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
208. **Sellers, T. F., J. Schulman, C. Bouvier, R. McCune, and E. D. Kilbourne.** 1961. The influence of influenza virus infection on exogenous staphylococcal and endogenous murine bacterial infection of the bronchopulmonary tissues of mice. J. Exp. Med. **114:**237–256.
209. **Shayegani, M., F. S. Lief, and S. Mudd.** 1974. Specific and nonspecific cell-mediated resistance to influenza virus in mice. Infect. Immun. **9:**991–998.
210. **Siegert, R., and P. Braune.** 1964. Pyrogens of myxoviruses. II. Resistance of influenza A pyrogens to heat, ultraviolet and chemical treatment. Virology **24:**218–224.
211. **Siem, R. A., H. Ly, D. T. Imagawa, and J. M. Adams.** 1960. Influenza virus infection in pregnant mice. J. Neuropathol. Exp. Neurol. **19:** 125–129.
212. **Simpson, R. W., and W. J. Bean.** 1975. The biologically active proteins of influenza virus: influenza transcriptase activity of cells and virions, p. 125–143. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
213. **Small, P. A., R. H. Waldman, J. C. Bruno, and G. E. Gifford.** 1976. Influenza infection in ferrets: role of serum antibody in protection and recovery. Infect. Immun. **13:**417–424.
214. **Smith, H.** 1972. Mechanisms of virus pathogenicity. Bacteriol. Rev. **36:**291–310.
215. **Smith, H.** 1977. Host and tissue specificities in virus infections of animals, p. 1–49. *In* G. Poste and G. L. Nicolson (ed.), Virus infection and the cell surface. Cell surface reviews, vol. 2. Elsevier, Amsterdam.
216. **Smith, H.** 1978. The determinants of microbial pathogenicity, p. 13/1–13/32. *In* J. R. Norris and M. H. Richmond (ed.), Essays in microbiology. John Wiley and Son, Chichester.
217. **Smith, H.** 1980. The little-known determinants of virus pathogenicity. Scand. J. Infect. Dis., in press.
218. **Smith, W., C. H. Andrewes, and P. P. Laidlaw.** 1933. A virus obtained from influenza patients. Lancet **ii:**66–68.
219. **Smorodintseff, A. A., and S. M. Ostrovskaya.**

1937. The distribution of influenza virus in experimentally infected mice. J. Pathol. Bacteriol. 44:559-566.

220. **Stringfellow, D. A., E. R. Kern, D. K. Kelsey, and L. A. Glasgow.** 1977. Suppressed response to interferon induction in mice infected with encephalomyocarditis virus, Semliki Forest virus, influenza A_2 virus, *Herpesvirus hominis* type 2, or murine cytomegalovirus. J. Infect. Dis. 135:540-551.
221. **Stuart-Harris, C. H.** 1965. Influenza and other virus infections of the respiratory tract. Edward Arnold (Publisher) Ltd., London.
222. **Stuart-Harris, C. H.** 1979. Epidemiology of influenza in man. Br. Med. Bull. 35:3-8.
223. **Stuart-Harris, C. H., and G. C. Schild.** 1976. Influenza. The viruses and the disease. Edward Arnold (Publisher) Ltd., London.
224. **Sullivan, J. L., R. E. Mayner, D. W. Barry, and F. A. Ennis.** 1976. Influenza virus infection in nude mice. J. Infect. Dis. 133:91-94.
225. **Suzuki, F., N. Ishida, M. Suzuki, T. Sato, and S. Suzuki.** 1975. Effect of the interferon inducer, dextran phosphate, on influenza virus infection in mice. Proc. Soc. Exp. Biol. Med. 149:1069-1075.
226. **Suzuki, F., J. Ohya, and N. Ishida.** 1974. Effect of anti-lymphocyte serum on influenza virus infection in mice. Proc. Soc. Exp. Biol. Med. 146:78-84.
227. **Sweet, C., R. A. Bird, D. Cavanagh, G. L. Toms, M. H. Collie, and H. Smith.** 1979. The local origin of the febrile response induced in ferrets during respiratory infection with a virulent influenza virus. Br. J. Exp. Pathol. 60: 300-308.
228. **Sweet, C., R. A. Bird, G. L. Toms, C. G. Woodward, and H. Smith.** 1977. Thermal stability and interaction with ferret inflammatory exudates of two clones of influenza virus of differing virulence for both ferrets and man. Br. J. Exp. Pathol. 58:635-643.
229. **Sweet, C., D. Cavanagh, M. H. Collie, and H. Smith.** 1978. Sensitivity to pyrexial temperatures: a factor contributing to virulence differences between two clones of influenza virus. Br. J. Exp. Pathol. 59:373-380.
230. **Sweet, C., D. Cavanagh, M. H. Collie, and H. Smith.** 1978. Yields of a virulent and an attenuated clone of influenza virus from organ cultures of ferret nasal tissue: divergence with time of incubation. FEMS Microbiol. Lett. 4: 191-194.
231. **Sweet, C., M. H. Collie, G. L. Toms, and H. Smith.** 1977. The pregnant guinea-pig as a model for studying influenza virus infection *in utero*: infection of foetal tissues in organ culture and *in vivo*. Br. J. Exp. Pathol. 58:133-139.
232. **Sweet, C., J. Stephen, and H. Smith.** 1974. Purification of influenza viruses using disulphide-linked immunosorbents derived from rabbit antibody. Immunochemistry 11:295-304.
233. **Sweet, C., G. L. Toms, and H. Smith.** 1977. The pregnant ferret as a model for studying the congenital effects of influenza virus infection *in utero*: infection of foetal tissues in organ culture and *in vivo*. Br. J. Exp. Pathol. 58:113-123.
234. **Swift, P. G. F., and J. L. Emery.** 1973. Clinical observation on response to nasal occlusion in infancy. Arch. Dis. Child. 48:947-951.
235. **Takeyama, T.** 1966. Virological studies on experimental infection of pregnant mice with influenza virus. Tohoku J. Exp. Med. 89:321-340.
236. **Tateno, I., S. Suzuki, S. Nakamura, and A. Kawamura.** 1965. Rapid diagnosis of influenza by means of fluorescent antibody technic. I. Some basic informations. Jpn. J. Exp. Med. 35:383-400.
237. **Taylor-Robinson, D.** 1973. Damage of ciliated cells by mycoplasmas, p. 196-200. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. Sixth International Symposium on Aerobiology. Oosthoek Publishing Co., Utrecht.
238. **Toms, G. L., R. A. Bird, S. M. Kingsman, C. Sweet, and H. Smith.** 1976. The behaviour in ferrets of two closely related clones of influenza virus of differing virulence for man. Br. J. Exp. Pathol. 57:37-48.
239. **Toms, G. L., J. A. Davies, C. G. Woodward, C. Sweet, and H. Smith.** 1977. The relation of pyrexia and nasal inflammatory response to virus levels in nasal washings of ferrets infected with influenza viruses of differing virulence. Br. J. Exp. Pathol. 58:444-458.
240. **Toms, G. L., I. Rosztoczy, and H. Smith.** 1974. The localization of influenza virus: minimal infectious dose determinations and single cycle kinetic studies on organ cultures of respiratory and other ferret tissues. Br. J. Exp. Pathol. 55: 116-129.
241. **Toms, G. L., C. Sweet, and H. Smith.** 1977. Behaviour in ferrets of swine influenza virus isolated from man. Lancet i:68-71.
242. **Tyrrell, D. A. J.** 1959. Interferon produced by cultures of calf kidney cells. Nature (London) 184:452-453.
243. **Tyrrell, D. A. J.** 1973. Cilia in infection, p. 183-185. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. Sixth International Symposium on Aerobiology. Oosthoek Publishing Co., Utrecht.
244. **Tyrrell, D. A. J.** 1979. Viral pathogenesis and virulence. Nature (London) 280:194.
245. **Tyrrell, D. A. J., and J. W. G. Smith.** 1979. Vaccination against influenza A. Br. Med. Bull. 35:77-85.
246. **Vallbracht, A., B. Flehmig, and H.-J. Gerth.** 1979. Influenza virus: appearance of high mouse-neurovirulent recombinants. Intervirology 11:16-22.
247. **Virelizier, J. L., A. C. Allison, and G. C. Schild.** 1979. Immune responses to influenza virus in the mouse, and their role in control of the infection. Br. Med. Bull. 35:65-68.
248. **von Magnus, P.** 1951. Propagation of the PR-8 strain of influenza A virus in chick embryos. II.

The formation of "incomplete" virus following inoculation of large doses of seed virus. Acta Pathol. Microbiol. Scand. **28**:278-293.

249. **von Magnus, P.** 1951. Propagation of the PR-8 strain of influenza A virus in chick embryos. III. Properties of the incomplete virus produced in serial passages of undiluted virus. Acta Pathol. Microbiol. Scand. **29**:157-181.
250. **Walsh, J. J., L. F. Dietlein, F. N. Low, G. E. Burch, and W. J. Mogabgab.** 1961. Bronchotracheal response in human influenza. Arch. Int. Med. **108**:376-388.
251. **Warr, G. A., G. J. Jakab, T. W. Chan, and M. F. Tsan.** 1979. Effects of viral pneumonia on lung macrophage lysosomal enzymes. Infect. Immun. **24**:577-579.
252. **Warshauer, D., E. Goldstein, T. Akers, W. Lippert, and M. Kim.** 1977. Effect of influenza viral infection on the ingestion and killing of bacteria by alveolar macrophages. Am. Rev. Respir. Dis. **115**:269-277.
253. **Webb, H. E., and J. G. Hall.** 1972. An assessment of the role of the allergic response in the pathogenesis of viral diseases. Symp. Soc. Gen. Microbiol. **22**:383-414.
254. **Webster, R. G., V. S. Hinshaw, and W. J. Bean.** 1977. Antigenic shift in myxoviruses. Med. Microbiol. Immunol. **164**:57-68.
255. **Webster, R. G., and W. G. Laver.** 1975. Antigenic variation of influenza viruses, p. 269-314. *In* E. D. Kilbourne (ed.), The influenza viruses and influenza. Academic Press, Inc., London.
256. **Wells, M. A., P. Albrecht, S. Daniel, and F. A. Ennis.** 1978. Host defence mechanisms against influenza virus: interaction of influenza virus with murine macrophages *in vitro*. Infect. Immun. **22**:758-762.
257. **Wells, M. A., F. A. Ennis, and S. Daniel.** 1979. Cytotoxic T-cell and antibody response to influenza infection of mice. J. Gen. Virol. **43**:685-690.
258. **Westerberg, S. C., C. B. Smith, B. B. Wiley, and C. G. Jensen.** 1972. Pathogenesis of influenza virus infection in mouse tracheal organ cultures. Proc. Soc. Exp. Biol. Med. **140**:846-851.
259. **Whitaker, A. N., I. Bunce, and E. R. Graeme.** 1974. Disseminated intravascular coagulation and acute renal failure in influenza A_2 infection. Med. J. Aust. **2**:196-201.
260. **Wilkinson, P. J., and R. Borland.** 1972. Persistent infection of human lung cells with influenza virus. Nature (London) **238**:153-155.
261. **Williams, K., and J. S. Mackenzie.** 1977. Influenza infections during pregnancy in the mouse. J. Hyg. **79**:249-257.
262. **Wilson, A. B., D. N. Planterose, J. Nagington, J. R. Park, R. D. Barry, and R. R. A. Coombs.** 1976. Influenza A antigens on human lymphocytes *in vitro* and probably *in vivo*. Nature (London) **259**:582-584.
263. **Woodruff, J. J., and J. F. Woodruff.** 1976. Influenza A virus interaction with murine lymphocytes. I. The influence of influenza virus A/Japan 305 (H_2N_2) on the pattern of migration of recirculating lymphocytes. J. Immunol. **117**:852-858.
264. **Woodruff, J. J., and J. F. Woodruff.** 1976. Influenza A virus interaction with murine lymphocytes. II. Changes in lymphocyte surface properties induced by influenza virus A/Japan 305 (H_2N_2). J. Immunol. **117**:859-864.
265. **Wyde, P. R., and T. R. Cate.** 1978. Cellular changes in lungs of mice infected with influenza virus: characterization of the cytotoxic responses. Infect. Immun. **22**:423-429.
266. **Wyde, P. R., R. B. Couch, B. F. Mackler, T. R. Cate, and B. M. Levy.** 1977. Effects of low- and high-passage influenza virus infection in normal and nude mice. Infect. Immun. **15**:221-229.
267. **Yealland, S. J., and R. B. Heath.** 1978. The significance of secondary gram-negative coliform infection of the lungs of mice with influenzal pneumonitis. Br. J. Exp. Pathol. **59**:48-51.
268. **Yilma, T., Y. C. Zee, and J. W. Osebold.** 1979. Immunofluorescence determination of the pathogenesis of infection with influenza virus in mice following exposure to aerosolized virus. J. Infect. Dis. **139**:458-464.
269. **Zee, Y. C., J. W. Osebold, and W. M. Dotson.** 1979. Antibody responses and interferon titers in the respiratory tracts of mice after aerosolized exposure to influenza virus. Infect. Immun. **25**:202-207.