PATHOGENESIS OF INFLUENZA[1]

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More is known about the pathogenesis of influenza than of any other viral respiratory disease. Nevertheless, there are important gaps in our knowledge. These deficiencies become increasingly apparent as one attempts to follow in detail the sequence of events that occur during an infection.

Early Stages of Infection

Let us stipulate that an infection begins when the virus reaches the respiratory tract via the airway. Two questions immediately arise. In what form is the virus upon entering the airway and where does it lodge first? Specifically, one would like to know whether the virus enters relatively naked, or is it incorporated in large, small, wet or dry droplets, and whether the upper or lower portion of the respiratory tract is involved earliest. Obviously the state of the virus upon entry must influence the probability that it will lodge in either locus, since the larger and more dense droplets would be expected to fall out of the air stream after a shorter traverse.

Now I am told that formerly, when these questions were being investigated more intensively than in recent years, opinion ran high and lifelong friendships were almost disrupted when the proponents of the large wet upper respiratory tract school of thought clashed with the small-dry or wet-droplet lower respiratory tract champions. The data that lent fuel to the flames of contention were derived from experiments on survival of infectious virus in various physical states and on infection of animals with such materials. It is not my intent to review that controversy. Rather, focusing our attention upon man, we know with certainty that infection with influenza viruses can be induced in volunteers by dropping infected fluids in the nasopharynx (17), by inhalation of finely atomized liquid suspensions (23) or of dry powders containing lyophilized virus, and even by insufflating the latter into the nostrils (30). The exposure need not be as direct as these statements imply, for infection can be induced by breathing room air into which virus has been sprayed (7). Thus infection can be initiated in man by virus in the wet or dry state, in small or in large aggregates, and when lodged on the upper as well as on the lower respiratory passages. Yet this information does not answer the question: What is the usual event in natural infection? Unfortunately, at present, such important questions remain unanswered. We shall return to them later, for obviously any consideration of control of influenza by altering or treating the atmosphere must rest upon precise knowledge of these factors.

Once lodgment of virus occurs, the picture becomes somewhat clearer. For convenience in presentation and because precise information is lacking, the condition is adopted that virus settles first in the upper respiratory passages, i.e., on the mucous film covering the respiratory epithelium of the nasopharynx or bronchi or larger bronchioles. The exact site of lodgment is of secondary importance since, as will be seen, virus can ultimately reach the remotest segments of the lung. When virus lodges on the mucous covering of respiratory epithelium, a number of reactions can take place. First in likelihood is combination with an α-type inhibitor of hemagglutination. α Inhibitors are heat-stable mucoproteins found in serum and in a wide variety of tissues and secretions including those of the lung. They are believed to be structurally analogous to the erythrocyte and tissue receptors for influenza virus. The combination is a specific one effected through the mutually complementary molecular configurations of virus hemagglutinins and α inhibitors.

However, if the hemagglutinin of influenza viruses did not possess another important property, it seems likely that the most common outcome would be extrusion of virus, since ciliary action would favor movement of the potential

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invader on the mucous film toward the exterior, as it would an inert particle of the same size and density. The enzymatic property of the hemagglutinin apparently tips the balance in favor of invasion. Gottschalk (10) has recently shown that the neuraminidase activity of influenza viruses rapidly lowers the viscosity of mucus. Conversion of this viscous material to a watery fluid lays bare cellular surface receptors and promotes the spread of virus by flow of virus-containing fluids to lower portions of the pulmonary apparatus.

Virus enzyme action not only liquefies mucus but, as Hirst (14) originally demonstrated, results in release of virus in a fully active state from combination with inhibitors. Hence, the virus can repeat the cycle of combination, liquefaction, and dissociation from mucus as many times as are necessary to reach susceptible cell surfaces. The stage is now set for penetration of virus. Hirst (14) proposed that penetration of virus through cell walls was mediated through viral enzyme activity. However, Fazekas de St. Groth (4) concluded that viral enzyme activity was not essential for penetration since virus heated sufficiently to inactivate the enzyme was apparently engulfed by chorioallantoic cells. He called that process "viropexis," implying that intact virus particles were taken into cells much as are colloidal dyes. Schäfer (22) and colleagues have developed the thesis that virus disrupts at the cell surface and that only a proportion of the liberated subunits enters cells. The problem of penetration of influenza viruses into cells requires further study.

Factors in Resistance to Infection

The scheme of attachment, liquefaction of mucus, and penetration of cells described here can be interrupted by either of two humoral factors. The first is a heat-labile proteinaceous substance called β inhibitor, and the second is specific antibody. The former substance is present in serum at low concentrations and presumably by diffusion may come to bathe the surfaces of respiratory epithelium and mix with mucus. β Inhibitor is capable of inactivating in vitro the infectivity of low concentrations of influenza viruses (2). It may be the same or a different substance from properdin or the viral inactivating principle found by Rose in the sputum of humans (21). The role that such substances play in the course of infection is conjectural. Conceivably they could prevent an infection by inactivating virus shortly before lodgment and attachment to cells.

More certain is the function of anti-influenza antibodies. Francis (6) demonstrated in 1941 that antibodies can diffuse from the plasma across the mucous membrane to appear in respiratory secretions. A gradient of about ten to one was found. Neutralization of virus will occur if a sufficient concentration of antibody reaches virus prior to penetration of cells, and under these circumstances, infection will be prevented.

Yet, the antibody mechanism of resistance can apparently be partially overcome if the challenge is severe enough. Convalescent or vaccinated ferrets with high antibody levels can be brought down with fever and upper respiratory symptoms of influenza by instilling virus into the nose. However, in this case the extent of the infection is limited since antibody prevents the development of pneumonia.

Cellular factors may also condition the outcome of exposure to virus although they are difficult to pin down. Francis and Stuart-Harris (5) noted that ferrets convalescent from a first infection with influenza viruses were for a short time refractory to reinfection despite the absence of circulating antibody. At this stage the respiratory epithelium is morphologically altered and is resistant not only to viral but also to chemical injury. Perhaps in man, temporary anatomical and functional alterations of the respiratory mucous membranes, induced by other viruses or by physical or chemical injuries, can render the respiratory epithelium temporarily resistant to infection. The same authors found that reinfected ferrets exhibit an accelerated rate of repair of cellular injury. Further, upon re-exposure after multiple infections, the respiratory epithelium appeared partially or completely resistant to injury by virus. These phenomena did not correlate exactly with the presence of circulating antibody. The possibility exists then that cellular responses conditioned by past infections can of themselves limit the capacity of influenza viruses to invade man.

Clearly the invading virus does not have everything its own way but must run the gauntlet between the influences that favor infection and those which can forestall it. Assuming that virus escapes inactivation and initiates an infection,

it multiplies and eventually emerges from the invaded cells. Possibly viral enzyme plays a role in the escape of virus from cells by liberating it from combination with cellular materials in or on the surface of cells. At this point the gauntlet must be run again, but at this stage the conditions have changed.

The inflammatory process which follows cell injury results in an increased diffusion of plasma constituents to the area of invasion owing to capillary dilatation and increased capillary permeability. Now if the diffusate contains a sufficient amount of virus-inactivating substances, the infection will be aborted and the extent of invasion limited. If not, the outpouring of fluid may favor dispersion of virus and hence increase the extent and severity of the infection. That result would be analogous to the experimental findings of Taylor (27), who demonstrated that sublethal infections of mice with influenza virus could be converted to lethal ones by instilling fluids via the nares into the lungs. Are there other factors that can limit the extent of viral invasion? This is not a new question. Rickard and Francis (20) in 1938 attributed the resistance of mice, given influenza virus intraperitoneally 2 days before an ordinarily lethal intranasal challenge, to operation of the interference phenomenon. Recent studies of Isaacs and Hitchcock (15) have renewed interest in the role of interference in limiting the extent of infection and in recovery from virus infections. These investigators demonstrated that the concentration of interferon, a substance apparently formed in infected cells that contributes to cellular resistance, increases rapidly in the lungs of infected mice and remains at high levels for several days before specific antibody can be detected either in serum or in lung extracts. Their findings and the earlier ones raise the possibility that production of interferon is a nonspecific mechanism of resistance which can limit viral multiplication until levels of specific antibody reach effective concentrations at a later period of time. Whether interference or interferon may play a role in infections of humans is not yet established but is an important question to answer. From these considerations, it is apparent that the course of infection of humans constitutes a dynamic process in which the relative importance of the changing forces in operation can result at several points either in spread of virus or in its containment. The outcome may be a case of variable severity, a subclinical infection, or a thwarted one, depending presumably upon the amount and probably the location of the respiratory epithelium involved.

Pathology of Influenza

The pathology of influenza in humans is well documented in the monographs of Winternitz, Wason, and McNamara (28) and of Hers (12). It is now certain that although the early stage of release of virus from infected cells is not accompanied by visible manifestations of cell injury, the end result of viral infection is necrosis and desquamation of respiratory epithelium. The process may extend to the basement membrane. The nasopharynx, trachea, bronchi, and bronchioles are involved to a variable extent in different cases. The injury and the reaction to it are focally distributed in both lungs. In the special case of influenza-virus pneumonia, the fixed alveolar cells may show cytopathic changes. There may be capillary thrombosis and necrosis with focal leukocytic exudate. Capillary aneurysms and capillary hemorrhages may be present. In some cases hyaline membranes are seen. These changes are considered the characteristic criteria of influenza virus pneumonia by Hers, Masurel, and Mulder (13), whose description is quoted. It is not clear how commonly the alveolar changes occur. Possibly they are present only in overwhelming infections and in a later stage of the disease. The complication of bacterial pneumonia adds to the complexity of the pathological picture but is so well known that it will not be considered here.

Limiting the inquiry to the effects of virus, the question arises, what is the mechanism of cellular injury? A precise answer cannot be given although a number of possibilities can be suggested. As obligate parasites, viruses could injure cells by competing for energy and metabolites. Since the mass of virus synthesized is so small in comparison with the mass of the invaded cell, it seems unlikely that injury results from deprivation of either. Currently, viruses are thought of as disturbers of intracellular harmony. They stimulate cells to make more viruses, to make virus subunits, and to make newly formed materials that are unrelated to virus but may or may not be related to normal cell components. These virus-induced activities may uncouple normal

metabolic sequences (1) and lead to the formation of an unnatural or toxic substance or failure to prevent the harmful accumulation of normal by-products of cellular metabolism. Speculative variation on these simple themes could be endless. In the last analysis it must be admitted that none of them can be wholly discounted nor is it possible to assess how much each contributes to the final outcome. Moreover, it is not quite clear that the processes involved in viral multiplication are the direct cause of the injury sustained. Active preparations of influenza viruses can exert a direct toxic effect upon cells, which is not apparently dependent upon multiplication of infectious virus within them (25, 26). Similar phenomena have been described with Newcastle disease virus (3, 9). High doses are required to demonstrate these effects, suggesting that the injury is a surface phenomenon that is concentration dependent. It has not been established whether the toxic properties of influenza viruses play a role in the pathogenesis of influenza in man. However, it is tempting to speculate that the alveolar and capillary injuries found in influenza-virus pneumonia may result from the toxic activity of those influenza viruses which may accumulate in the dependent portions of the lung as virus-rich fluids flown down from higher segments.

Distribution of Virus in Man

Thus far the pathology of influenza has been described as the result of viral action on the respiratory tract. Indeed, most accounts either state or imply that in natural infection the distribution of virus is limited to the cells lining that system. However, this may not be true.

In mice, viremia and isolation of virus from the spleen, liver, and kidney have been shown repeatedly (11, 24). Zakstel'skaya (29) reported in 1953 that she was able to isolate influenza virus from the urine of children during an outbreak of influenza A-prime. To explain this result it would be most reasonable to assume that virus reached the urine by way of the circulation.

Last year Dr. Hennessy, Dr. Maassab, and Miss Minuse of our virus laboratory had the opportunity to collect and test throat swabs and urine samples obtained from three infants less than 2 years of age who contracted Asian influenza. To our surprise virus was isolated in embryonated eggs and in tissue culture from two of the three cases, using as inoculum the resuspended pellet obtained after centrifuging the urine samples in a high-speed centrifuge. At present we cannot exclude the possibility that the urine samples were contaminated by the patient or an attendant during the process of collection. We hope to resolve this question in future studies.

Oseasohn, Adelson, and Kaji (19) described the isolation of virus from extrapulmonary tissues obtained at autopsy from fatal cases of Asian influenza. One case, a 10-year-old child, yielded virus from tracheobronchial lymph nodes and from the spleen. In another case, virus was found in the liver, kidney, spleen, and heart. That patient also suffered from chronic alcoholism and portal cirrhosis. In a third instance, virus was isolated from the tonsil of a 4-year-old child found dead in bed. To date confirmatory evidence is lacking and for that reason these results may relate either to unrecognized contamination of specimens, to the occasional antemortem existence of viremia, or to an unusual agonal event.

In 1957 we tried to obtain further information on the question of viremia in man. Blood specimens were drawn at frequent intervals from 21 university students ill with Asian influenza. The clots were ground in a small amount of serum and injected into eggs. The results of our attempts to isolate virus from the blood were negative.

Hence, at present, it must be concluded that convincing evidence to support the hypothesis that viremia occurs in natural infection is lacking. What a pity, for it would be quite convenient if one could establish that viremia was a common feature of influenza. There would then be a ready explanation for the constitutional symptoms that accompany illness, i.e., the fever, chills, headache, eye symptoms, muscle aches, and severe asthenia. The alternative explanation that these symptoms all result from respiratory tissue damage is not altogether satisfying, since it is not certain that similar injuries do not accompany other infections, yet the symptoms of influenza are so characteristic that they are pathognomonic. Moreover, we would be in a much better position to evaluate occasional reports of isolation of virus from tissues other than the lung and to develop more accurate concepts about the significance of the unusual cases in

which clinical correlations have been made between influenza and the occurrence of encephalitis, myocarditis, pericarditis, or other unproved manifestations of influenza. Finally, it would be much easier to comprehend the antibody response to infection, for it stretches the imagination a bit to account for antibody increase as the exclusive result of the activity of that amount of lymphoid tissue to which the virus would have most direct access, i.e., the nasopharyngeal components of Waldeyer's ring and other scattered subepithelial nests of lymphoid cells.

Perhaps the results of recent experiments conducted in our laboratory by Dr. Anna Inglot can be used to suggest a mechanism whereby virus can be distributed from the lungs throughout the body. Dr. Inglot confirmed the studies of Boand, Kempf, and Hanson (1*a*) with fluorescent antibody, demonstrating that leukocytes phagocytize influenza viruses. In addition, she showed that leukocytes phagocytize the hemagglutinin subunits of influenza viruses obtained after ether treatment. Furthermore, Dr. Inglot has found that leukocytes exposed to virus in vitro are far more efficient carriers of influenza virus than are red blood cells. Suspensions of leukocytes take up more virus per cell and more virus per unit of cell surface than do suspensions of erythrocytes. The cell-associated virus of leukocytes is resistant to neutralization by immune serum, whereas that attached to red blood cells is readily neutralizable.

Thus, leukocytes could function to ingest virus or degraded virus in the lung and to transport it, after movement into the circulation, to any portion of the body, even in the presence of circulating antibody. Earlier observations of Merchant and Morgan (18) on the effect of influenza viruses on leukocytes demonstrated that treatment with virus inhibited phagocytosis of bacteria. This effect might not only favor the development of bacterial pneumonia in individual patients but could protect the leukocyte that had ingested virus by preventing it from phagocytizing those bacteria capable of destroying it. Dissemination of virus would thus be favored.

Although the hypothesis that viremia does occur in man may seem fanciful, the tantalizing bits of information cited and the possible usefulness of the hypothesis warrant keeping it alive.

Transmission and Environmental Pathogenic Factors

Ultimately, to perpetuate the chain of infection, virus must escape from the infected host in a form or forms that allow transmission. As stated earlier, we are ignorant about the details of this phase of transmission. Conceivably, liquefaction of mucus by viral enzyme may uniquely affect the physical and chemical properties of the droplets that emanate from the infected person. In that case some of the concepts that we have heard about at this Conference may not apply. However, it is to be hoped, from the papers presented previously, that techniques have been or are being developed that will make it possible to determine what the usual infective form of influenza virus is.

In that connection I again call attention to influenza as an excellent model to use for the detailed study of airborne viral infections (8). These viruses can be handled in the laboratory with relative ease and at a reasonable cost. Volunteers could readily be infected with strains of either full or attenuated virulence. Diagnosis can be made with adequate precision using clinical, virological, and serological criteria. It seems reasonable to expect that useful information would come from exposure of human volunteers to well-defined atmospheres. The same system could be used to test the efficacy of physical or chemical measures designed to render noninfectious air contaminated by infected persons or by artificial means. Clearly, information on these subjects is needed if we are to make effective progress in air sanitation.

Finally, I wish to raise some questions about pathogenic factors in the environment (using that word in its epidemiological sense) which are of importance to the pathogenesis of influenza. How can we identify and cope with the reservoir of infection? If it is in man, as seems most probable, how can we isolate it to prevent recurrence of epidemics? If it is extrahuman, where is it and how can we contain or eliminate it? What is the "winter factor" which in the temperate zones appears to facilitate spread of influenza virus from person to person? Is it physiological, perhaps an alteration in respiratory membranes, or in the level of natural immunity or in some as yet unidentified factor in resistance? Is it sociological? When schools open after the summer holidays, it is true that the pupils are congregated and their contacts thereby enlarged. Simultaneously, the social tempo increases and adults tend to meet together more frequently. Mingling with one's fellow man can be hazardous when respiratory pathogens are about. Exposure to children is especially risky, since the attack rate

is highest in childhood. Is the "winter factor" in some way connected with our housing? Does central heating lower resistance by irritating mucous membranes or does the dry atmosphere of our homes, our schools, and our work places foster epidemics by favorably influencing the persistence of influenza virus in room air in an infectious state (16)? Other pathogenic factors could be mentioned, but these are sufficient to make the point that epidemics of influenza are generated by multiple influences, of which the air we breathe is but one.

In conclusion, an attempt has been made in this presentation to emphasize questions in place of restating the obvious. It is hoped that when the next Conference is held on this subject, there will be fewer questions and more answers.

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