Owing to the great variety of influences simultaneously involved in the epidemic course of influenza, the basic epidemiology has been elusive. An animal model was used to elucidate the role and significance of different variables and the results are presented in this summary. The meaning of these findings for human influenza are discussed.

THE USE OF AN ANIMAL MODEL TO STUDY TRANSMISSION OF INFLUENZA VIRUS INFECTION

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UNDERSTANDING of much of the basic epidemiology of human influenza has been an elusive goal to achieve, partly because of the great variety of host, environmental, and viral influences simultaneously modifying the epidemic course of the disease. The multiplicity of these factors has made it difficult to assess the relative importance of each in affecting the spread of infection through human populations, thus limiting investigators of the disease to mere conjecture concerning the significance of many of the variables which may be operative.

Seasonal Variations

An outstanding example of this dilemma is seen in the variety of explanations that have been offered for the striking seasonal variation in the occurrence of epidemic influenza. These include wintertime crowding, the opening of school, decreased indoor ventilation during the winter, the effect of low humidity on the virus, and environmental stresses affecting the host.

All these factors, as well as a multitude of others, provide plausible hypothetical explanations for the "winter factor" in influenza, but it is difficult to assess the significance of any one of them under natural conditions.

For many reasons, mice are an ideal experimental mammalian host to study the transmission of influenza virus infection under controlled conditions. Mice are notably susceptible to infection with viruses of human and swine influenza. Unadapted strains of virus readily multiply in mouse respiratory tissues, and strains of virus that have been adapted by serial passage in mice multiply to high titer and induce typical pulmonary lesions.

Experiments on Mice

Despite this unique susceptibility of mice to influenza virus infection, early experimental attempts to effect transmission of infection from mouse to mouse were only irregularly successful.

For the past seven years, we have been working in our laboratory with an experimental model, studying transmission of influenza virus infection in mice. The details of this experimental model are described elsewhere,[1] but the essential procedures may be described here. Mice are infected by exposure to an aerosol spray of influenza A_2 virus. Twenty-four hours later, contact between

infector mice and previously uninfected animals is established according to one of two experimental designs. In the first of these, two infector mice and two contact mice are housed together for 24 hours in each of a series of small stainless steel cages. The contact animals then are removed, and 48 hours later their lungs are tested for the presence of infectious virus.

The particular time period for contact, 24 hours after the initiation of infection in infector mice, was chosen because it was found in a series of experiments that virtually all transmission of infection occurred during this period, despite the persistence of high titers of virus in the tracheas and lungs of infector mice for several days longer. In addition, throat swabs obtained from infector mice were positive for infectious virus long after these mice no longer were capable of spreading infection to other mice. We believe that these observations illustrate the possible error involved in assuming that the period of infectiousness is identical to the interval during which the infecting organism can be demonstrated in the appropriate tissues or secretions. Certainly, the presence in high titer of the infecting organism in respiratory tract secretions may be essential for transmission to occur, but the release and expulsion of such organisms into the environment may be significantly affected by the nature of the host reaction to infection.

We have also found that some mice can transmit infection more readily than others. The upper half of Table 1 summarizes a series of 28 experiments in which the fates of 511 pairs of contact mice, exposed to the same infectors, were examined. Three possible combinations of results were possible: both contacts infected, one of two infected, and neither infected. Using binomial expansion, predictions could be made regarding the expected frequency of each of the possibilities for each experiment. More pairs in which both or neither contact animal acquired infection were found than were predicted.

Similarly, the lower half of Table 1 summarizes the results when one infector and three contacts were placed in each

Table 1—Expected and observed incidences of possible combinations of results among pairs and triplets of susceptible mice exposed to transmitted influenza virus infection

Two infectors and two contacts in each cage

Both mice infected		One of two mice infected		Neither mouse infected	
Exp.*	Obs.	Exp.	Obs.	Exp.	Obs.
22.7%	30.6%	41.3%	25.6%	36%	43.8%

1,022 mice (511 pairs) $P<0.01$

One infector and three contacts in each cage

Three infected		Two of three infected		One of three infected		None infected	
Exp.†	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
15.9%	36.8%	40.5%	21.1%	34.2%	10.5%	9.4%	31.6%

57 mice (19 triplets) $P<0.001$

* $P^2+2PQ+Q^2=1$.
† $P^3+3P^2Q+3PQ^2+Q^3=1$.

cage. Again, many more triplets were found where all or none of the contacts were infected than were predicted.

Our interpretation of these results is that some mice are effective transmitters while some are poor and that, in the presence of a good transmitter, all of the exposed contacts tend to be infected.

Moreover, when the virus titers of lungs and tracheas of infector mice were measured at the end of the contact period, no differences between good and poor transmitters were found. Once again, therefore, this experimental model provides evidence that infectiousness is not simply a function of the amount of virus present in respiratory tract tissues.

In the second experimental design, larger groups of infectors and contacts were placed in a closed chamber through which the ventilation could be regulated and varied experimentally from one experiment to another. We found that the rate of transmission decreased proportionally as the rate of transmission increased.[2]

In a second series of experiments in the closed chamber model, air flow was kept constant, but some contacts were physically separated from infectors by a double mesh-wire screen while other contacts were allowed to freely mingle with the infector mice on the same side of the screen. The frequencies of acquired transmitted infection in the two groups of contact mice are shown in Table 2. It is apparent that there was no appreciable difference in the likelihood of acquiring transmitted infection related to physical separation of contacts from infectors.

The results of the closed chamber experiments—that transmission is inversely related to air flow and not affected by separation—are consistent with the hypothesis that transmission in this model is principally by small air-borne droplet nuclei, since spread by larger droplets predictably would be influenced by separation of infectors and contacts and would not be affected by ventilation. Furthermore, in more recent experiments, we have been able to demonstrate small particle air-borne virus in the air surrounding infector mice during the period of their infectiousness.[3]

The profound effects of relative humidity on transmission have also been consistent with the hypothesis of transmission by air-borne droplet nuclei. In the first series of experiments, we observed virtually no transmission during the summer months.[4] In later experiments, when year-round controls of temperature and humidity were achieved in the animal quarters, transmission during summer months was almost as frequent as that seen during winter months. However, wintertime transmission was still significantly greater.[4] In other experiments, where relative humidity was experimentally manipulated, significantly greater rates of transmission were observed at lower relative humidities than at higher ones.[2]

Another variable that we have examined is the relative transmissibility of different strains of virus, comparing the results with other indications of mouse virulence such as peak pulmonary virus titers and the production of lung lesions. Table 3 summarizes the results with a number of different strains. It can be seen that transmissibility can be sepa-

Table 2—Effect of separation on incidence of transmitted influenza virus infection in mice

	Contacts physically separated from infectors*	Contacts not separated from infectors
No. Pos.	39/110	48/110
%	35.5	43.6

* Separation produced by two wire screens, ¾ inches apart.

rated from the other attributes of host virulence. Thus, the FM1 strain of influenza A_1 multiples to much higher titer and produces much more pneumonia than the $A_2/AA_2/60$ strain of A_2. However, the FM1 strain is far less readily transmitted. It is also apparent that the A_2 or Asian strains are more readily transmitted than strains of the other subtypes. This observation leads to speculation that the pandemic which resulted following the introduction of the A_2 subtype in 1957 was not only a consequence of the antigenic distinctiveness of the new virus. It might also have been related in part to a greater potentiality for spread inherent in viruses of the A_2 subtype.

In another series of experiments, we have examined the effects on transmission following immunization of infector mice by a variety of procedures.[5] One somewhat unexpected result observed in these experiments concerns the effects of parenteral administered inactviated A_2 virus, the same agent employed in commercial vaccines for humans. Mice immunized with this material had hemagglutinating inhibiting antibody to A_2 virus, and were less readily infected by aerosols of A_2 virus. Moreover, 48 hours following challenge with large doses of virus, their lung virus titers were considerably lower (2.2 $\log_{10}$) than those of control animals. Later in the course of infection, less extensive pneumonia was seen in the immunized animals. When such mice immunized with inactivated A_2 vaccine were employed as contacts in a transmission experiment, they were less frequently infected than control contacts. However, these immunized mice when challenged with A_2 virus infection and employed as infectors were as capable as unimmunized infectors of transmitting infection to normal contacts. Thus, immunization with inactivated vaccine engendered a state of increased resistance in the recipients of the vaccine. Nevertheless, these partially

Table 3—Comparison of transmissibility of different strains of influenza virus

Virus	Infector mice: Pulmonary virus titer 48 hr*	Infector mice: Lung lesions day 7†	Contact mice: No. infected	Contact mice: %
S-15 (Swine)	7.8	45	2/20	(10)
PR8 (A_0)	7.5	42.5	1/20	(5)
NWS (A_0)	7.6	65	3/40	(7.5)
FM-1 (A_1)	8.7	65	2/20	(10)
Lee (B)	6.9	20	1/20	(10)
RI/5+ (A_2)‡	6.8	2.5	6/20	(30)
Jap 305 (A_2)	7.6	60	25/40	(62.5)
$A/A_2/60$ (A_2)	7.1	20	11/20	(55)
Bethesda (A_2)	6.8	12.5	7/20	(35)
Rockville (A_2)	6.7	125	8/20	(40)

* EID/50, $\log_{10}$.
† Extent of lung lesions (per cent).
‡ Unadapted (by serial passage) to mice.

protected animals are capable of infection and following infection, despite lower virus titers in the lung, can transmit infection as readily as nonimmune animals. In contrast, we have found that immunity following infection results not only in a decreased susceptibility to the acquisition of infection, but also in a decreased ability to transmit infection.

Human Transmission and Susceptibility

We believe that if human populations respond in a similar way, these different consequences of immunization with inactivated vaccine and with live virus infection would have important epidemiologic implications. The additional effects on "herd immunity" involved with the use of a vaccine that affects the ability to transmit as well as susceptibility to infection could be of great significance. We have used a simple stochastic model based on assumptions derived from our data to describe hypothetical epidemics. We found that the use of an immunization procedure that affects transmission as well as susceptibility would prevent an epidemic under circumstances in which another vaccine —equally effective in terms of reducing likelihood and severity of infection but without effects on transmission—would not abort the epidemic.

Another experimental manipulation which affected transmission as well as susceptibility was the use of the viral chemoprophylactic agent rimantadine. In our model we found very little effect with amantadine, but its analogue rimantadine reduced the susceptibility of mice to aerosols of influenza A_2 virus and diminished the ability of infector mice to transmit infection to normal contacts.

Once again, this potential to act not only on the likelihood of infection and severity of infection in the recipients of the drug, but also on the likelihood of spread of infection from a treated infector, could have important epidemiologic implications.

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