INFECTION AND IMMUNITY, Feb. 1976, p. 438-447

Vol. 13, No. 2
Printed in U.S.A.

Influenza Virus Population Dynamics in the Respiratory Tract of Experimentally Infected Mice

EDGAR W. LARSON,* JOSEPH W. DOMINIK, ALAN H. ROWBERG,[1] AND GLEN A. HIGBEE

United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

Received for publication 3 July 1975

Virus population dynamics in the lungs, trachea, and nasopharynx of Swiss-ICR mice were studied after respiratory challenge with mouse-adapted preparations of strain A2/Aichi/2/68 influenza virus. Markedly higher doses of virus were required to produce infection with nasopharyngeal challenge than with bronchoalveolar challenge. In all of the infections, the highest virus concentrations were observed in the lungs. Peak concentrations in the trachea were lower than in the lungs but higher than in the nasopharynx. Decreasing virus levels were observed by 120 h after challenge and were generally below detectable levels by the end of 10 days. A compartmental model of a single mathematical form was developed which provided close fits of the virus concentration measurements regardless of the challenge dose, site of initial deposition, or respiratory tissue considered. The model includes seven compartments with five associated rate parameters. The application of compartmental modeling techniques and expression of the virus population dynamics in mathematical terms is regarded as a new approach to the study of the pathogenesis of infections.

Gerone et al. (5) appear to be the only investigators to report on the quantitative virus concentrations in the respiratory tissues of any experimental animals, i.e., mice, as a function of time after influenza virus challenge. The objectives of their work were such that they titrated only the lungs of the mice and challenged only with small-particle aerosols of the kind that would initially deposit the challenge virus primarily in the lungs. Other investigators have studied the pathogenesis of influenza infections by examination of the histopathological and cytopathological changes observed in infected respiratory tissues. Hers and Mulder (8) described the histopathological changes seen in the respiratory tissues from fatal cases of human influenza. Their observations bore similarities to many of those described by Loosli (11) in a report on the serial histopathological changes in mice after aerosol challenge with influenza virus.

We have attempted to add to the base of information on the pathogenesis of this viral respiratory infection by partitioning and assaying the total respiratory tract of experimentally infected mice and by examining the influence, if any, of initially depositing the virus at different sites in the respiratory tree. We further postulated that we could develop mathematical models to describe the virus population dynamics; the characteristics of these models might then lead to hypotheses about the mechanisms involved in the host and virus relationships.

[1] Present address: 2615 Midway Street, Falls Church, Va. 22046.

MATERIALS AND METHODS

Virus suspension. Mouse-virulent preparations of strain Aichi/2/68 (H3N2) influenza virus were used. Increased virulence was achieved in nine serial mouse passages, each consisting of the intranasal (i.n.) instillation of fluid supernatants from homogenates of infected mouse lungs. Final products were produced in 9- to 11-day-old embryonated eggs that were incubated for 48 h at 35 C after virus inoculation. The virus-containing allantoic fluids were harvested, pooled, and stored as small samples at −70 C. The thawed product contained approximately $10^{8.7}$ egg median infectious doses (EID_{50})/ml. Desired dosages in mice, challenged by the respiratory route, were achieved by diluting the virus in heart infusion broth (HIB) containing 250 U of penicillin G and 250 μg of streptomycin per ml. Antigenic composition of the virus was verified by the Communicable Disease Center, Atlanta, Ga.

Mice. Six- to seven-week-old Swiss-ICR mice, weighing 20 to 25 g each (procured from Microbiological Associates, Walkersville, Md.), were used throughout.

Respiratory challenges. Three methods of respiratory challenge were compared. These included i.n. instillation, small-particle aerosol (SPA) inhalation, and large-particle aerosol (LPA) inhala-

tion. With i.n. challenge, the mice were lightly anesthetized with ether and the virus contained in 0.05 ml of liquid was delivered to the nose pad, encompassing both nares, using a 50-μl micropipette (Oxford Sampler, Oxford Laboratories). A Collison spray device connected to a Henderson aerosol apparatus (7), modified by the incorporation of an animal exposure box, was used to expose mice to SPA; mice placed in the exposure box were permitted to inhale the appropriate concentrations of the virus aerosols for periods of 10 min. Calibration trials indicated that 50% of the virus in these aerosols was contained in particles <2.3 μm in diameter; 90% was in particles <4.5 μm in diameter (1). A modified May spinning-top spray apparatus (13) was used with the modified Henderson tube for LPA exposures. Aerosol particles with this system were of a mass median diameter of approximately 8.0 μm with 90% of the particles <11.0 μm (13). All aerosols were sampled with all-glass impinger samplers (4) to estimate virus aerosol concentrations. HIB with 100 U of penicillin G and 100 μg of streptomycin per ml was used as the sampler collecting medium. Presented aerosol dosages were estimated on the basis of measured aerosol concentrations using a mouse breathing rate of 25 ml/min (6).

Mouse necropsies. Immediately after challenge all mice were caged in stainless-steel pans (25.4 by 50.8 by 15.2 cm deep) at a maximum density of 20 per cage and given water and ration pellets ad libitum. Groups of mice were withdrawn from the cages periodically after exposure for purposes of measuring the virus concentrations in respiratory tissues. Tissues were collected frequently during the first 24 h and daily thereafter through 10 days (240 h). The mice were killed by cervical dislocation and immediately necropsied. Three specimens of respiratory tissues were collected aseptically from each mouse including (i) the lungs, taken as individual lobes and pooled; (ii) tracheal samples, including the mainstem bronchi, portions of the secondary bronchi, the trachea and epiglottis; and (iii) nasopharyngeal tissues, taken by trimming the head to exclude the mandible, tongue, and cranium, leaving the nasal turbinates, soft palate, and pharynx. Specimens were homogenized in tissue grinders (Ten Broeck) with measured volumes of HIB containing 250 U of penicillin G per ml and 250 μg of streptomycin per ml.

Virus assays. All assays of virus harvest, virus spray suspensions, aerosol samples, and mouse respiratory tissue homogenates were performed in 9- to 11-day-old embryonated eggs. Eggs were inoculated with 0.1 ml of appropriate sample dilutions in HIB with antibiotics and incubated for 48 h at 35 C. Allantoic fluids were then tested for viral hemagglutinin activity using guinea pig erythrocytes.

Modeling techniques. Linear compartmental analysis procedures as described by Berman et al. (2) and later reviewed by Ottaway (12) were used. The Simulation Analysis and Modeling (SAAM) program, developed by the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md. (3), was used.

An updated user's manual, SAAM 25 version, dated 4 April 1974 (unpublished draft), was obtained from the Laboratory of Theoretical Biology (National Cancer Institute) for use as the primary program reference. The program was processed in the digital computer (Univac 1108) of the National Bureau of Standards, Rockville, Md.

The virus concentrations measured in the various respiratory tissues of mice after i.n. and SPA challenge with a mean dose of $10^{3.6}$ EID_{50} of virus were used as the main data base for model development. Inspection of the log-linear plots of log virus concentrations as functions of time after challenge served as the point of departure for constructing initial models. These were tested for adequacy on the basis of the goodness of fit of the models to the applicable data observations. Iterative evaluations, constrained by rational considerations regarding host and virus relationships, were pursued in deriving a final method.

RESULTS

Ancillary studies. Studies were conducted to determine the sites of initial virus deposition in the respiratory tracts of mice and the lethal dose response characteristics as a function of method of respiratory exposure. The deposition properties of LPA were previously reported (13). This challenge method met the criterion of initially depositing the virus almost exclusively in the nasopharynx i.n., using an inoculum volume of 0.05 ml, and SPA challenges were similar with respect to deposition properties, and both represented primarily lung exposures. Immediately after exposure, approximately 70% of the recoverable virus was found in the lung with these challenge methods, whereas from 12 to 16% was found in both the tracheal and nasopharyngeal tissue.

Lethal dose response studies with LPA challenges did not provide estimates of median lethal doses because no mouse deaths were observed at the highest dose presented ($10^{5.0}$ EID_{50}). Measured median lethal dose values with i.n. and SPA challenges were $10^{3.0}$ and $10^{2.8}$ EID_{50}, respectively.

Virus population dynamics with i.n. and SPA challenges at high dose levels. An initial data base was created with i.n. and SPA challenges at dose levels that would uniformly infect all of the mice (mean dose = $10^{3.6}$ EID_{50}; range, $10^{2.8}$ to $10^{4.3}$). Three replicate experiments were completed with each method of challenge. At least 50 mice were exposed in each experiment with intensive serial sampling through 10 days of postexposure. Three mice were necropsied at each sampling period; the respiratory tissues among mice were pooled before assay for virus. Pooling to minimize variability was considered justifiable in the

case of these uniformly infected mice. In a separate experiment involving individual lung assays of 69 mice (23 groups of 3) the average bias due to pooling, as determined by the differences between arithmetic and geometric means, amounted to 0.16 (standard deviation = 0.19) $\log_{10}$ EID_{50}.

The mean virus recoveries of the three experiments as a function of time after exposure are presented graphically in Fig. 1 and 2. Among all three tissues and with both i.n. and SPA challenges, virus levels decreased in the first 4 h after exposure. Virus concentrations then increased exponentially in the period from 4 through 24 h. Peak concentrations were reached, usually, by 48 h and, routinely, in the period from 48 through 96 h. Virus concentrations gradually decreased after 120 h and reached undetectable levels by 240 h.

Analyses of variance were computed on various parameters of the virus populations to compare methods of challenge and to examine the differences among tissues as a function of challenge method. These parameters included (i) the initial virus concentrations, (ii) the 4-h virus levels, (iii) the exponential growth rates in the period from 4 through 24 h, (iv) the 48- through 96-h virus levels as indicators of peaks, and (v) the log-linear virus disappearance rates in the period from 120 through 240 h.

In no analyses were the differences between challenge methods significant, but the differences among tissues were highly significant ($P < 0.005$) whenever they were compared on the basis of virus levels, i.e., initially, at 4 h, and the virus peaks. The highest virus concentrations were observed in the lungs, whereas virus levels in the trachea were intermediate; lowest virus titers were measured in nasopharyngeal tissues. None of the exponential virus replication rates among tissues and challenge method tested significantly different from a mean of 0.23 $\log_{10}$ EID_{50} per h. On the average, therefore, the virus concentrations increased by $10^{4.6}$ EID_{50} in each tissue in the period from 4 through 24 h.

Virus levels in the period from 48 through 96 h were affected significantly ($P < 0.01$) by tissue and sampling times, but not challenge method. Not only were the highest titers observed in the lungs, but these titers remained high through 96 h postchallenge; tracheal and nasopharyngeal tissue titers decreased by various amounts during this period.

Virus disappearance rates in the tissues of survivor mice in the period from 120 through 240 h were not significantly affected by either challenge method or tissue. Differences among tissues approached significance, however, and the mean values suggested a somewhat faster disappearance from the lung than from the trachea; in turn, virus disappearance from the trachea was somewhat faster than from the nasopharynx.

A degree of bias is acknowledged for the estimates of virus disappearance rates because of

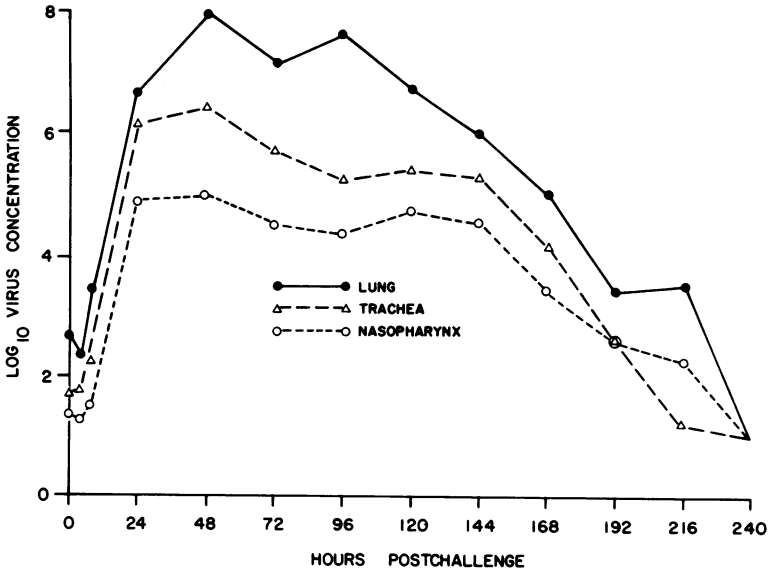

FIG. 1. *Influenza virus recoveries from lung, trachea, and nasopharynx of mice after i.n. instillation of* $10^{3.6}$ EID_{50} *of virus.*

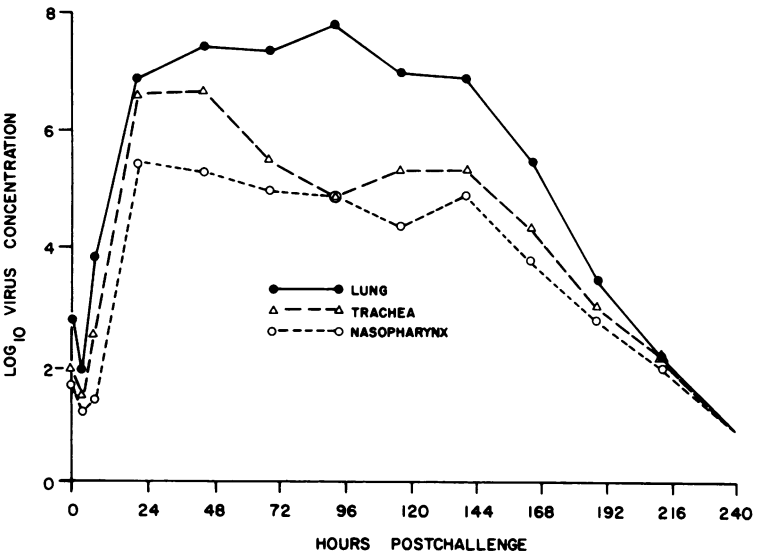

FIG. 2. *Influenza virus recoveries from lung, trachea, and nasopharynx after SPA inhalation of* $10^{3.6}$ EID_{50} *of virus.*

early mouse deaths. In none of these high-dose experiments did mice which were not killed survive beyond 240 h. In some experiments, 240-h measures of virus concentrations were precluded for lack of surviving mice. Among all experiments using both SPA and i.n. challenge, 64 mice died. A very few mice died as early as 120 and 144 h after challenge. Most deaths occurred in the period from 144 to 192 h. The geometric mean time to death for all 64 mice was 173 h.

Deep red areas of consolidation appeared on the lungs of mice which were killed after 96 h. Approximately 25% lung consolidation was seen at 96 and 120 h; 50 to 75% consolidation was present at 144 and 168 h and 75% to essentially complete consolidation was frequently seen by 192 and 216 h. Among mice that died and were necropsied, lung consolidation appeared to be complete.

Virus population dynamics after sublethal i.n. and SPA challenge. Additional studies were conducted to examine the virus population dynamics after sublethal i.n. and SPA challenges of virus ($10^{2.0}$ EID_{50} or approximately 0.1 mouse respiratory median lethal dose). Sixty mice were exposed to an SPA in a single trial. Sixty additional mice were challenged by i.n. instillation of 0.05 ml of collected aerosol sample containing the same amount of virus as was presented by aerosol. Five mice of each group were necropsied for each postexposure period. Infection rates were determined by screening individual mouse lungs for the presence of virus. Thereafter, all tissues from three mice, shown to be infected, were removed from frozen storage and titrated individually for virus.

Lung screening indicated that 94% of the mice challenged with SPA and 89% of the mice challenged by i.n. instillation were infected. The mean virus levels among the three mice from each exposure period are depicted graphically as a function of challenge method and tissue in Fig. 3 and 4.

The virus populations tended to develop more slowly with these sublethal challenges than with the lethal challenges. The 8-h virus titers were only similar to or lower than the 4-h recoveries observed at the higher challenge doses. Exponential growth rates were equal to or lower than those seen with the higher challenge doses; in the nasopharyngeal tissues, the virus populations developed especially slowly. Except for the tracheal tissues, mean recoveries in the interval from 48 through 96 h postexposure were from 1 to 2 $\log_{10}$ less than those observed with higher doses. Also, peak virus levels tended to occur later. Virus disappeared from the trachea and lungs of these sublethally challenged mice at about the same rate as in mice given lethal doses of virus. However, the virus in nasopharyngeal tissues, while reaching minimal peaks, remained at these levels through 10 days of experimentation.

Deaths among these mice challenged with a low dose of virus were minimal. Among 40

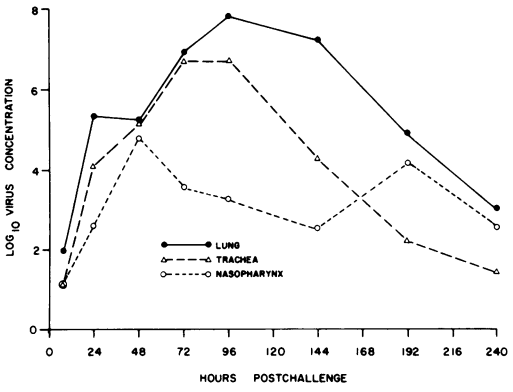

FIG. 3. *Influenza virus recoveries from lung, trachea, and nasopharynx after i.n. instillation of* $10^{2.0}$ *EID*$_{50}$ *of virus.*

mice which were excess to those required for tissue virus titrations, only four died, including two each with SPA and i.n. challenge. All four mice died between 168 and 192 h postchallenge. First lung consolidation, amounting to approximately 25%, was observed in mice which were necropsied at 144 h. Fifty percent lung consolidation was the highest level reached, and it was observed after 192 and 240 h.

Virus population dynamics after LPA challenge. Mice were challenged with presented doses of $10^{3.2}$ and $10^{5.0}$ EID$_{50}$ in these studies. Because of the difficulty of establishing infections with LPA exposure, it was considered unreasonable to challenge mice with large particles at a dose any lower than about $10^{3.0}$ EID$_{50}$ of virus. The respiratory tissues of three mice at each postexposure period were titrated individually for virus concentration.

Table 1 presents the mean log virus concentrations in each respiratory tissue as a function of time after challenge with each dose level. The infection rate among all of the mice challenged at the lower dose, based on virus recoveries from one or more respiratory tissues, was 40%; the infection rate at the high dose was 80%. Among the infected mice at either dose, highest virus concentrations developed first in the nasopharynx and trachea and later in the lungs. Ultimately, however, virus concentrations in the lungs reached levels that were at least equal to those seen in the nasopharynx and trachea. The mean virus concentration of $10^{2.2}$ EID$_{50}$ from the lungs of two mice immediately after the high dose challenge was regarded as a chance observation, which probably resulted from assay errors or sample contamination. The result was contradictory to an earlier and broader base of information; i.e., only trace recoveries in 3 of 20 samples of lung homogenates (13).

There were no mouse deaths among the 40 mice which were excess to those required for tissue virus titrations in these experiments. Maximum lung consolidation, amounting to 50%, was infrequently seen in high-dose mice necropsied after 168 and 192 h.

Modeling studies. The object of these studies was to develop a model of a single form that would have a minimum number of parameters and could be applied uniformly to describe the virus population dynamics for all experimental conditions of dose, site of deposition, and respiratory tissue. The final model consisting of seven compartments and five rate parameters (proportionality constants) is depicted in Fig. 5, using the notation of Berman et al. (2). Because there was no evidence that the inclusion of the early virus measurements (to the time when exponential growth commenced) would produce useful information, these data were dropped from consideration for model development.

The significant features of the model are twofold. First, as might have been predicted from subjective analysis of the host and virus system and retrospectively was the case, the model provides for three primary contributions to the net virus in a given tissue at any point in time after challenge (accumulator, compartment 4, Fig. 5). These include (i) the effective inoculum, reflection of challenge dose, represented by compartment 3 with the associated parameter P_1, (ii) exponential virus replication represented by compartments 1 and 2 with the associated parameter P_2, and (iii) virus inhibition, inactivation and clearance factors repre-

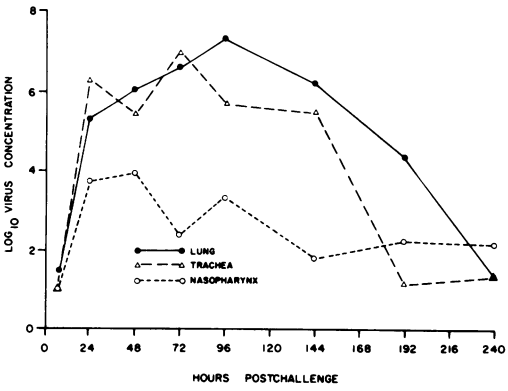

FIG. 4. *Influenza virus recoveries from lung, trachea, and nasopharynx after SPA inhalation of* $10^{2.0}$ *EID*$_{50}$ *of virus.*

TABLE 1. *Influenza virus recoveries from mouse respiratory tissues after LPA challenge with $10^{3.2}$ and $10^{5.0}$ EID_{50} of virus*

Postchallenge (h)	Log_{10} EID_{50}/whole tissue					
	Challenge dose ($10^{3.2}$ EID_{50})			Challenge dose ($10^{5.0}$ EID_{50})		
	Lung	Trachea	Nasopharynx	Lung	Trachea	Nasopharynx
0	—[a]	—	1.44 (2)[b]	2.22 (2)	1.35 (2)	3.64 (2)
4	—	—	1.27 (2)	—	—	—
8	—	—	—	1.53 (3)	1.27 (1)	2.27 (2)
24	1.27 (1)	—	—	2.56 (2)	3.27 (3)	3.13 (2)
48	—	—	—	4.40 (3)	4.85 (2)	4.40 (2)
72	—	—	—	6.67 (2)	5.47 (2)	—
96	3.19 (2)	4.60 (2)	5.25 (2)	5.69 (3)	5.43 (1)	4.57 (2)
120	—	3.43 (1)	3.60 (1)	6.80 (3)	5.43 (3)	5.29 (2)
144	—	4.17 (1)	5.52 (1)	6.60 (1)	5.17 (3)	5.29 (2)
168	5.80 (2)	5.80 (2)	3.68 (3)	5.40 (3)	5.07 (2)	6.75 (3)
240	—	—	—	—	—	4.44 (2)
SE[c]	NC[d]	NC	NC	0.35	0.48	0.36

[a] —, Virus, if any, below assay sensitivity of 1.10 EID_{50}.
[b] Numbers in parentheses, number of mice with virus out of three assayed.
[c] Pooled standard error (SE) among all postchallenge periods for $n = 3$.
[d] NC, Standard errors were not computed for lack of sufficient data.

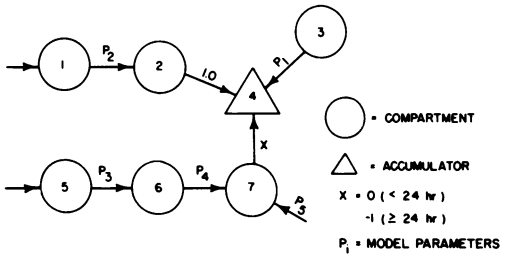

FIG. 5. *Compartmental virus population model using the notation of Berman et al. (2) with compartment 3 and associated parameter P_1 reflecting effective virus inoculum, compartments 1 and 2 with associated parameter P_2 reflecting exponential virus replication, and compartments 5, 6, and 7 with parameters P_3, P_4, and P_5 reflecting net virus population losses.*

sented by compartments 5, 6, and 7 with the associated rate parameters P_3, P_4, and P_5.

This last contribution, which is presumed to encompass the whole complex of host defenses and limiters of virus growth, makes a negative contribution to the net virus in the tissue by virtue of its negative multiplier ($X = -1$). The contribution is not only biologically but mathematically complex. Moreover, our experimentation to date does not permit association of specific compartments with specific host defense mechanisms; rather the total contribution must be regarded as a net effect due to such factors as possible tissue saturation, induced interferon, possible cell-mediated immunity, and complexing of virus with specific antibody.

The second notable feature of the model is that of the time delay associated with the negative contribution due to host defenses and any limitations of virus growth (see Fig. 5, $X = 0$ at <24 h and $X = -1$ at ≥24 h). The requirement for such a time delay indicated unrestricted virus replication in the period before 24 h.

The SAAM program is designed in such a way that the computer searches for those values of the model parameters which will yield a least squares solution for the data observations. This is accomplished by iterative procedures which require provisional, initial estimates of not only the parameter values but also the inputs for compartments 1 and 5 (see Fig. 5). Because the virus growth was log-linear through 24 h after challenge, initial estimates of P_1 (intercept) and P_2 (slope) were computed by least squares linear regression analysis and these were entered as fixed parameters for model solution. A value equal to $-P_2$ was used as fixed input for compartment 1. The negative sign simply denotes the direction of flow into the compartment. Hence, the computed values of tissue virus concentrations in the period through 24 h after challenge are given by the log-linear solution of a simple exponential equation of the form: $\log C_t = P_1 + P_2 (t)$ where C represents tissue virus concentrations, t is the time in hours after challenge, and P_1 and P_2 are the intercept ($\log_{10} C_0$) and

slope, respectively, of the simple exponential.

After 24 h, when X goes from 0 to -1, the right hand side of the above equation takes on a subtractive factor to account for virus losses. The value of the amount subtracted at any time t is determined by the solution of a differential equation describing the contents of model compartment 7. In turn, the solution of that differential equation is based on the solutions of differential equations describing the contents in compartments 5 and 6.

Although the computer readily calculates the virus population losses for any time after 24 h, statement of an algebraic equation which would permit a numerical solution by manual methods is beyond the scope of this paper. Initial estimates and limits for P_3, P_4, and P_5 were derived by trial and error until experience suggested stable values that satisfied the program. Initial estimates of 0.2 for P_3 (with limits between 0 and 1.0), 0.05 for P_4 (limits between 0 and 1.0) and 0.002 for P_5 (limits from -1.0 to 1.0) generally satisfied the program for converging on final values giving minimal sums of squares of deviations from observed data.

A value equal to $-P_3$ was used as input for compartment 5. Therefore, this input converged to a final value with P_3. In addition, while parameters P_3, P_4, and P_5 operate in the period before 24 h, the accumulated contents of compartment 7 are cleared at 24 h. Then as X takes on the value of -1, compartment 7 becomes operative and is heavily influenced by the accumulation in compartment 6 which was not cleared.

Figure 6 shows, conceptually, how each of the five rate parameters contribute to the model in providing a mathematical form consistent with the characteristics of the virus population dynamics. Again, parameters P_1 and P_2 are the intercept and slope, respectively, of the early, exponential increases in virus concentrations. Parameters P_3, P_4, and P_5 first affect the net virus in the tissue at 24 h and operate thereafter from the exponentially increasing virus population. Simplistically, one may consider P_3 to reflect a virus population plateau when it is equal to P_2 and given the opposite sign by multiplier $X = -1$. Parameters P_4 and P_5 provide successive adjustments reflecting virus disappearance.

Because of the paucity of data, the model was not applied to the LPA, low-dose results. Final parameter values resulting from iterative fits of the model to the data obtained in the remaining experiments are listed in Table 2. These values provide information on the characteristics of the virus population dynamics in

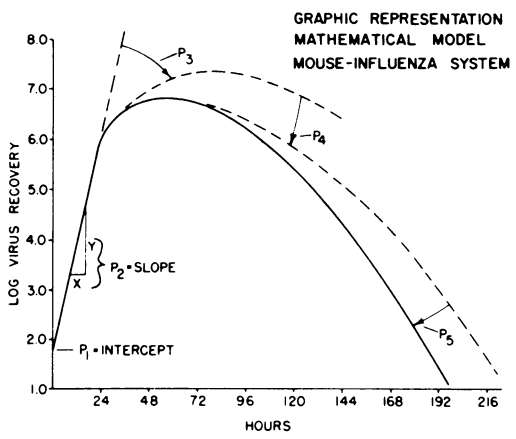

FIG. 6. *Compartmental model contrasts among SPA and LPA particle aerosol challenges in (A) lung, (B) trachea, and (C) nasopharynx of infected mice.*

the various respiratory tissues as functions of exposure method and challenge levels. SPA and i.n. challenges with $10^{3.6}$ EID_{50} of virus (high dose) yielded similar parameter values. Zero-hour intercepts (P_1) consistently decreased between lung and trachea and between trachea and nasopharynx. Ascending slopes (P_2) were similar among tissues, indicating that the recovery level differences observed at zero hours remained relatively constant through 24 h. The values of P_3 were similar to those of P_2 (ascending slopes), suggesting the peaking of virus concentrations relatively soon after 24 h. Values of P_4 were less stable from tissue to tissue and from one exposure method to another than were the values of the other parameters, suggesting variability in the form of the descending (virus disappearance) curves. Values of P_5 representing, primarily, measures of the curvature associated with virus disappearance were uniformly low. A negative sign for this parameter indicated downward curvature.

When mice were challenged with a low dose of virus ($10^{2.0}$ EID_{50}) by i.n. and SPA challenges, the parameter values were less consistent than those observed at the higher dose. P_1 values for the nasopharynx at the lower dose were equal to or higher than the P_1 values at the higher dose, but the P_2 values were lower, indicating slow development of virus in this upper respiratory tissue. Again, the P_3 values were generally similar enough to P_2 values to suggest early peaking of virus after 24 h. With one exception, namely the lung with i.n. challenge, the values of P_4 were low by comparison with those seen at the higher challenge level and reflected slower rates of virus disappearance. Values of P_5 remained uniformly low,

TABLE 2. *Effects of challenge method and dose on the parameters of the compartmental virus population model applied to influenza virus recoveries from the respiratory tissues of infected mice*

Challenge	Tissue	Parameter[a] values				
		P_1	P_2	P_3	P_4	P_5
i.n. ($10^{3.6}$ EID_{50})	Lung	1.468	0.222	0.231	0.294	−0.00091
	Trachea	0.587	0.233	0.235	0.114	−0.00113
	Nasopharynx	0.193	0.198	0.190	0.262	−0.00104
SPA ($10^{3.6}$ EID_{50})	Lung	1.366	0.232	0.211	0.424	−0.00200
	Trachea	0.682	0.259	0.279	0.457	−0.00132
	Nasopharynx	−0.024	0.231	0.227	0.352	−0.00140
i.n. ($10^{2.0}$ EID_{50})	Lung	0.435	0.204	0.162	0.288	−0.00257
	Trachea	−0.355	0.186	0.265	0.0150	0.00051
	Nasopharynx	0.420	0.0890	0.112	0.0216	0.00120
SPA ($10^{2.0}$ EID_{50})	Lung	−0.480	0.243	0.211	0.0868	−0.00198
	Trachea	−1.530	0.329	0.339	0.0855	−0.00050
	Nasopharynx	−0.225	0.166	0.196	0.0648	0.00103
LPA ($10^{5.0}$ EID_{50})	Lung	0.906	0.072	0.0425	0.0165	−0.00869
	Trachea	0.270	0.125	0.103	0.0301	−0.00318
	Nasopharynx	1.840	0.0540	0.0204	0.0336	−0.00999

[a] P_1, intercept at $t = 0$; P_2, = slope of virus formation ($\log_{10}$ EID_{50}/h); P_3, P_4, and P_5, virus disappearance parameters.

with a positive sign indicating slight upward curvature of the virus disappearance profiles in three of six cases.

A different pattern of parameter values was seen with LPA challenge. The highest virus population intercept was observed for the nasopharynx followed by the lung and trachea. The virus populations developed especially slowly as indicated by the low values of P_2. In this respect it is important to note that a change of the model to the extent of delaying the time interrupt from 24 to 48 h was required for an adequate fit of the lung recoveries. This indicated that in the case of this tissue the virus population tended to increase exponentially for a longer period of time than in either the trachea or nasopharynx. As with i.n. and SPA challenges, values of P_3 were similar to P_2. Low values of both the P_4 and P_5 parameters suggested slow virus disappearance rates which were nearly linear.

Fits of the model to the observed data from selected experiments of both high- and low-dose challenges with SPA and the high-dose challenge with LPA are illustrated by Fig. 7. Despite marked virus population profile differences among tissues and from one exposure treatment to another, the computed estimates of the virus concentrations closely fit the observed data.

The effects of respiratory tissue and challenge treatment, both with respect to dose level and site of initial virus deposition, on tissue virus levels are readily apparent. The late development (low ascending slopes) of the

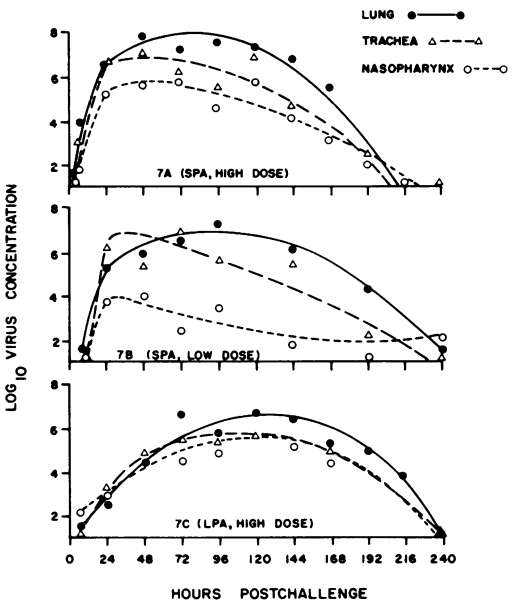

FIG. 7. *Fit of compartmental model to observed data from selected experiments showing influenza virus recoveries in mouse respiratory tissues after SPA and LPA challenges.*

virus populations with LPA challenge is confirmed by comparing the curves of Fig. 7C with those in 7A and 7B. Also, it is seen that in the case of the lung with LPA exposure, virus recoveries tended to increase exponentially for a longer period of time than in either the trachea or nasopharynx.

The same virus population curves, contrasted again in Fig. 8, permit the convenient comparison of challenge treatments within respiratory tissue. The slow rates at which the virus populations developed in all tissues with LPA challenge can be readily discerned. Also, the lower virus levels measured with the low level SPA challenge by comparison with the high-level challenge are apparent.

DISCUSSION

We have described and presented the results of a series of experiments designed to measure the influenza virus concentrations in various respiratory tissues of mice as a function of time after challenge. Exposure methods aimed at selectively depositing the challenge virus at different levels within the respiratory tract were used. Our studies have demonstrated that i.n. instillation of 0.05 ml of virus provided for a pattern of penetration and deposition in the respiratory tract which was similar to that with SPA inhalation; both methods of challenge represented primarily bronchoalveolar exposures. This indicated that with i.n. instillation a major part of the virus inoculum was aspirated into the lung in the process of administration. Ancillary studies (unpublished data) have indicated that when the inoculum volume is reduced to a level of 0.001 ml, aspiration to the lung is largely avoided and the inoculum remains in the nasopharynx.

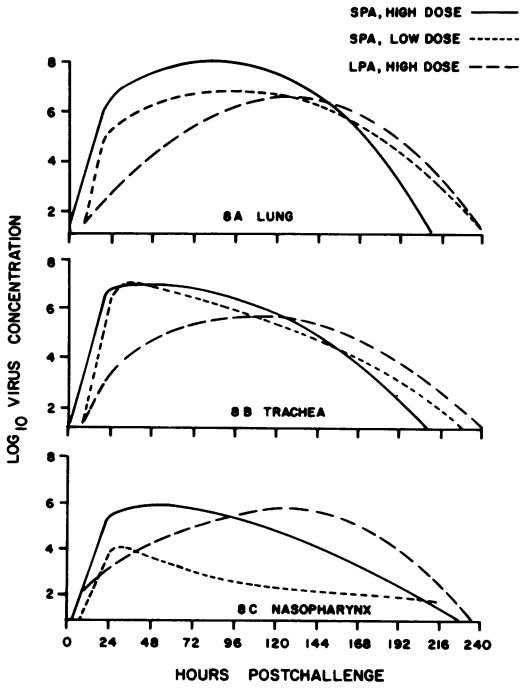

FIG. 8. *Conceptual representation of influenza virus model in mice showing parameter contributions to the graphic form of virus population profiles.*

These studies demonstrated also that high lung virus concentrations were characteristic of the infections regardless of the site of initial virus deposition. Even when the primary site of initial deposition was the nasopharynx, if the challenge was sufficient to produce infection, high concentrations of virus were ultimately found in the lung. On the other hand, not only were high doses required to produce uniform infections in mice when the virus was initially deposited in the nasopharynx, but the virus populations in the lungs developed later and at a slower rate than with bronchoalveolar challenges. These observations suggested a process of delayed lung inoculation with aspirated virus originating in the upper respiratory tract.

Our results in mice would support a hypothesis that viral infections of the lung in human cases of influenza may occur more frequently and be of greater importance than has previously been considered. Knight and Kasel (10) in a review of influenza have stated that "the development of pneumonia (in humans) due to influenza virus, with or without superimposed bacterial infection, is uncommon but is the most serious complication of influenza." They further state that "the extent of spread of the virus down the respiratory tract in apparently uncomplicated cases of influenza is not known" and cite the evidence from Johanson et al. (9) indicating frequent transient interference with oxygenation which could have resulted in part from viral infection in the lung.

We regard our modeling studies as a novel approach for identifying interacting variables between the host and infecting microorganism in infectious disease research. Perhaps the present studies will stimulate other investigators to pursue the concepts of modeling. As our model provided reasonable fits of the data observations for a relatively wide range of experimental conditions, the model is regarded as predictive for influenza in mice. That is, with the specification of the challenge dose and the initial site of deposition, Table 2 can be entered to find values of the model parameters for estimating the expected virus concentrations in a given tissue at any time after challenge.

The work to date does not permit predictions regarding the influence of such factors as preexisting immunity, postexposure therapy, or, for that matter, another host with either influenza virus or another respiratory pathogen. We would suggest, however, that our model might

serve as a point of departure for further study of these factors.

ACKNOWLEDGMENTS

The contributions of George Scott in performing influenza virus passages in mice and preparing the virus suspensions used in these studies and of Robert Magruder and Robert Stockman for dedicated technical assistance are gratefully acknowledged. Also acknowledged are the efforts of W. R. Dowdle, Communicable Disease Center, for verification of the virus antigens.

LITERATURE CITED

1. Berendt, R. F. 1974. Simian model for the evaluation of immunity to influenza. Infect. Immun. 9:101-105.
2. Berman, M., M. F. Weiss, and E. Shahn. 1962. Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems. Biophys. J. 2:289-316.
3. Berman, M., and M. F. Weiss. 1967. Simulation analysis and modeling program manual. U.S. Public Health Service Publication no. 1703. U.S. Government Printing Office, Washington, D.C.
4. Brachman, P. S., R. Ehrlich, H. F. Eichenwald, V. J. Gabelli, T. W. Kethley, S. H. Madin, J. R. Maltman, G. Middlebrook, J. D. Morton, I. H. Silver, and E. K. Wolfe. 1964. Standard sampler for assay of airborne microorganisms. Science 144:1295.
5. Gerone, P. J., T. G. Ward, and W. A. Chappell. 1957. Combined infections in mice with influenza virus and *Diplococcus pneumoniae*. Am. J. Hyg. 66:331-341.
6. Guyton, A. C. 1947. Measurement of respiratory volumes of laboratory animals. Am. J. Physiol. 150:70-77.
7. Henderson, D. W. 1952. An apparatus for the study of airborne infection. J. Hyg. 50:53-68.
8. Hers, J. F. Ph., and J. Mulder. 1961. Broad aspects of the pathology and pathogenesis of human influenza. Am. Rev. Respir. Dis. 83:84-97.
9. Johanson, W. G., Jr., A. K. Pierce, and J. P. Sanford. 1969. Pulmonary function in uncomplicated influenza. Am. Rev. Respir. Dis. 100:141-146.
10. Knight, V., and J. A. Kasel. 1973. Influenza viruses, p. 87-123. *In* V. Knight (ed.), Viral and mycoplasmal infections of the respiratory tract. Lea and Febiger, Philadelphia.
11. Loosli, C. G. 1949. The pathogenesis and pathology of experimental air-borne influenza virus A infections in mice. J. Infect. Dis. 84:153-168.
12. Ottaway, J. H. 1971. Compartmental analysis and optimisation procedures, p. 332-349. *In* L. G. Whitby and W. Lutz (ed.), Principles and practice of medical computing. Churchill Livingstone, Edinburgh.
13. Young, H. W., E. W. Larson, and J. W. Dominik. 1974. Modified spinning top homogeneous spray apparatus for use in experimental respiratory disease studies. Appl. Microbiol. 28:929-934.