

AD-A090 039

ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/5
EFFECT OF PRIOR INFLUENZA VIRUS INFECTION ON SUSCEPTIBILITY OF --ETC(U)
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
	AD-A090039	
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED	
Effect of Prior Influenza Virus Infection on Susceptibility of AKR/J Mice and Squirrel Monkeys to Respiratory Challenge by <u>Legionella pneumophila</u>	Interim rept.	
6. AUTHOR(s)	7. PERFORMING ORG. REPORT NUMBER	
with <u>Legionella</u> RICHARD F. BERENDT PhD		
8. PERFORMING ORGANIZATION NAME AND ADDRESS	9. CONTRACT OR GRANT NUMBER(s)	
US Army Medical Research Institute of Infectious Disease, Aerobiology Division (SGRD-UIA-E), Fort Detrick, Frederick, Maryland 21701	(11) 000	
10. CONTROLLING OFFICE NAME AND ADDRESS	11. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
US Army Medical Research and Development Cmo. Office of the Surgeon General, Department of the Army, Frederick, Md 21701	3M762776A841 A841-00-050	
12. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	13. REPORT DATE	
(12) (13)	30 Jul 1980	
	14. NUMBER OF PAGES	
	12	
	15. SECURITY CLASS. (of this report)	
	UNCLASSIFIED	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	

16. DISTRIBUTION STATEMENT (of this Report)

Cleared for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

A

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be submitted for publication in the Infection and Immunity Section
on Pathogenic Mechanisms.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

SEQUENTIAL RESPIRATORY; INFECTION ANIMAL MODELS; INFLUENZA and
LEGIONNAIRES DISEASE

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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to a sequence of influenza virus and Legionella pneumophila than
to either agent alone.

AD A 090039

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80 10 6 158

Legionella pneumophila

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ABSTRACT

AKR/J mice and squirrel monkeys show greater mortality when exposed to a sequence of influenza virus and Legionella pneumophila than to either agent alone.

As knowledge of Legionnaires' disease has accumulated, the evidence suggests that many infections occur in individuals with underlying disease. Since Legionella pneumophila appears to spread by the airborne route (5, 6, 8, 9), we have initiated a study of respiratory virus infection as a predisposing factor for L. pneumophila infection in experimental animals.

The virus selected was a mouse-adapted variant of the Aichi/2/68 strain of type A influenza virus (H3N2). The virus was propagated in embryonated eggs (10). The virus titer in the allantoic fluid that was harvested was estimated to be $10^{8.2}$ median egg infectious doses (EID_{50})/ml.

The Philadelphia-1 strain of L. pneumophila was grown on charcoal-yeast extract (CYE) agar as previously described (3). Enumeration of bacteria was accomplished by spreading 0.1 ml of the appropriate dilution on CYE agar and counting colonies after incubation.

Two hosts were chosen for study: the AKR/J mouse, which has been reported to be susceptible to L. pneumophila challenge by Hedlund et al. (7), and the squirrel monkey, which demonstrates a mild clinical response to intratracheal instillation with either influenza virus (2) or L. pneumophila (unpublished observation).

For infection with both disease organisms mice were lightly anesthetized with halothane and inoculated intranasally (i.n.) with 0.05 ml of the appropriate dilution of organisms. Preliminary titrations with graded doses established the median lethal dose (LD_{50}) of influenza virus by this route to be $10^{4.8}$ EID_{50} and the LD_{50} of L. pneumophila, 1.1×10^8 organisms. Fifteen mice in each of three groups were then treated as follows: one group received $10^{4.0}$ EID_{50} of influenza virus in 0.05 ml of heart-infusion broth (HIB); 3 days later, the mice were

given 0.05 ml of tryptose saline diluent. A second group was inoculated with virus and 3 days later were inoculated i.n. with 10^6 L. pneumophila. The third group received 0.05 ml of HIB followed 3 days later by 10^6 L. pneumophila. Results are shown in Table 1. The mortality rate among the mice given the sequence of virus followed by bacteria was significantly higher than that of either of the single organism control groups.

Confirmatory experiments with monkeys were restricted in scope because of cost and availability. The reaction of squirrel monkeys to influenza virus has been described previously (2). Preliminary observations on groups of four monkeys each given 10^6 L. pneumophila either by intratracheal (i.t.) instillation or aerosol are described in Figure 1. Four additional monkeys served as controls. The technique for i.t. instillation has been described elsewhere, as have the procedures for aerosol exposure and subsequent dosage calculation (1). No monkeys died or were even markedly ill after challenge by either route. Such clinical signs as dyspnea, coughing, sneezing, nasal crusting and lethargy were present at various times, but were inconsistent and not considered as reliable indicators of infection. All i.t. instilled monkeys showed significant leukocytosis, anorexia, weight loss and increased respiratory rate; responses were somewhat less marked in aerosol-exposed monkeys. Table 2, which presents the serum microagglutination titers carried out by the method of Farshy et al. (4), indicates that the serological response was greater in i.t. infected monkeys.

To determine the effect of sequential respiratory infection 8 monkeys were inoculated i.t. with 10^7 EID₅₀ of influenza virus, and four with sterile HIB. Three days later four of the eight influenza-infected monkeys

and the four HIB-instilled monkeys were exposed to an aerosol dose of 10^7 L. pneumophila. The four remaining influenza-infected animals were reserved as controls with no further treatment. Two of the four sequentially infected monkeys died, one on day 5 and one on day 7. The lungs of both contained at least 10^7 L. pneumophila. Unfortunately, histopathologic examination was not carried out. None of the monkeys in this experiment, including those that died, had fever higher than 103°F . The values for the four parameters discussed previously are shown in Figure 2. In the study case of the sequentially infected monkeys, these data are subject to bias because only two monkeys survived. Although the small numbers preclude statistical analysis, it appears that the response of sequentially-infected monkeys was more severe.

The sequence of influenza followed by Legionnaires' disease may be relatively uncommon in nature because of the differing seasonal patterns of the two diseases. What these data suggest, however, is the possibility that respiratory viruses may enhance host susceptibility to subsequent L. pneumophila infection.

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TABLE 1. Response of AKR/J mice to intranasal instillation of $10^{3.0}$
EID₅₀ of influenza virus followed by 10^5 *Legionella pneumophila*

Treatment	Geometric mean time to death (days)	Dead/Total	P
Influenza virus alone on day 0	8.4	5/14	0.025 ^a
Influenza virus on day 0 + <u>L. pneumophila</u> on day 3	7.1	13/15	0.005 ^b
<u>L. pneumophila</u> alone on day 3	NA	0/15	

^aVirus control vs. sequentially-infected mice, χ^2 test with Yates correction.

^bBacterial control vs. sequentially-infected mice, χ^2 test with Yates correction.

TABLE 2. Serum microagglutination (MA) titers in squirrel monkeys
exposed to 10^6 Legionella pneumophila

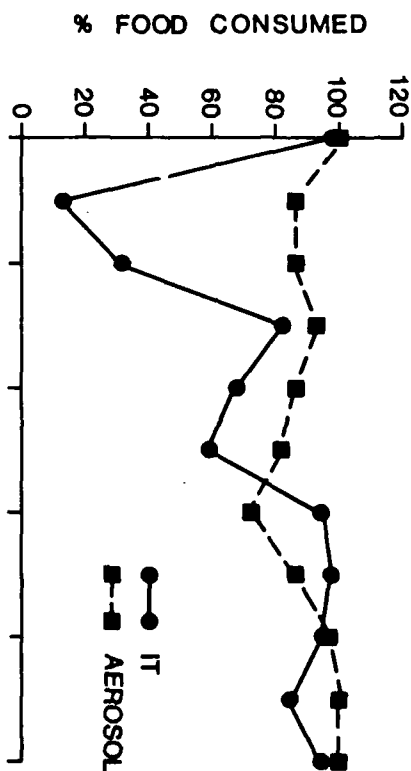
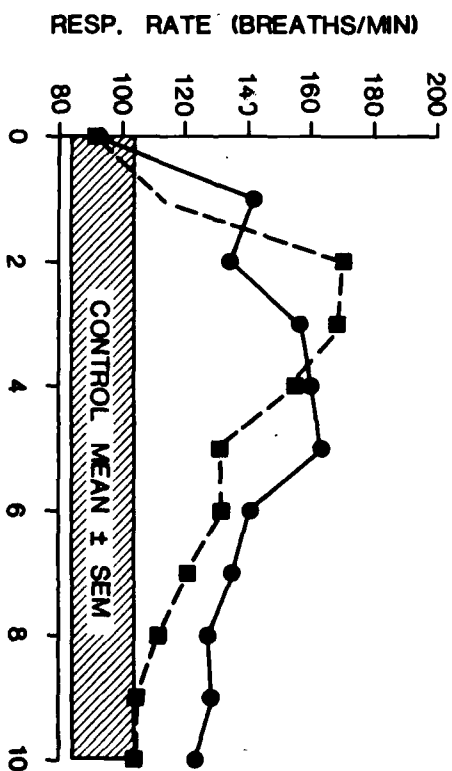
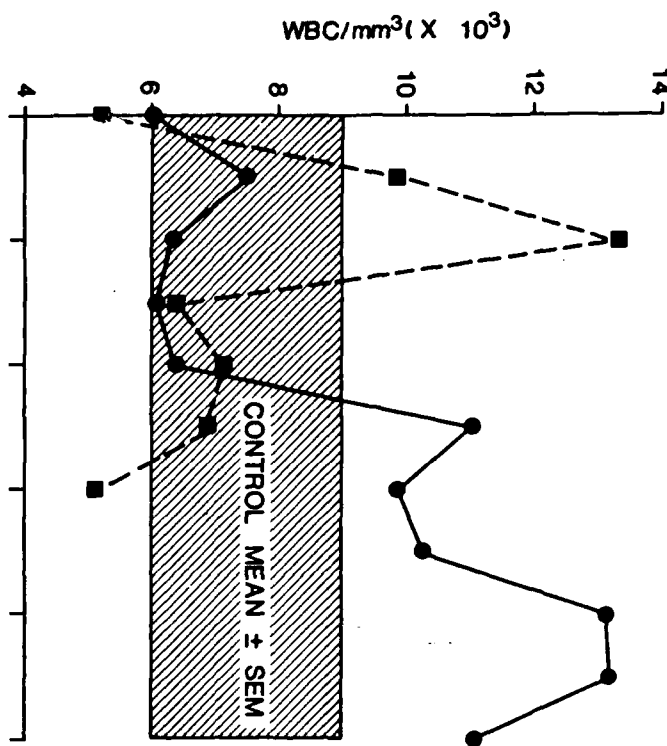
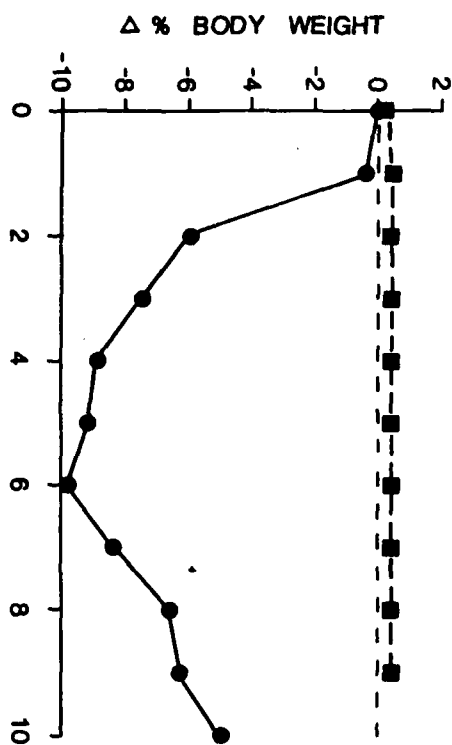
Days after infection	Geometric mean reciprocal MA titer	
	Intratracheal exposure (n = 6)	Aerosol exposure (n = 6)
0	< 8.0	< 8.0
14	64.0	32.0
28	256.0	45.3

FIGURE LEGENDS

FIG. 1. Response of squirrel monkeys to intratracheal or aerosol challenge with 10^6 Legionella pneumophila. Cross-hatched band is mean + SEM for uninfected controls. Control data are not shown for weight change (negligible) or food consumption (invariably 100%).

FIG. 2. Response of squirrel monkeys to sequential infection with influenza virus and L. pneumophila.

DAYS AFTER INFECTION



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AEROSOL

