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COMPARISION OF AEROSOL AND INTRAMUSCULAR KANAMYCIN TREATMENT OF--ETC(U)
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REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* COMPARISON OF AEROSOL AND INTRAMUSCULAR KANAMYCIN TREATMENT OF RESPIRATORY KLEBSIELLA PNEUMONIAE INFECTION IN A SUBHUMAN PRIMATE	5. TYPE OF REPORT & PERIOD COVERED Interim 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) R. F. BERENDT, M. A. SCHNEIDER, F. R. FROLA, H. W. YOUNG, M. C. POWANDA* *Biochem. Br, USA Inst. Surg. Rsch., Brooke Army Medical Ctr, Ft Sam Houston, TX 78234	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research Institute of Infectious Diseases SGRD-UIA-E Fort Detrick, Frederick, Maryland 21701	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3M762776 A841-00-050
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command, Office of the Surgeon General, Department of the Army, Frederick, MD 21701	12. REPORT DATE 6 June 1978 13. NUMBER OF PAGES 27
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

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18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be submitted for publication in Infection and Immunity.

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

Primate pneumonia
Aerosol therapy
Kanamycin therapy
Klebsiella pneumonia

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

The objective of this study was to compare the efficacy of i.m.- and aerosol-administered kanamycin for the treatment of pneumonia due to Klebsiella pneumoniae in the squirrel monkey. Both routes were equally effective in preventing mortality, inhibiting bacteremia and pharyngeal shedding of organisms and in reducing fever. Aerosol treatment seemed to cause tachypnea in infected monkeys, whereas anorexia was more notable in some of the monkeys treated i.m. In view of their approximately equal effect, the i.m. must be the preferred route of treatment rather than the aerosol.

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SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

(6) Comparison of Aerosol and Intramuscular Kanamycin Treatment of Respiratory *Klebsiella pneumoniae* Infection in a Subhuman Primate,

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(16) 3M762776A841

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Running Head: THERAPY OF PRIMATE *KLEBSIELLA* INFECTION

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

The objective of this study was to compare the efficacy of i.m.- and aerosol-administered kanamycin for the treatment of pneumonia due to Klebsiella pneumoniae in the squirrel monkey. Both routes were equally effective in preventing mortality, inhibiting bacteremia and pharyngeal shedding of organisms and in reducing fever. Aerosol treatment seemed to cause tachypnea in infected monkeys, whereas anorexia was more notable in some of the monkeys treated i.m. In view of their approximately equal effect, the i.m. must be the preferred route of treatment rather than the aerosol.

Recently, we showed that aerosols of kanamycin more effectively prevented mortality in mice infected with Klebsiella pneumoniae than did intramuscular (i.m.) injection of the same antibiotic [1]. Respiratory K. pneumoniae infection of mice is primarily a bronchopneumonia [1]. More recently, we found that intratracheal inoculation of K. pneumoniae in squirrel monkeys (Saimiri sciureus) produced lobar pneumonia [2], and, therefore, this species might be a better host for study than the mouse since human pneumonia caused by this organism is also primarily lobar [3].

This report presents a comparison of aerosol with i.m. therapy. Emphasis is placed upon changes in percent mortality and also upon several clinical and laboratory findings.

MATERIALS AND METHODS

Test Organism. Techniques for growing, storing and enhancing the virulence of the A-D strain of type 1 K. pneumoniae have been described previously [1]. Inocula for infection were prepared as previously described [4] and contained from 600-800 viable organisms.

Test Animals. Healthy, juvenile, male squirrel monkeys, weighing from 0.5 to 1.0 kg were used. They were housed individually in wire-bar cages and were allowed free access to commercial monkey chow and water. Their diet was supplemented with fresh fruit several times weekly. During experiments fruit was eliminated and each monkey was limited to six biscuits daily to facilitate estimation of food consumption.

Intratracheal Inoculation. The intratracheal inoculation procedure (i.t.) has been described previously [4]. Previous experimentation indicated that the dose administered was approximately 1 LD_{50} [4].

Clinical Determinations. Once daily, 0.75 ml of blood was obtained from the saphenous vein for determination of total and differential leukocyte concentration, hematocrit and bacteremia. Bacteremia was determined by spreading 0.1 ml of blood on the surface of a trypticase soy agar plate, incubating for 18 hr at 37C and counting the colonies produced. The presence of Klebsiella in the pharynx was similarly determined with a sample obtained with a cotton swab moistened with trypticase

soy broth (TSB). Additional daily observations included rectal temperatures, respiratory rate, weight, activity, sneezing or coughing, dyspnea and food consumption. Each of these parameters was measured 3 days before and 7 days after infection.

Plasma Protein Determinations. On two of the pre-exposure days, as well as on days one, two, three and six post-exposure, the volume of the daily blood sample was increased to 1.5 ml to permit the determination of plasma lysozyme, α_1-antitrypsin, ceruloplasmin and haptoglobin. Plasma lysozyme activity was determined by the procedure of Osserman and Lawlor [5]. The other proteins were determined by radial immunodiffusion procedures using kits obtained from Behring Diagnostics (American Hoeschst Corp., Somerville, NJ). Preliminary testing indicated that sufficient cross-reactivity between squirrel-monkey and human plasma was present to permit use of these kits, even though they were manufactured for determinations using human plasma.

Therapy Techniques. All kanamycin solutions were prepared from bulk drug (78% kanamycin base, obtained from Bristol Laboratories, Syracuse, NY) dissolved in 0.085 M sodium citrate solution adjusted to pH 4.5 with 35N sulphuric acid.

Intramuscular therapy was accomplished by injecting 15 mg of kanamycin/kg of body weight/day, divided into two equal doses administered twice daily. Each injection was 0.5 ml.

Aerosol therapy with the same dose required fabrication of a special exposure box. This box (figure 1) was constructed

of acrylic plastic and was divided by a partition into two compartments each 32 cm wide by 25 cm deep by 40 cm high. Perch and exercise bars and a tray for collection of urine and feces are shown. Four monkeys could thus be exposed to aerosols simultaneously. Aerosols were disseminated into the mixing tube at the top and exhausted through ports near the bottom of each compartment. Ports on the side of the box provided for collection of aerosol samples for the determination of aerosol concentration of antibiotic. A port on the top of the box, fitted with a pressure gauge, permitted measurement of pressure differential between the box and the safety cabinet in which it was housed. This pressure differential was equal to 0.5 inch of water so that the exposure box was maintained at a lower pressure than the safety cabinet in the system was housed.

Kanamycin was disseminated into the system from a Collison nebulizer [6] at a rate of 0.22 ml of liquid/min. The aerosol particles were entrained in air flowing at a rate of about 40 l/min. One-half of this volume was directed into each exposure compartment. Figure 2 shows the entire aerosol-treatment system including the apparatus required to maintain an equal air-flow to the two compartments as well as to equilibrate the air-balance during dissemination and sampling.

All-glass impingers [7] containing distilled water were used for sampling the kanamycin aerosols that were collected at the middle of each exposure period.

Aerosols were disseminated from a 100 mg/ml solution of kanamycin for 120 min twice daily in order to administer 15 mg/kg/day. Inhaled dose was calculated on a basis of 300 ml of aerosol inhaled per min [8] and 50% retention of inhaled particles [9].

Kanamycin Assay. Kanamycin concentration was determined by microbiological assay as previously described [1].

Experimental Plan. Kanamycin, whether by i.m. or aerosol, was given twice daily for a total of 10 treatments during 5 days (a total of 75 mg/kg). Therapy was initiated either when illness was first apparent (24 hr after infection) or when it was well established in all animals (30 hr after infection). Limitations on the availability of monkeys and on the number that could be treated at one time in the aerosol-therapy apparatus were significant experimental constraints. There were eight experimental groups: one group was sham-infected with sterile TSB (sham controls), one group was sham-infected and treated by i.m. injection of kanamycin beginning at 24 hr (uninfected kanamycin controls - i.m.). A third group was sham-infected and treated with aerosols of kanamycin beginning at 24 hr (uninfected kanamycin controls - aerosol). The fourth group was infected with K. pneumoniae and received no further treatment (K. pneumoniae controls). The fifth and sixth groups were infected and received kanamycin by i.m. injection at 24 or 30 hr, respectively. The last two groups were infected and given the antibiotic by aerosol at either 24 or 30 hr.

Sham control and uninfected kanamycin control monkeys that were given i.m. therapy were reused in later experiments. The uninfected monkeys that received aerosols of kanamycin were not reused because kanamycin has been shown to persist in the lungs of rodents following aerosol administration [1, 10, 11].

The number of monkeys employed as well as the various treatment groups are outlined in table 1. K. pneumoniae-control monkeys were distributed throughout the experiment so that three to five of them were evaluated at the same time as a group that received therapy. The uninfected controls that received i.m. antibiotic were similarly distributed. Since the treatment box held only four monkeys, this approach was not possible with the aerosol-control monkeys, so they were evaluated in consecutive weeks.

RESULTS

Mortality. The dose of Klebsiella administered in these experiments was intended to produce approximately 50% mortality in untreated monkeys. The rationale for this dose was that the effect of therapy upon both mortality and severity of illness in survivors could be studied in a relatively small group of monkeys. The death rate was 67% in the untreated controls (table 1). Only one monkey died in the treated groups (in the group receiving i.m. treatment at 30 hr) indicating that all times and routes of antibiotic were equally effective with respect to preventing mortality.

Clinical Response. These experiments were designed to determine the effect of two routes of therapy upon morbidity as well as upon mortality. Therefore, several clinical and laboratory parameters were investigated to determine whether either of the routes of therapy might be more effective in reducing the severity of illness than the other. Generally, when therapy was initiated at 24 hr, the severity of illness was less marked than when it was started at 30 hr. Also, the values for the various parameters that were measured in the three uninfected-control groups did not differ significantly (analyses of variance), so the data obtained from these monkeys were combined for purposes of illustration in the various figures that are presented. Statistical comparison of the results obtained from treated groups with the untreated-infected controls was not carried out because of the possibility that the survivors from the latter group would not be representative of the population from which they were drawn.

Figure 3 illustrates the frequency with which Klebsiella were isolated from the blood and pharynx of the various groups. The effect of therapy was marked, particularly in the clearance of bacteremia, but differences in response due to the route of therapy were not obvious. When initiated at 24 hr, i.m.-treatment appeared to promote better clearance from pharynx and blood than the aerosol, but when initiated at 30 hr, the aerosol therapy seemed to be more effective than the i.m. The differences were not statistically significant, however, when tested at the $P \leq 0.05$ level (χ^2 test, Yates' correction).

Figure 4 shows respiratory rate and rectal temperature curves. With treatment initiated at 24 hr, the respiratory rate of the aerosol-treated group was significantly greater than the i.m.- treated monkeys on four of the six post-treatment days (days two, five, six, seven). This indicated that kanamycin aerosols may have had an adverse effect on infected monkeys. The uninfected monkeys that received aerosols did not show this effect.

It is of interest to note here that all infected monkeys showed labored inspiratory and expiratory patterns beginning on day one after infection and continuing through day seven, regardless of treatment (not shown). On the other hand, only the i.m. therapy significantly reduced the frequency of coughing (not shown), but coughing in the aerosol-treated group seemed to be related to inhalation of aerosol, and usually stopped within a few hours after the monkey was removed from the treatment box.

Kanamyc'n treatment significantly reduced fever in monkeys, regardless of route of administration (figure 4). However, i.m. therapy initiated at 30 hr seemed to reduce fever more rapidly than the aerosol, since the difference between the two routes was highly significant ($p < .0.005$).

Figure 5 shows the food consumption and weight changes during infection and treatment. Although sporadic anorexia was noted in all infected monkeys, only the group that was given i.m.-treatment beginning at 30 hr showed significant persistent loss of appetite. All infected monkeys lost weight, and there was evidence that treatment beginning at 24 hr terminated the weight loss about 48 hr later. Treatment beginning at 30 hr was ineffective in this regard. The data obtained on the two 30-hr groups indicated that not all of the loss of weight was due to anorexia. Although the animals that were treated with aerosols regained their appetite within 48 hr after treatment was initiated, they lost weight as rapidly as the i.m.-treated groups in which anorexia was more prolonged.

Plasma protein levels. We have previously reported that the concentration of several serum proteins was increased during respiratory K. pneumoniae infection and speculated that determination of these proteins would have prognostic value as well as provide information on biochemical pathogenesis [2, 12]. The effect of antibiotic therapy upon several of these proteins is given in figures 6 and 7. The concentrations were normalized to the baselines.

The effect of aerosol therapy on concentrations of α_1-antitrypsin was not different from that of i.m. therapy initiated at 24 or 30 hr. The values for treated monkeys did not deviate significantly from those of the uninfected controls when therapy was initiated at 24 hr, and were only slightly higher in the group treated initially at 30 hr. The pooled values of noninfected controls were about 25% greater than baseline, indicating that either intratracheal inoculation of sterile fluid or handling stimulated this particular response; albeit to a lesser degree than infection.

Figure 6 also shows the changes that occurred in haptoglobin concentration during infection. The effect of therapy was manifested only in the return to baseline values at 6 days in all treated groups in contrast to the infected-control monkeys. No effect of route of treatment was seen.

The lysozyme activity found in the various groups is presented in figure 7. Marked variation was observed between monkeys within each group, and the values between groups were not significantly different.

Ceruloplasmin concentrations seemed to be affected by aerosol therapy (figure 7). At both 24 and 30 hr after treatment was initiated, the concentrations in aerosol-treated monkeys were lower than those in i.m.-treated animals.

DISCUSSION

In this report we present evidence that 15 mg/kg/day of kanamycin, given either i.m. or by aerosol, were equally effective in preventing mortality in K. pneumoniae-infected squirrel monkeys. Generally, the effect of the two routes on the degree of morbidity also was equal. In view of these observations, there seems to be no basis for the use of aerosols in the treatment of acute respiratory K. pneumoniae infection in squirrel monkeys. It is possible, however, that a dose-response determination would favor the aerosol because kanamycin is complexed with DNA in the lungs [13] and thus persists in effective concentration for a longer time after aerosol than after i.m. administration. Thus the aerosol could be useful in those patients in whom antibiotic toxicity is a problem.

In a previous publication [1] we stated that aerosolized kanamycin was more effective in the treatment of K. pneumoniae infection in mice than was an equal dose injected i.m. Although direct comparisons between species are risky, it is true that K. pneumoniae causes bronchopneumonia in mice [1] and lobar pneumonia in monkeys [2]. If the difference in effect of the two routes is due to the kind of pneumonia involved, then, in the monkeys with lobar pneumonia, aerosol would not be expected to penetrate readily to the involved alveoli in the presence of large amounts of fluid and cellular material. On the other hand, the blood supply to the bronchi is not as voluminous as to the alveoli. Therefore, i.m. kanamycin would not be present in bronchial tissue

in as high concentrations as the aerosolized antibiotic, and aerosols would be more effective for the treatment of bronchopneumonia.

Several of the observations made during these experiments are worthy of special mention. The rise in respiratory rate in monkeys first treated with aerosols at 24 hr suggested possible pulmonary irritation resulting from inhalation. That tachypnea did not occur in the uninfected control that was given aerosol indicates that an interaction with infection had occurred. On the other hand, no evidence of bronchial irritation has been reported during aerosol treatment of human patients [14, 15]. The difference between the humans and monkeys might be due to differences in the amount of antibiotic inhaled. Resolution of this possibility would require execution of dose-response experiments.

Another example of interaction of infection and treatment occurred in monkeys first treated i.m. at 30 hr. This treatment caused marked anorexia, yet weight loss in the aerosol-treated monkeys was as extensive, thus suggesting that much of the loss of weight must be attributed to factors other than reduced food intake.

The effect of therapy on several metabolic phenomena is of interest. Both routes of treatment inhibited the rise in α_1-antitrypsin levels after infection. However, since the role of this protease inhibitor in infectious disease is poorly understood [16] the significance of the effect is unknown. In addition, it is not

known whether the therapy-related inhibition of increase in blood levels reflects an effect on production rate, turnover rates or of distribution. Resolution of this question would require the determination of tissue concentrations and kinetic studies procedures that were not feasible during these experiments.

Finally, an objective of this study was to determine whether changes in plasma protein concentration might provide indices of prognostic value. Of the four proteins included in our study, only α_1-antitrypsin seems to be of value in this regard.

Both intramuscular and aerosol therapy, therefore, effectively prevent mortality in monkeys suffering from pneumonia due to K. pneumoniae. Neither of the routes of administration seems to offer an advantage over the other in reducing morbidity. In view of the greater simplicity of administering the i.m. therapy it must remain the treatment of choice.

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Table 1. Mortality of squirrel monkeys infected with Klebsiella pneumoniae after treatment with kanamycin

Description	n	Dead	%
Uninfected kanamycin controls (aerosol)	7	0	0
Uninfected kanamycin controls (i.m.)	15	0	0
Sham controls	15	0	0
K. pneumoniae controls	21	14	67
Infected-i.m. treatment at 24 hr	15	0	0
Infected-i.m. treatment at 30 hr	14	1	7.1
Infected-aerosol treatment at 24 hr	8	0	0
Infected-aerosol treatment at 30 hr	7	0	0

Figure Captions

Figure 1 - Box for the exposure of monkeys to aerosols of antibiotics (right door removed for clarity).

A - aerosol sampling tube; B - left door; C - exercise bar; D - sampling port; E - exhaust port; F - refuse tray; G - perch; H - partition.

Figure 2 - Schematic diagram of aerosol therapy system.

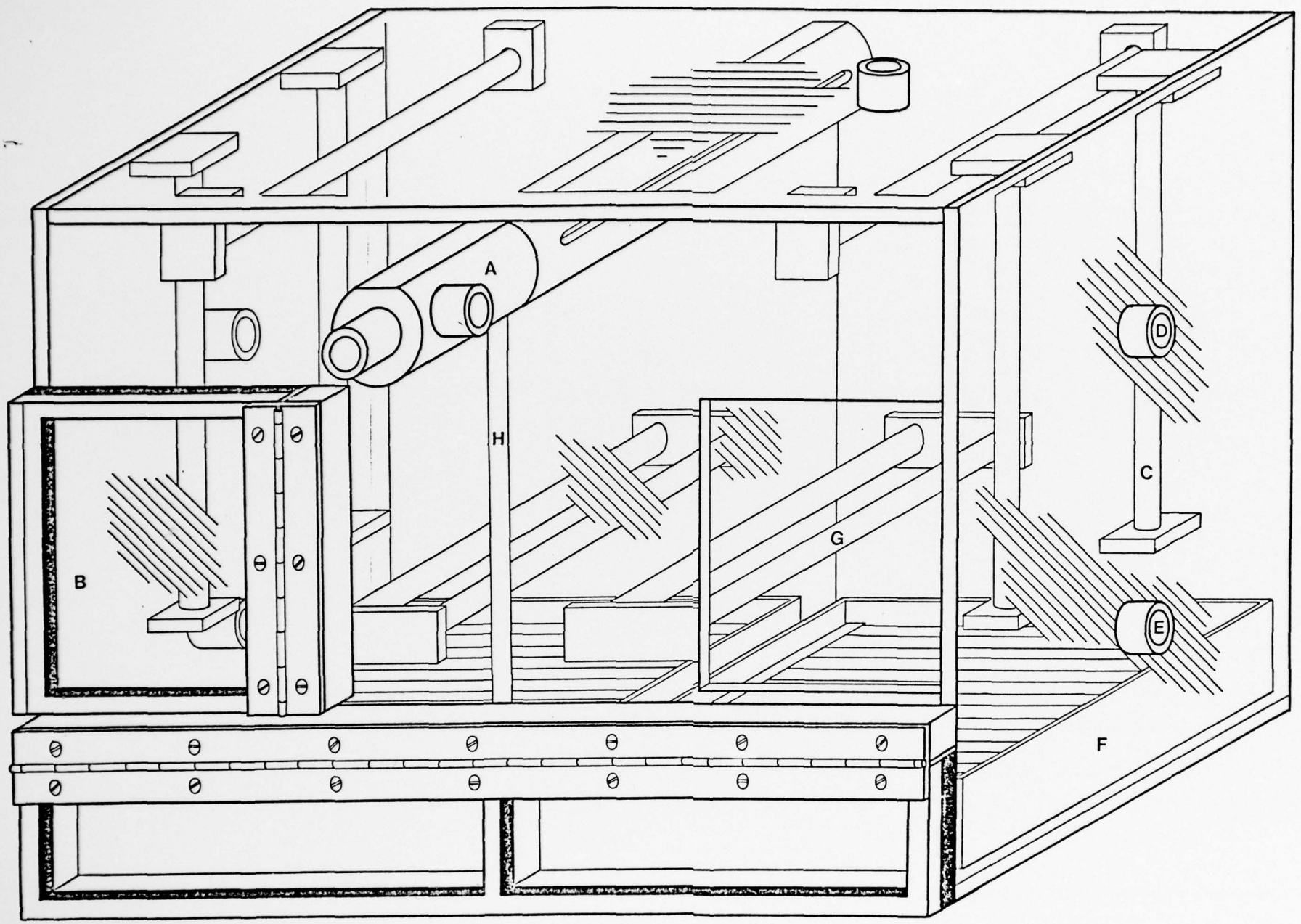
Figure 3 - Recovery of K. pneumoniae from blood and oropharynx

Figure 4 - Respiratory rate and temperature of treated monkeys. Cross-hatched bars represent the mean of the pooled controls ± standard error of the mean.

Figure 5 - Food consumptions and weight change in treated monkeys. Cross hatched bars represent the mean of the pooled controls ± standard error of the mean.

Figure 6 - α_1-antitrypsin and haptoglobin levels. Cross hatched bars represent the mean of the pooled controls ± standard error of the mean.

Figure 7 - Lysozyme and ceruloplasmin levels. Cross-hatched bars represent the mean of the pooled controls ± standard error of the mean.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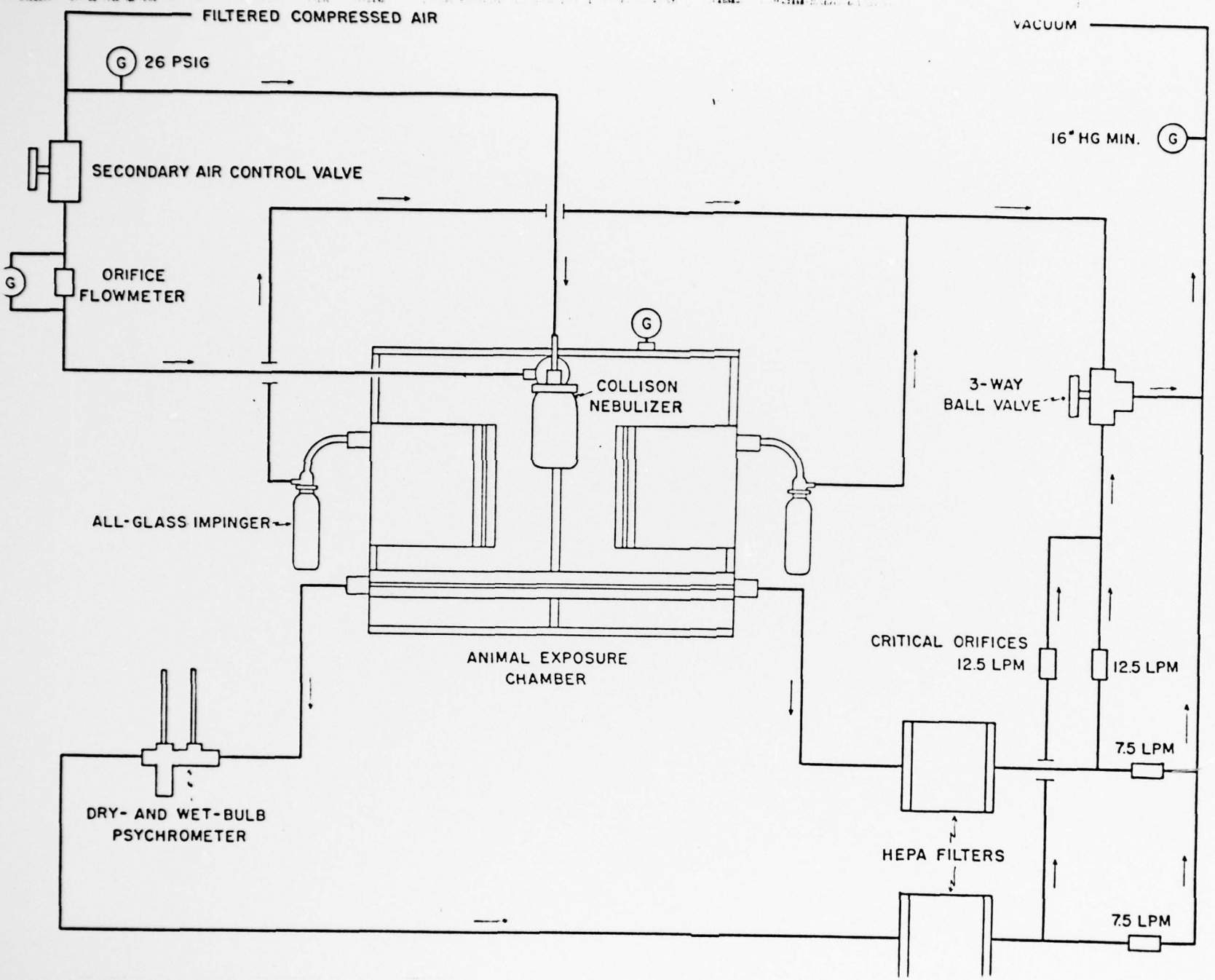
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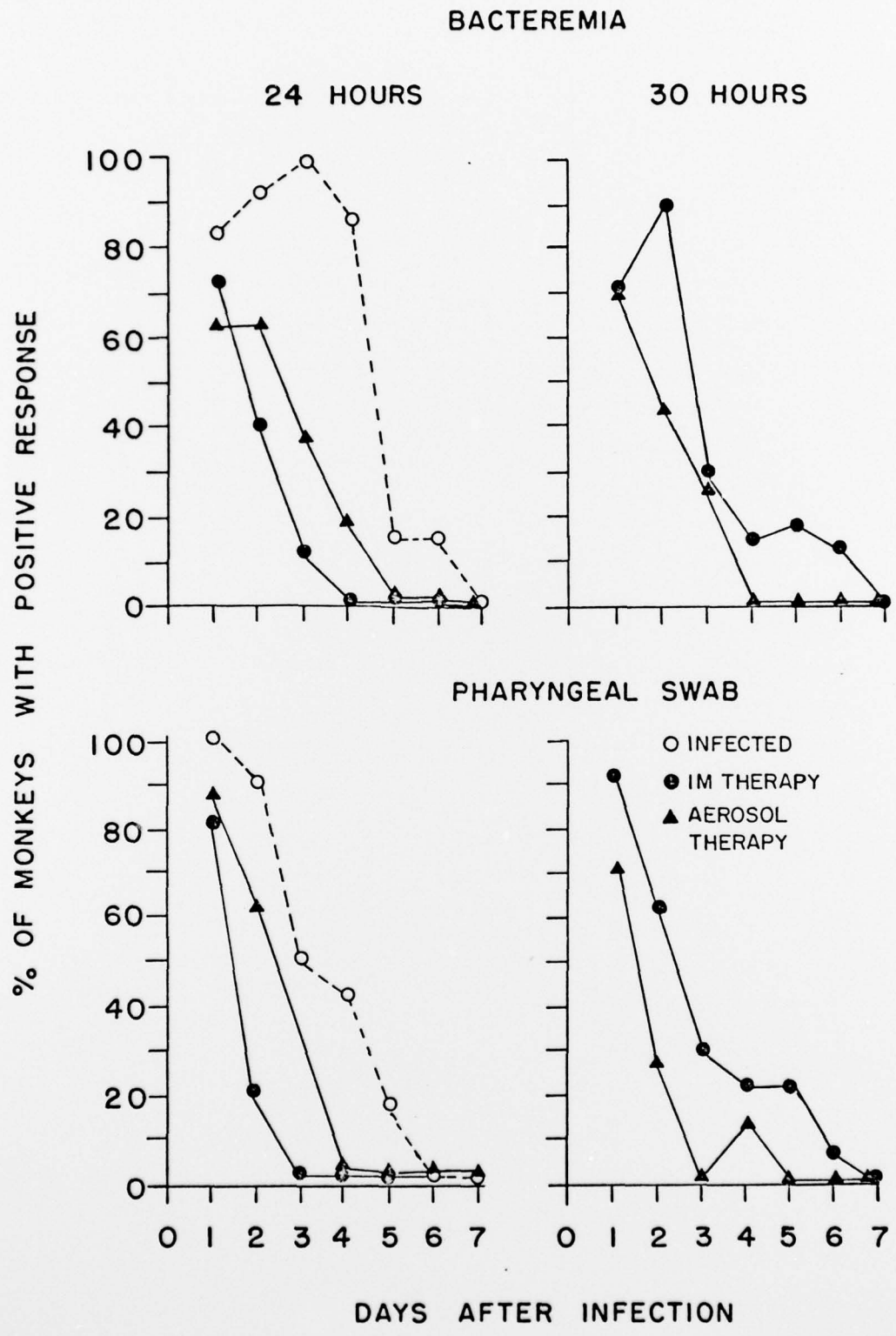
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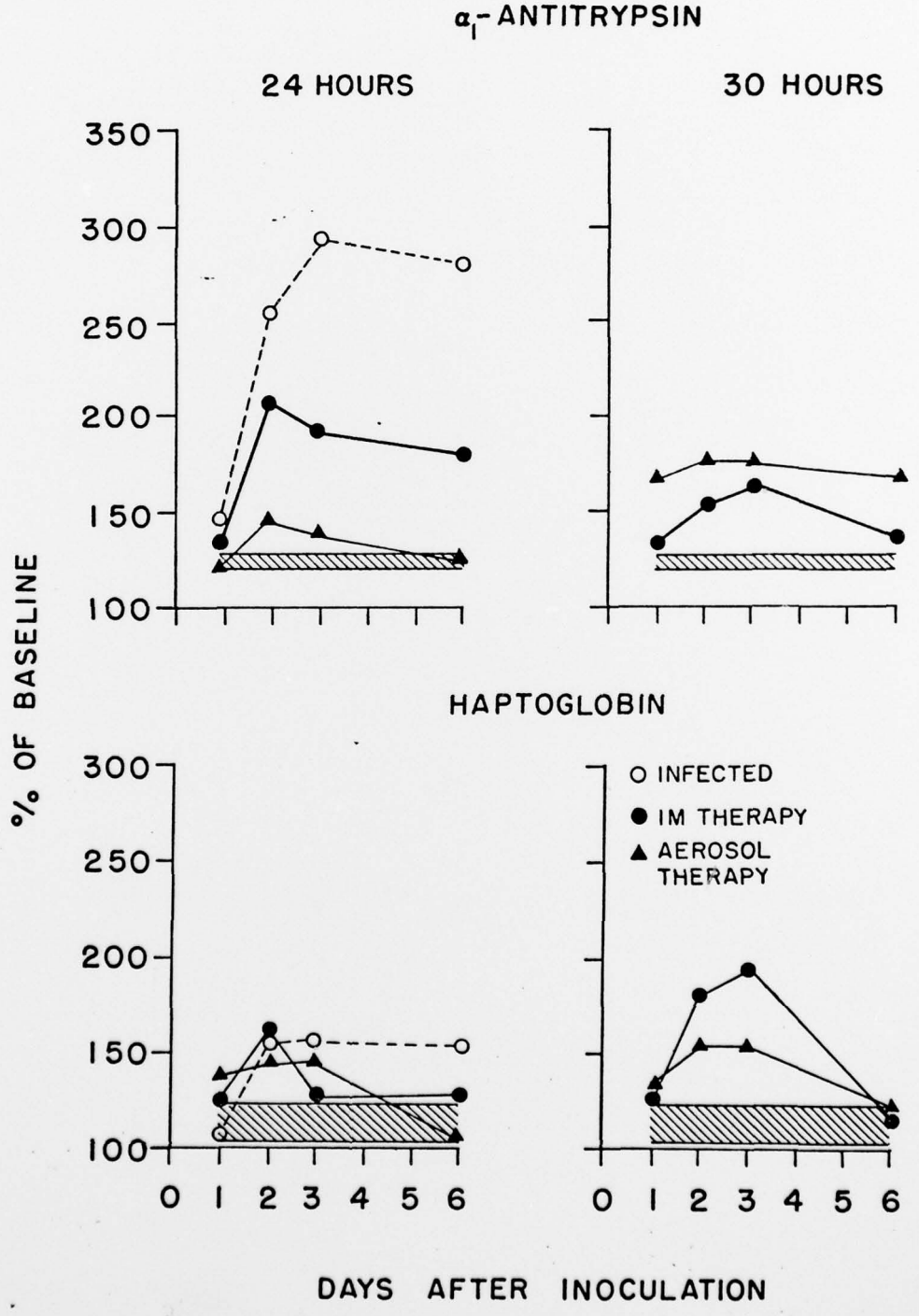
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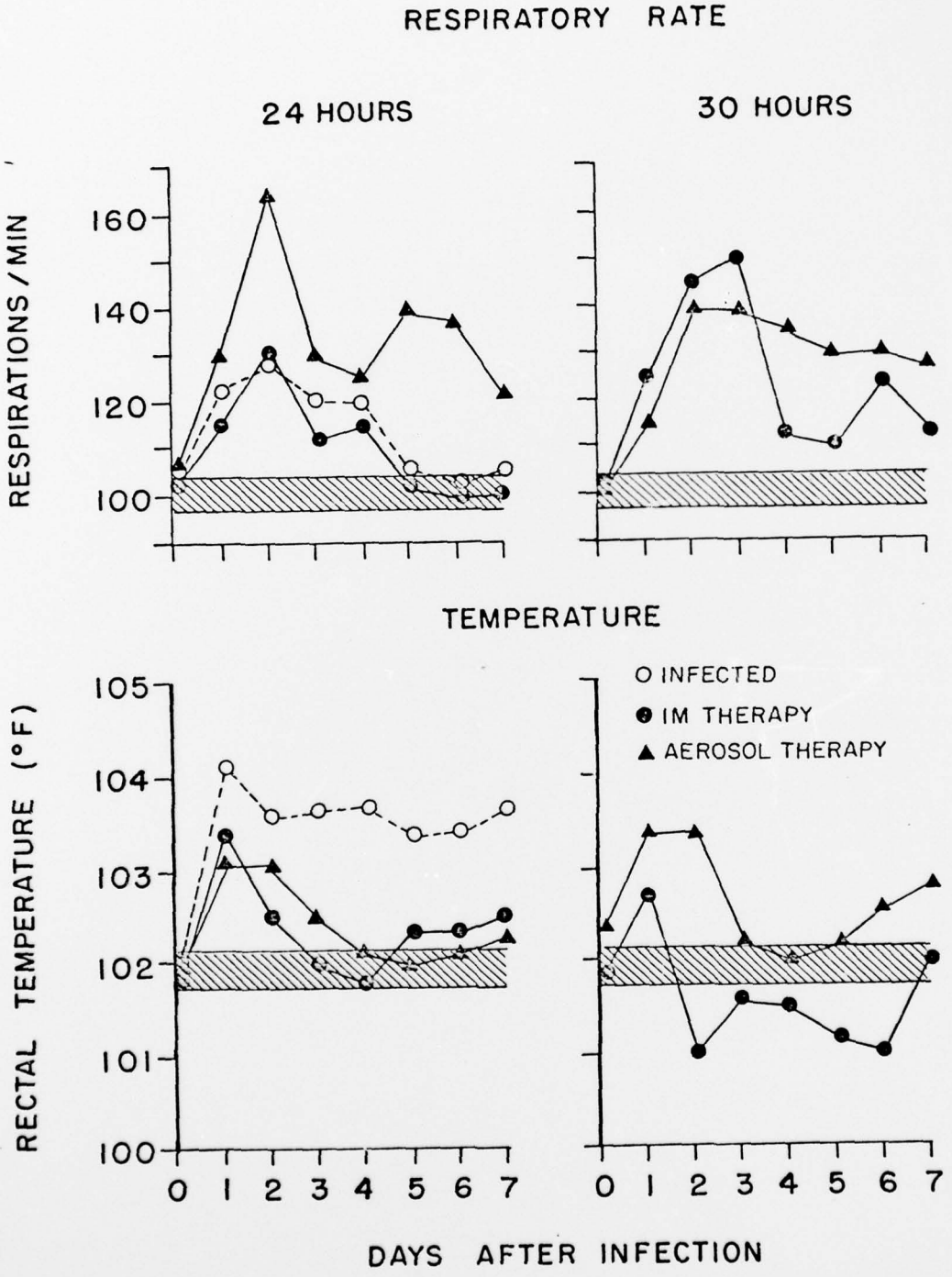
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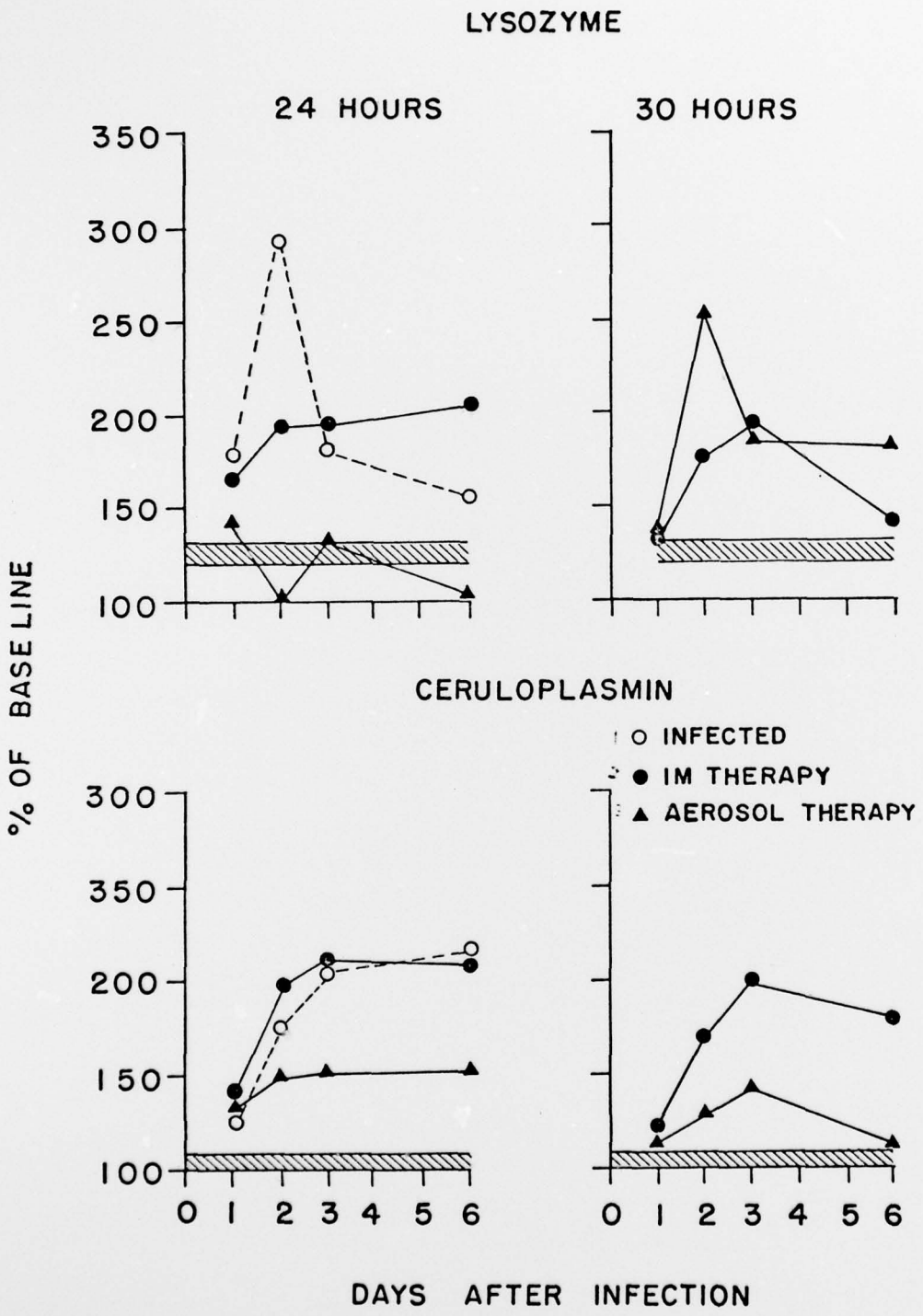
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