have a more solid foundation on which to discern real and not apparent results since the intracerebral route could be counted upon to induce lethal encephalitis in every instance among controls. It therefore follows that what was concluded from the experiments with mice and the intracerebral route of inoculation might not correspond with the results of tests on larger laboratory animals, such as guinea pigs and monkeys, and on the use of peripheral (nonneural) route of inoculation of virus. Then, of necessity, smaller numbers of animals have to be used and the peripheral route of viral exposure is not always disease-producing in the control animals.

What is significant is that in the present investigation a certain dosage of antiserum which could prevent infection at one particular stage during the course of infection, when the virus was even actively multiplying, was ineffective in another, when virus was increasing, or remained stationary at a higher level, Either the antiserum was incapable of neutralizing the virus at its place of multiplication[4] or an amount of serum might have been needed that in terms of blood volume of the host was impractical to use, or changes in the tissues progressed to a degree which could no longer be influenced by serotherapy. It should be stressed, however, that the stage during which antiserum was ineffective was one in which the treated animals could still appear to be normal to the observer.

Summary. If 2 cc of rabbit hyperimmune antiserum of high titer were given intraperitoneally to mice of about 17 g weight—a prodigious amount since it represents twice the total blood volume of the host—encephalitis was prevented from developing in animals receiving intracerebrally ordinarily lethal doses of the virus of Western equine encephalitis. The preventive effect was noted when the virus was multiplying but had not as yet reached its maximal titer. Thus, if animals were treated with antiserum 2 hours before, at the same time, or from 4-16 hours after the virus was inoculated, it was effective in preventing an attack of encephalitis. Even if serum was administered 24, 36 or 48 hours after the virus certain but not all mice survived without recognizable signs of the disease. At 72 hours, however, only an exceptional mouse survived after serum treatment. Finally, at 73 or more hours after virus inoculation, when definite manifestations of experimental Western equine encephalitis could be observed, injection of antiserum was found to have no influence on the course of the lethal infection.

[4] Rivers, T. M., *J. Am. Med. Assn.*, 1948, **136**, 291.

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Penicillin Aerosol in the Treatment of Experimental Pneumococcus Pneumonia.*

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Inhalational therapy with penicillin mist, or aerosol, in acute and chronic respiratory infections in man has recently been receiving the attention of clinical investigators both here and abroad.[1-10] In fact, the clinical

* Work done under a grant from the Medical Division of the Chemical Warfare Service, Edgewood Arsenal, Md.

[1] Garthwaite, B., and Barach, A. L., *Am. J. Med.*, 1947, **3**, 261.

[2] Olsen, A. M., *Proc. Mayo Clinic*, 1945, **20**, 184.

[3] Segal, M. S., and Ryder, C. M., *New Eng. J. Med.*, 1945, **233**, 747.

application of this method of administration of the drug has outdistanced controlled animal experiments designed to prove its effectiveness as a prophylactic or therapeutic measure. Barach and co-workers[10] were the first to show, in experiments on rats infected intraperitoneally with a fatal dose of hemolytic streptococci, that inhalations of penicillin aerosol considerably reduced the percentage of mortality as compared with that of an untreated group. The degree of protection was not so great, however, as that conferred by intramuscular injection of the drug. Wilson and collaborators[11] in studies on experimental pneumonia which were concurrent with ours, reported that inhalations of penicillin aerosol at 6, 12, and 24 hours after intrabronchial infection of rats with pneumococci caused survival rates which compared favorably with those resulting from intramuscular injection of similar doses of the drug (97% and 100% respectively). We briefly reported[12] the results of experiments in which the prophylactic and therapeutic effect of penicillin aerosol on pneumococcus pneumonia in rats was determined. The present report includes a description of methods used by us for the production of pneumonia in rats and for the administration of the aerosol, as well as an elaboration of the results of treatment at varying times both before and after infection.

Methods. Method of Producing Lobar Pneumonia in Rats. The method of Nungester and Jourdonais[13] for intrabronchial inoculation of mucin-suspended pneumococci was used, with some modifications which in our experience simplified the technique. The inoculations were made with a 3-inch 16-gauge Ingall cannula, to the proximal end of which was attached a Fordyce handle.† The distal end of the cannula was bent over about one-quarter inch from the tip at an angle of 4° from the axis. Albino rats, weighing between 200 and 250 g, were deeply anaesthetized with ether and the cannula was inserted through the mouth into larynx and trachea. We found that with sufficient practice this could be accomplished by the sense of touch, rather than visually with the aid of a spot-light reflected into the throat, as recommended by Nungester. The degree of anesthesia was found to be highly important, since a lightly anesthetized animal showed a greater degree of spasm of the laryngeal muscles when the cannula made contact. This was not true of deeply anesthetized rats. When the tip of the cannula has entered the trachea the operator can feel the "slip" of the cartilaginous rings over the cannula's blunt, well-rounded tip. This is in contrast to the "smoothness" felt when the cannula enters the esophagus. A verification test of the position of the cannula was, however, always made by attaching to the cannula a piece of rubber tubing (fitted to an adaptor) and observing a bubble of air rising through water when the rats exhaled. After checking on the cannula's position in this manner it was gently pushed further into the trachea until greater resistance was felt at the bronchial bifurcation. At this point the handle was rotated through 90° to the right, so that the bent tip would follow into the right bronchus. Dissection of the tracheobronchial area in trial animals then revealed the tip of the cannula lying either at the entrance to the right-middle or lower lobe, or within the cardiac lobe. A previously-filled

[4] Knott, F. A., and Clark, W. H., *Lancet*, 1945, **1**, 468.

[5] Vermilye, H. N., *J. Am. Med. Assn.*, 1945, **129**, 250.

[6] Humphrey, J. H., and Joules, H., *Lancet*, 1946, **2**, 221.

[7] Southwell, N., *Lancet*, 1946, **2**, 225.

[8] Morse, F. W., *J. Am. Med. Assn.*, 1946, **132**, 272.

[9] Abramson, H. A., *An. Allergy*, 1946, **4**, 440.

[10] Barach, A. L., Silberstein, F. H., Oppenheimer, E. T., Hunter, T., and Soroka, M., *Ann. Int. Med.*, 1945, **22**, 485.

[11] Wilson, C. E., Hammond, C. W., Byrne, A. F., and Bliss, E. A., *Bull. Johns Hopkins Hosp.*, 1945, **77**, 411.

[12] Hadley, F. P., Hadley, P., McIlroy, A. P., and Laurent, A. M., *J. Bact.*, 1946, **51**, 612 (abstract).

[13] Nungester, W. J., and Jourdonais, L. F., *J. Bact.*, 1935, **29**, **34**, and *Science*, 1935, **81**, 74.

† Supplied through the courtesy of Becton, Dickinson and Company, Rutherford, N. J.

tuberculin syringe, containing the proper inoculum and with adjustment for the air volume already present in the cannula itself, was attached to the cannula base and the injection of mucin-suspended pneumococci completed. The rats tolerated this manipulation well, showing only a few minutes of dyspnea following inoculation.

A Type I strain of pneumococcus ("Bailey" strain, obtained from Dr. Eleanor Bliss) was used in all experiments. The culture was maintained in beef-infusion broth containing 5% rabbit blood and 0.1% agar. It was passed through a mouse and re-isolated on the day preceding each major experiment. Frequent tests of virulence in mice revealed a constant M.L.D. equivalent to a dosage of 10^{-8} cc (2 to 4 organisms per cc), injected intraperitoneally. However, approximately 600 organisms, suspended in mucin, were necessary to kill rats when inoculated by the intrabronchial route. In the following experiments intrabronchial inoculations were made with 60,000 mucin-suspended organisms, or 100 M.L.D. by this route (0.15 cc of a 10^{-3} dilution of a 16 hour blood broth culture). The mucin used was a 6% aqueous suspension of Wilson's granular mucin, Type 1701 W, autoclaved at 15 pounds for 15 minutes and then adjusted to pH 7.3.[14]

Under the conditions outlined above typical lobar pneumonia resulted in the untreated rats within 18 hours of inoculation. This was accompanied by septicemia and consolidation of the inoculated lobe, and a high percentage of mortality resulted. For example, in one experiment in which 30 rats were inoculated intrabronchially with mucin-suspended pneumococci, 100% of the animals died with an average survival time of 42 hours. At autopsy all animals showed consolidation of a portion or all of the inoculated lobe, and varying degrees of congestion and edema of the remaining lobes. Sections revealed acute pneumonitis, with congestion, edema, fibrin and leucocytic infiltration of the alveoli. One of these 30 animals showed fibrinous pleurisy, a condition which we found to occur more frequently in those rats which survived longer than 48 hours.

Method of Producing and Administering Penicillin Aerosol. For each experiment 20 rats were usually employed, all of which were inoculated as described. Ten were then given treatment and 10 used as untreated controls. The time after inoculation at which treatments were begun, as well as the frequency of treatments, varied in different experiments.

The rats to be treated were placed together in a wooden box, 8 x 16 x 16 inches, having a capacity of about 30 liters of air. The box was covered by a loosely-fitting glass plate through a hole in which a rubber tube was inserted as an air vent. The tip of the Vaponephrin nebulizer entered an opening at one side of the box 1½ inches below the top, while the lower end of the nebulizer was attached by a rubber connection to the L-14 oxygen flowmeter which measured the air flow, in liters per minute, through the nebulizer. This in turn was attached to an air pressure gauge and to the air pressure line. The measured penicillin solution (sodium or calcium penicillin, Pfizer), usually in a concentration of 40,000 units per cc of saline, was placed in the nebulizer as needed. Before the rats were admitted to the chamber the penicillin was sprayed into it for 5 minutes at an air-flow rate of 4 liters per minute. The chamber was thereby filled with the nebulin to such an extent that a heavy fog was visible throughout the box and could be seen escaping through the airvent. This was taken as a good indication of the rapid dissemination of the nebulin through the treatment chamber itself. Treatment periods consisted of 15 minute exposures. After the first minute or 2 the rats were at rest and appeared to be little disturbed by the effects of inhaling the penicillin mist.

Estimation of amount of penicillin inhaled by rats. The approximate number of units of penicillin inhaled by each rat was estimated by multiplying the tidal air for rats,‡ in cc

[14] Miller, C. P., and Castles, R., *J. Infect. Dis.*, 1936, **58**, 263.

‡ Formula of Navy Med. Res. Unit No. 1[15] for tidal air of albino rats:

$V = 212\ W^{3/4}$ where V is the volume of air inhaled, in cc per minute, and W is the weight of rat in kg.

TABLE I.
Prophylactic Effect of Penicillin.
(Administered previous to intrabronchial inoculation of pneumococci.)

Menstruum for organisms	Treatment		Results			
	Route of administr.	Total units (estimated)	No. of rats	No. survived	% survival	Avg survival time (hr)
Mucin	Inhalation	5600 to 7600	20	1	5	56
	Intramusc.	5000	10	0	0	41
	Controls	0	30	0	0	42
Broth	Inhalation	3600 to 4600	20	19	95	40
	Intramusc.	5000	10	10	100	—
	Controls	0	30	3	10	42

per minute, by the number of drug units in each cc of air leaving the atomizer (estimated from the air and fluid delivery of the atomizer). Thus the *estimated* inhalational dose of penicillin for a 200 g rat (tidal air of 63 cc/min.), during a 15 minute exposure to a mist produced from a solution of 40,000 units/cc, with an air-flow through the atomizer of 4 liters per minute, was approximately 1500 units (0.16 cc of penicillin was nebulized per minute).

We recognize that this estimation can give only an approximation of the actual dosage, since it is based on two assumptions,—first, that after the preliminary "saturation" of the chamber, the concentration of nebulin within the chamber maintained a fairly constant equilibrium, and that this concentration was the same as that of the nebulin upon leaving the nozzle of the atomizer. Considering the small volume of the chamber, and the constant flow of nebulin through the chamber and out the vent, the concentration in the chamber is believed not to have varied sufficiently to disturb the results. The *second* assumption was that the full amount of penicillin inhaled was retained in the lungs. We know this cannot be true but it is not possible at the present time to estimate that fraction which was again exhaled. Lyons and coworkers[16] have reported an experiment in which only about 20% of inhaled radioactive chromic phosphate was retained in the lungs of a monkey exposed to a mist of this substance for 40 minutes. With a variable of this order it is impossible at this time to present for our own experiments more accurate figures on the inhalational dosage. It seems certain, however, that our *estimated* drug dosage is in excess of the amount actually received and retained by the rats. Therefore, in evaluating the therapeutic effect of penicillin aerosol on pneumococcus pneumonia in rats the actual amount of the drug which gave protection or resulted in cure was less than our figures indicate.

Results. Prophylactic Effect of Penicillin on Pneumococcus Pneumonia. It was considered desirable to learn whether penicillin would prevent the development of pneumonia, when administered by either the inhalational or the parenteral route *previous to* the intrabronchial inoculation of pneumococci. A comparison was also desirable between the effect of such treatment on animals inoculated with mucin-suspended and broth-suspended organisms. The rats receiving treatment by the inhalational route were exposed to penicillin aerosol for two 15-minute periods at 4 hours and at one hour previous to the inoculation of pneumococci. The parenterally-treated rats received, by intramuscular injection, 2500 units of penicillin at each of the same two periods. The results are shown in Table I.

Of 20 rats receiving mucin-suspended pneumococci following exposure to penicillin by inhalation, only one survived. Ten additional rats in the same group, which received penicillin intramuscularly, showed no survivals. All 30 control untreated rats died. Thus, neither the inhalational nor the parenteral adminis-

[15] Personnel of U. S. Navy Med. Res. Unit No. 1, and Kleiber, M., *Science*, 1944, **99**, 542.

[16] Personnel of Naval Lab. Res. Unit No. 1, and Lyons, W. R., *Am. J. Med. Sc.*, 1944, **207**, 40.

tration of penicillin, previous to the inoculation of mucin-suspended pneumococci, prevented death from pneumonia. The average survival time (56 hours) of the group receiving inhalations was somewhat higher than that of the parenterally-treated group (41 hours) and the controls (42 hours).

On the other hand, when broth-suspended organisms were inoculated, following similar administration of the drug, the survival rates were high. In the group receiving inhalational therapy 95% survived; of those receiving intramuscular injections 100% survived, as compared with 10% survival among the controls.

The mechanism by which a prophylactic dose of penicillin, given by inhalation or parenterally, prevents infection by pneumococci suspended in broth, while it does not prevent infection by organisms suspended in mucin, is not clear. Untreated control animals in both groups showed similar high fatality rates (100% for the mucin group, 90% for the broth group), as well as the same average survival time (42 hours). It is true, as Nungester[17] has pointed out, that broth-suspended pneumococci, injected intrabronchially, cause a pulmonary infection showing less consolidation than when mucin-suspended organisms are injected. In harmony with this observation we found that of 30 control rats injected with broth suspensions approximately 50% showed, at death, only a severe pulmonary congestion, while about 50% showed some degree of consolidation, 20% of the latter group involving more than half of one lobe, and 30% involving less than half. On the other hand, among the 30 control rats receiving mucin suspensions, all showed some degree of consolidation at death, 83% showing more than half of one lobe consolidated. All animals showed septicemia at death.

One possible explanation of the failure of survival of animals receiving mucin-suspended organisms following prophylactic administration of penicillin is that the mucin formed around the organisms a protective barrier against penetration by the penicillin. On the other hand, a chemically-inhibiting effect of mucin upon small amounts of penicillin is not out of the question, although a preliminary experiment carried out *in vitro* indicated that mucin has no inhibiting effect upon penicillin. We have also verified Nungester's observation that mucin does not support the growth of pneumococci, *in vitro*. We believe that, until further study has been conducted, it is not possible to reach a conclusion on the causes for the difference in effect of penicillin (given prophylactically) on broth suspensions and mucin suspensions of pneumococci.

Therapeutic Effect of Penicillin Inhalations. In these experiments penicillin inhalations were given at various intervals following intrabronchial inoculation of *mucin-suspended* pneumococci. In Table II are presented 2 groups of experiments in which comparisons are made between the therapeutic effects of penicillin inhalations begun soon after inoculation, and those begun after a delay of 18 hours. In the first group (designated "Early" in the table) it may be noted that when 4 exposures to penicillin aerosol (each of 15 minute duration) were given, at 1, 3, 5 and 7 hours after inoculation 22 (73%) of the 30 rats so treated survived. When 8 treatment periods were given at 1, 3, 5 and 7 hours, and at 22, 24, 26 and 28 hours following inoculation, 100% of 20 rats survived. These survival rates are to be contrasted with those of the control untreated group (controls for all of these experiments are combined in the table) in which 9% of 78 rats survived.

In the "delayed" treatment group the first treatment was not given until 18 hours after inoculation. We had shown previously in untreated rats sacrificed at 18 hours, that consolidation of the inoculated lobe had already begun by this time, and septicemia had taken place. Treatment started at this time was therefore a severe test of the therapeutic effectiveness of the drug. Five treatments at 2-hour intervals beginning 18 hours after inoculation resulted in the survival of 69% of 16 rats; and 10 treatments at intervals from 18 to 72 hours caused a 70% survival

[17] Nungester, W. J., and Jourdonais, L. F., *J. Infect. Dis.*, 1936, **59**, 258.

TABLE II.
Therapeutic Effect of Penicillin Inhalations.
(Administered after intrabronchial inoculation of mucin-suspended pneumococci.)

	Treatments						
Group	No.	Hr after infection	Total estimated units	No. of rats	No. survived	% survival	Avg survival time (hr)
Early	4	1-3-5-7	5,000	30	22	73	73
	8	1-3-5-7; 22-24-26-28	12,000	20	20	100	
Delayed	5	18-20-22-24-26	7,500	16	11	69	85
	10	18 to 72	16,750	20	14	70	58
Control	0		0	78	7	9	48

among 20 rats. The survival time for those treated rats which died was in all cases higher than that for the fatal control cases, as seen in Table II.

These results indicate that penicillin, administered solely by inhalation of the aerosolized solution, has a definite therapeutic effect upon pneumococcus pneumonia in rats. In these experiments, approximately 70% of the animals were saved by either one series of four 2-hourly treatments, beginning one hour after inoculation; or by one series of five 2-hourly treatments, beginning 18 hours after inoculation. And 100% of animals (in 2 experiments involving 10 rats each) were saved by a combination of early and late treatments, *viz.*, four 2-hourly treatments beginning one hour after inoculation, followed by four 2-hourly treatments beginning 22 hours after inoculation. Only 9% of 78 control untreated rats survived.

Summary. 1. The prophylactic effect of penicillin, whether administered by inhalation or parenterally, upon experimental pneumococcus pneumonia in rats, was negligible if the infection (intrabronchial) was produced with pneumococci suspended in mucin. If, however, the organisms were suspended in broth the prophylactic effect was such as to determine a 100% survival rate.

2. When penicillin inhalations were given at intervals of 1, 3, 5 and 7 hours following intrabronchial infection with 100 M.L.D. of mucin-suspended pneumococci, a 73% survival rate resulted (9% for untreated controls) and the survival time of all treated rats which died was lengthened compared with that of the controls.

3. If the penicillin treatment by inhalation was delayed for 18 hours after infection (permitting the establishment of lung consolidation and septicemia), and then given from the 18th to the 26th hour, at 2-hourly intervals, the survival rate of the treated animals was 69%.

4. When penicillin inhalations were given in 2 series of treatments, at 2-hourly intervals from the 1st to the 7th hour after infection and again at 2-hourly intervals from the 22nd to the 28th hour, 100% of the animals survived compared with a 100% fatality rate for controls in this experiment.

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