SULFONAMIDE CHEMOTHERAPY OF COMBINED INFECTION WITH INFLUENZA VIRUS AND BACTERIA*‡

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Most of the deaths during the pandemic of influenza in 1918–19 were probably due to secondary bacterial pneumonia superimposed on a primary viral infection of the lung (3–5). Recent progress in the chemotherapy of bacterial pneumonia (6–8) has been sufficiently encouraging to suggest that such treatment may be effective in controlling secondary pneumonia in influenza pandemics. The present investigations have concerned the study of sulfonamide chemotherapy in combined viral and bacterial infections produced experimentally in laboratory animals under carefully controlled conditions. The results of these experiments indicate that the sulfonamide drugs act as effective antibacterial agents even in the presence of active infection with influenza virus.

Methods and Materials

Virus.—The PR8[1] and Weiss[2] strains of Type A influenza virus and the Lee[2] strain of Type B influenza virus were passed through mice and suspensions of infected mouse lung were stored in a cabinet containing solid carbon dioxide.

In the preparation of virus for inoculation of rats, infected mouse lungs were ground without abrasive and a 10 per cent suspension was prepared as described in previous experiments with rats (9).

For inoculation of mice, infected mouse lungs were ground with fine sterile sand, 10 per cent suspensions were prepared with broth,[3] and aerobic cultures were made on blood agar plates. Such suspensions were stored in the cabinet containing solid carbon dioxide until the results of cultures were obtained. All batches containing bacteria were discarded and the uncontaminated lots were thawed and subjected to horizontal centrifugation. The supernatant fluid of each batch was distributed after centrifugation in 0.2 ml. amounts and stored again in the cabinet containing solid carbon dioxide. Portions of the same batch were used both for determining the sublethal dilution and for the final experiment. At the time of use, a single tube was thawed and serial tenfold dilutions in broth were made using a separate pipette for each dilution.

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‡ Preliminary reports have been made on two phases of this investigation (1, 2).

[1] Kindly supplied by Dr. S. E. Sulkin.

[2] Kindly supplied by Dr. Thomas Francis, Jr.

[3] Tryptose or tryptose phosphate broth (Difco).

Bacteria.—Two strains of bacteria virulent for mice were used—the A5 strain of Type I pneumococcus (10, 11), and the C203 strain of group A hemolytic streptococcus.[4] The cultures were passed frequently through mice by intraabdominal inoculation in order to maintain virulence. Preservation of the bacteria was accomplished by keeping cultures in defibrinated rabbit blood in the refrigerator or the cabinet containing solid carbon dioxide.

In preparation for intrabronchial inoculation, the bacteria were allowed to grow for 6 to 12 hours in horse or beef infusion broth containing rabbit blood, and from the resulting culture serial tenfold dilutions in tryptose or tryptose phosphate broth were prepared. One of these dilutions was used for inoculation and two pour plates were made from 1 ml. each of the dilution 10^{-8} in order to calculate the number of bacteria in the inoculum.

For administration by inhalation of fine droplets, the bacteria were allowed to grow for 24 hours on blood agar slants, and from this growth a heavy suspension of bacteria in physiological salt solution was prepared.

Animals.—Adult white rats weighing 150 to 200 gm. were used in all experiments.

Swiss mice were of a single strain and obtained from the same dealer.[5] The ages of the mice varied from about 3 to 6 weeks.

Intrabronchial Inoculation of Rats and Mice.—Rats were inoculated intrabronchially by a method already described (12).

In preliminary experiments with mice intranasal inoculation under light ether anesthesia was carried out in the usual manner but inconsistent results were obtained. Therefore, the method of intrabronchial inoculation of rats was modified so that it could be used in mice in order to insure the inoculum reaching the lung in every instance.

For intrabronchial inoculation, the mice were anesthetized by intraabdominal injection of 0.4 to 0.5 ml. of a 2.5 per cent solution or chloral hydrate in physiological salt solution. The desired depth of anesthesia was regulated by variation in the amount of solution injected in different animals. When complete relaxation of the mouse was obtained the animal was held on its back on an inclined board by a rubber band passed over the upper incisor teeth. A small bulldog clamp was suspended from a superstructure by a rubber band and served to retract the tongue and keep the mouth open. The pharynx was then held widely open by the tips of a small Kelly clamp and illumination from a head mirror was used to visualize the larynx. The cannula employed for inoculation was made by filing off the point of a 20 gauge needle and bending the terminal 0.4 cm. to a slight angle. Under direct visualization the cannula was passed through the larynx with the tip pointing ventrad. After it was in the trachea, it was gently passed downward with rotation of the tip to the left and dorsad. Positive evidence that the cannula was in the trachea was always obtained by attaching it to a piece of rubber tubing connected to a small water manometer consisting of a glass tube with one end immersed in a flask of water. Oscillations of the level of water in the tube indicated respiratory excursions and it was found that there was a sharp decrease in their amplitude when the cannula entered the left main bronchus. When the cannula was in the bronchus, a tuberculin syringe was attached to it and the cannula removed after injection of the inoculum. The total amount of fluid injected was 0.1 ml., and, since the cannula itself held 0.05 ml. of fluid, the volume deposited in the bronchus was 0.05 ml. In order to prevent regurgitation after inoculation, the mice were suspended for 20 minutes by hooking the upper incisor teeth over rubber bands stretched between nails on a vertical board.

Determination of Sublethal Dose of Virus for Mice.—In order to determine the amount of virus that would produce non-fatal lesions, preliminary titration with tenfold dilutions of a given batch of virus was carried out by intrabronchial inoculation of mice and that dilution was chosen which allowed survival with gross pulmonary lesions. Another tube was then

[4] Kindly supplied by Dr. Rebecca Lancefield.

[5] Rockland Farms, New City, New York.

thawed and this dilution given to a larger number of mice to determine how near it came to the ideal in which all mice would survive for 14 days and yet show pulmonary lesions. With some suspensions, further modification of the dilution was made in order to achieve this end more closely. Table I shows tests of dilutions of batches of virus suspensions later used in the experiments. In nearly all animals the gross lesion involved part or all of the left lobe.

Inhalation of Fine Droplets Containing Bacteria.—Mice were made to inhale fine droplets of fluid containing bacteria by the use of a simple modification of apparatus devised by others for this purpose (13–15).

The mice were placed in a 5 gallon Pyrex glass jar containing some shavings and supported on its side. The mouth of the jar was 6 cm. in diameter and was tightly fitted with a rubber stopper held in place by a metal clamp attached to the neck of the jar. The stopper was perforated by two holes for glass tubes to be used for inlet and outlet. These tubes extended for 10 to 15 cm. into the jar and had perforations of their sides to prevent obstruction to the flow of air by the bodies of the mice.

The turbid suspension of bacteria was placed in an atomizer[6] (14) in 20 ml. amounts and the atomizer connected to a compressed air line. The pressure was maintained at 500 mm. of

TABLE I

Tests for Sublethal Dose of Influenza Virus in Mice

No. of experiment	Strain of virus	Dilution of virus	Mice inoculated	Mice surviving 14 days	Survivors showing lesions
1	PR8	10^{-7}	8	6	6
2	PR8	0.5×10^{-7}	13	8*	8
3	PR8	10^{-7}	8	8	8
4	PR8	10^{-7}	12	11	11
5	PR8	0.5×10^{-7}	15	14	13
6	Weiss	2×10^{-7}	10	8	6
7‡	Lee	10^{-4}	11	9	9

* Killed for observation of lesions on the 11th day.

‡ Done as a part of Experiment 4, Table VII.

mercury by observation of a mercury manometer connected by a side arm. Between the atomizer and the inlet of the jar was a small bottle serving as a trap to catch any large drops of fluid coming from the atomizer.

The outlet of the 5 gallon jar was connected to two bottles in series so that the air containing droplets with bacteria would bubble through 0.5 per cent cresol in both bottles. Over the outlet of the second bottle in series several layers of flannel were tied. A blood agar plate held over the flannel during operation of the system showed that this method prevented contamination of the air of the room with the pathogenic bacteria.

Sulfonamide Chemotherapy.—Rats were given sulfapyridine by gastric tube in amounts previously found to control experimental pneumococcal pneumonia (10). In the first and second experiments administration of the drug was started before inoculation with virus in order to minimize the possibility of secondary infection with other organisms before the injection of pneumococci. In these experiments, sulfapyridine was continued in those animals in which it was desired to give chemotherapy. In control rats, sulfapyridine was stopped and

[6] DeVilbiss Atomizer No. 180.

1½ hours before inoculation with pneumococci each animal was given para-aminobenzoic acid[7] in order to ensure the cessation of sulfonamide activity.

Mice were given sulfamerazine by gastric tube (16) in doses known to produce therapeutic levels in mice (17). 10 mg. of sulfamerazine were administered every day at 9 a.m. and 5 p.m. In two instances groups of mice were bled to death at the conclusion of treatment and the levels of sulfamerazine were determined by a standard method (18).

Observation of Results.—The development of pneumococcal infection in the rats was determined by daily cultures of blood taken from the tail (11) in animals still alive and by observation of gross lesions and cultures of the lung and blood of the heart at autopsy. Rats surviving 5 to 7 days after inoculation with pneumococci were killed with ether and similar observations made.

Evidence of bacterial infection in mice was obtained by mincing the left lobes of the lungs with scissors in a sterile Petri dish and preparing cultures of this tissue on blood agar plates. In all instances, the identity of pneumococci obtained in culture was confirmed by the Neufeld reaction with Type I rabbit antiserum and usually the number of organisms was so great in mice dead or dying of pneumococcal infection that demonstration of *Quellung* was readily made in direct preparations from minced portions of the left lung. Surviving mice were killed 14 days after original inoculation for inspection and culture of the lung.

For microscopic study of lesions in mice, the lungs were fixed by introducing Zenker's solution containing 10 per cent formalin into the trachea by a cannula as in experiments with rats (20, 11). Sections were stained with hematoxylin and eosin for study of the cellular reactions and by the Gram-Weigert method for demonstration of bacteria.

The statistical significance of the results has been confirmed by use of a formula for determination of the value of chi square with small numbers (19).[8]

EXPERIMENTAL PROCEDURES AND RESULTS

Sulfonamide Chemotherapy of Pneumococcal Pneumonia Superimposed upon Viral Pneumonia in Rats.—The demonstration of a mild infection of rats with influenza virus (9) made it possible to study combined viral and bacterial infections in this animal.

The viral infection was produced in rats by intrabronchial inoculation with heavy suspensions of pulmonary tissue of mice infected with the PR8 strain of influenza virus as in the earlier experiments, and 2 days later a suspension of Type I pneumococci was given by intrabronchial cannula with the same technique as that used for the virus in order to place the organisms as nearly as possible directly on the viral lesion. Half of the animals in each experiment were given sulfonamide chemotherapy and all were observed for the development of pneumococcal pneumonia and bacteremia. In rats developing pneumococcal infection death resulted in all animals, usually in 2 to 3 days. Extensive areas of dark red or gray consolidation were noted in the lungs of these rats and turbid fluid was frequently found in the pleural and pericardial cavities. Cultures of blood taken from the tail during life and from the heart at autopsy showed many pneumococci. Large numbers of organisms were demonstrated at autopsy also in the lung and the fluid of the pleural and pericardial cavities.

[7] 5 gm. of para-aminobenzoic acid were dissolved in 100 ml. of distilled water and the solution was neutralized with sodium hydroxide. 5 ml. of this preparation were given by gastric tube.

[8] We wish to acknowledge with thanks the advice of Dr. E. Gurney Clark concerning the statistical analysis.

The results of chemotherapy are seen in Table II and show that pneumococcal pneumonia was successfully treated with sulfapyridine in spite of the presence of infection of the same portion of the lung with influenza virus. The outcome of these experiments in rats seemed clear, but it was considered desirable to perform similar experiments in mice because of the relative mildness of the viral infection in rats.

Effect of Reinstillation of Fluid on Sublethal Viral Infection in Mice.—In planning experiments on combined viral and bacterial infection in mice, it was apparent that strict account would have to be taken of the phenomenon described by Taylor (21) who showed that the reinstillation of fluid into the respiratory passages of mice suffering from a non-fatal infection with influenza virus would convert the process into a fatal one.

TABLE II

Chemotherapy of Pneumococcal Infection Superimposed upon Influenza Viral Pneumonia in Rats

No. of experiment	Dose of pneumococci	Sulfapyridine chemotherapy	Development of fatal pneumococcal infection		
			Positive	Negative	Doubtful
1	24,000	Continued	0	12	
		Stopped*	5	5	
2	50,000	Continued	0	7	1
		Stopped*	6	2	0
3	900,000	Treated	0	8	3
		Not treated	10	0	

* Sulfapyridine was discontinued and para-aminobenzoic acid given in order to stop further sulfonamide action.

In order to test this observation of Taylor, mice were given sublethal doses of influenza virus by intrabronchial cannula and, 1 to 7 days later, were reinoculated with broth or physiological salt solution by the same method. The results shown in Table III confirm the observation concerning the effect of reinoculation of fluid on sublethal viral infection.

Chemotherapy of Combined Infection Produced by Inoculation of Mixtures of Virus and Bacteria in Mice.—The fact that an inoculation of fluid upon an existing sublethal viral infection causes it to become fatal poses a technical obstacle in the study of combined bacterial and viral infection in mice. It is apparent that inoculation of the fluid alone of a bacterial suspension can render the viral infection fatal.

In order to avoid the lethal effect of a second inoculation of fluid, suspensions of pneumococci or hemolytic streptococci were mixed with sublethal doses of

influenza virus and half of the mice were treated with sulfamerazine starting 6 hours before inoculation of the infectious agents. The outcome of these experiments is recorded in Table IV and it indicates that the combined infection was rapidly fatal in the controls while the treated mice survived and showed bacteria-free sublethal viral lesions when examined later.

The experiments of Table IV show that the severe bacterial infection was controlled by sulfonamide chemotherapy in spite of the coexistence of a sublethal viral infection of the lung. Since viral infection of this intensity did not interfere with the sulfonamide action, it became necessary to determine whether a severe lethal viral infection would alter the results of therapy of the bacterial infection. For this purpose, dilutions containing many lethal doses of virus were mixed with the suspensions of bacteria and inoculated intrabronchially. Sulfonamide chemotherapy was carried out as before. In these experiments,

TABLE III

Conversion of a Sublethal into a Lethal Infection with Influenza Virus by Reinoculation of Fluid

No. of experiment	Sublethal dose*	Tests of sublethal dose‡	Interval of reinoculation	Second inoculum	Reinoculated			Not reinoculated		
					No. reinoculated	Survivors	Survivors showing lesions	No. not reinoculated	Survivors	Survivors showing lesions
	dilution		*days*							
1	10^{-7}	1	3	Broth	21	0		12	7	7
2	0.5×10^{-7}	2	5	Broth	10	0		12	10	10
3	0.5×10^{-7}	3, 4	3	Saline	21	1	1	18	18	17

* PR8 strain.

‡ Numbers of experiments in Table I.

the results of treatment were evaluated by killing the surviving mice on the 5th day after inoculation and making cultures of their lungs. The results are presented in Table V and it is apparent that even such a severe viral infection did not interfere with the chemotherapeutic effect.

Bacterial Pneumonia Superimposed upon Viral Pneumonia by Inhalation of Fine Droplets.—In the preceding experiments, the chemotherapeutic effect took place in spite of severe viral infection. Nevertheless, inflammatory lesions from the viral infection were found to be absent 24 hours after inoculation and the possibility existed that interference with sulfonamide action on bacteria might depend on the existence of the inflammatory reaction to viral infection during the early hours of the bacterial infections.

In order to superimpose the bacterial infection at a time when the lesions from viral infection were at their height, it was necessary to find a way to administer bacteria after the inoculation of virus without instilling fluid. A num-

ber of preliminary experiments demonstrated that the inhalation of fine droplets of fluid did not cause the sublethal virus infection to become lethal. Furthermore, the inhalation of fine droplets of a heat-killed suspension of pneumococci did not make the viral infection become fatal in fifteen of sixteen mice.

When mice with sublethal viral infection were allowed to inhale fine droplets

TABLE IV

Chemotherapy of Infection Resulting from Inoculation of Mixtures of Bacteria and Sublethal Doses of Influenza Virus in Mice

No. of experiment	Influenza virus			Bacteria		Treated			Untreated		
	Strain	Sublethal dose	Tests of sublethal dose*	Culture	Dilution of culture	No. inoculated	Survivors	Survivors showing lesions	No. inoculated	Survivors	Survivors showing lesions
		dilution									
1	Weiss	2×10^{-7}	6	Pneumococci	10^{-3}	9	9	9‡	15	0§	
2	PR8	10^{-7}	1	Streptococci	1:50	14	14	14‡	14	1§	1

* Numbers of experiments in Table I.

‡ Cultures at autopsy showed no growth.

§ Pneumococci or streptococci recovered from the lungs of all dead mice.

TABLE V

Chemotherapy of Infection Resulting from Inoculation of Mixtures of Bacteria and Lethal Doses of Influenza Virus in Mice

No. of experiment	Influenza virus		Bacteria		Treated			Untreated	
	Strain	Lethal dose	Culture	Dilution of culture	No. inoculated	No. with positive cultures	No. showing lesions	No. inoculated	No. with positive cultures
		dilution							
1	PR8	10^{-5}	Pneumococci	10^{-4}	20	0*‡	18§	18	18‖
2	PR8	10^{-5}	Streptococci	1:25	14	0*	12§	14	14‖

* Killed for culture and observation on the 5th day after inoculation.

‡ Blood pooled from 8 of these mice showed a concentration of sulfamerazine of 31.8 mg. per 100 ml.

§ Two died before the 5th day and lesions could not be distinguished from post mortem changes.

‖ Died within 72 hours.

of a suspension of virulent pneumococci or hemolytic streptococci for 3 to 4 hours, they developed secondary bacterial pneumonia and died usually 2 to 6 days later. In contrast, normal mice placed in the jar as controls did not develop any obvious illness and survived (22–24). Table VI shows the results of experiments demonstrating the difference in susceptibility to inhaled bacteria of mice with sublethal viral infection compared with normal mice.

Histopathology of Combined Viral and Bacterial Pneumonia.—Observation of pulmonary lesions induced in mice by influenza virus alone 4 to 7 days after inoculation confirmed descriptions recorded in the literature (25–30). By this time, destruction of the bronchial epithelium had taken place and various stages of regeneration of the mucosa could be recognized. The lumina of the bronchi were either empty or contained large compact masses of polymorphonuclear leucocytes. Infiltrating cells in the peritruncal areas and in the lumina and walls of the alveoli were almost all mononuclear and closely packed collections of lymphocytes (probably lymphoid tissue) were more prominent than normal.

TABLE VI

Inhalation of Fine Droplets of Bacterial Suspension by Normal Mice and Mice with Sublethal Viral Infection

No. of experiment	Sublethal dose of virus*	Tests of sublethal dose‡	Interval between virus and bacteria	Species of bacteria	Period of inhalation	Sublethal viral infection			Normal	
						No. inoculated	Survivors§	Survivors showing lesions	No.	Survivors
	dilution		*days*		*hrs.*					
1	0.5×10^{-7}	2	4	Pneumococci	2	14	8	8	14	14
2	0.5×10^{-7}	2	4	Pneumococci	4	19	9	6	19	18
3	0.5×10^{-7}	2	7	Pneumococci	3	15	0		12	9
4‖	10^{-7}	3, 4	5	Pneumococci	4	14	1	1	15	15
5	10^{-7}	3, 4	6	Pneumococci	2	19	2	2	22	19
6	10^{-7}	3, 4	5	Streptococci	3	14	7	¶	14	13

* PR8 strain.

‡ Numbers of experiments in Table I.

§ Cultures of the lungs of dying mice showed pneumococci or streptococci except for 2 mice in experiment 2.

‖ Done as part of Experiment 1, Table VII.

¶ Not observed.

When mice with sublethal viral infection of this duration were allowed to inhale fine droplets of bacterial culture, the lungs showed all these lesions due to viral infection with the addition of infiltrations of polymorphonuclear leucocytes in various parts of the lung and edema fluid in some of the alveoli. There was considerable variation in the distribution of these polymorphonuclear leucocytes since they occurred in foci in some sections and more diffusely in others. The intensity of the polymorphonuclear infiltration varied from sections in which large densely packed masses of cells were present throughout to a few instances where they were in small enough number to be of doubtful significance. Gram-Weigert stain did not reveal bacteria in these lesions during the first 24 hours

after exposure to the organisms, but pneumococci were numerous in the lesions just before death.

In view of the possible importance of edema fluid in the bronchi, a review of the sections was made in order to estimate its frequency and amount. It was found that very few bronchi contained any edema fluid and that when it was present the amount was usually small.

Sulfonamide Chemotherapy of Combined Viral and Bacterial Pneumonia.—Administration of sulfamerazine was begun 24 hours after inhalation of the bacteria in order to allow the viral and bacterial lesions to coexist for as long a time as possible. The outcome of these experiments is shown in Table VII and it can be seen that treated animals survived and revealed bacteria-free viral lesions when examined 2 weeks later.

TABLE VII

Chemotherapy of Bacterial Pneumonia Superimposed upon Influenza Viral Infection in Mice

No. of experiment	Strain of virus	Sub-lethal dose of virus	Tests of sub-lethal dose*	Interval between inoculation of virus and bacteria	Bacteria	Period of inhalation	Treated				Untreated			
							No. treated	Survivors	Survivors showing lesions	Dying with positive cultures	No. untreated	Survivors	Survivors showing lesions	Dying with positive cultures
		dilution		*days*		*hrs.*								
1	PR8	10^{-7}	3, 4	5	Pneumococci	4	17	14	14‡	1	14	1	1	13
2	PR8	10^{-7}	3, 4	5	Pneumococci	3	20	15	14	2	20	7	7	12
3	PR8	10^{-7}	3, 4	5	Pneumococci	3	21	19	§	1	20	5	§	15
4	Lee	10^{-4}	7	6	Pneumococci	3	20	17	17	2	18	4	4	14
5	PR8	10^{-7}	3, 4	5	Streptococci	3	21	15	15	5	20	6	6	14

* Numbers of experiments in Table I.

‡ Cultures of 2 mice showed pneumococci.

§ Not observed.

DISCUSSION

The ineffectiveness of sulfonamide and sulfone chemotherapy in experimental influenza viral infections is now accepted (31–35) although some early reports indicated beneficial action (36–39). Sulfapyridine fails to alter the clinical course of uncomplicated influenza in human beings (40). Furthermore, neither penicillin nor any other chemotherapeutic agent has been shown to affect this viral disease (33, 34). The experiments reported in this paper confirm the observation that sulfonamide chemotherapy has no effect upon the pulmonary lesions caused by influenza virus. Even relatively mild, sublethal lesions in both rats and mice are unaffected by the drug. In view of these facts, it is probable that chemotherapy will not avert fatalities when the viral infection

itself is sufficiently severe to cause death. Although most of the clinical cases of uncomplicated influenza in pandemic and epidemic times are mild there is no way of evaluating the degree of severity of the viral infection in the fatal cases with simultaneous bacterial pneumonia, so the possibility remains that chemotherapy will fail in such circumstances. Fatal cases of influenza have been reported in which bacteria were not present in the lungs (41) or were probably not important (42).

The activating effect on sublethal virus infection of the reinstillation of fluid suggested to Taylor (21) that secondary bacterial pneumonia in human beings might bring about a similar fatal viral infection. The possibility of a potentiating effect of bacterial infection on the viral process is also suggested by the experiments of Bang (43, 44) who showed that combined infection of chick embryos with *H. influenza suis* and swine influenza virus resulted in a higher titer of virus and spread of it to portions of the embryo not reached in simple viral infections. The experiments recorded in Table VII tested the hypothesis that the secondary bacterial pneumonia would increase the severity of the viral infection. Bacterial pneumonia was allowed to coexist with sublethal viral infection for 24 hours and then the bacterial component of the combined infection was eliminated by chemotherapy. The survival of the animals with viral lesions indicated that no apparent change in the degree of severity of the viral process resulted from the bacterial pneumonia.

The effect of reinstilled fluid in mice suggests that the presence of fluid in the bronchi may be of more importance in determining the severity of the viral infection than the bacterial pneumonia *per se*. There appears to be no reason why endogenous fluid in the bronchi such as edema fluid in pneumococcal pneumonia (11, 45, 46) or pulmonary edema from any cause would not have the same effect as exogenous instillation of fluid. This concept would explain the failure of secondary bacterial infection to intensify the sublethal viral infection in the present experiments since study of sections of lungs of such mice showed that the bacterial pneumonia consisted chiefly of infiltrations of polymorphonuclear leucocytes, and that edema fluid was only rarely found in the bronchi.

The initial chemotherapeutic experiments here reported were done on combined infection in rats, and the virus failed to interfere with the sulfonamide effect on the bacterial infection. This finding suggested the possibility that the viral infection in the rat was not of sufficient severity to block the sulfonamide action; similar experiments, therefore, were carried out with mice. In this more susceptible animal, Taylor (21) has demonstrated that a tremendous multiplication of virus takes place in the first 24 hours. In spite of this fact, when sublethal and lethal doses of virus were mixed with fatal but curable doses of bacteria sulfonamide chemotherapy was found to be effective against the bacterial element of the combined infection. It must be concluded, therefore, that sulfonamide drugs exert their antibacterial action even in the presence of rapidly multiplying influenza virus.

Clinical experience (47) has shown that sulfonamide chemotherapy is not effective in the treatment of purulent foci and there is experimental evidence (48) indicating that this failure is at least partly due to the presence in pus of sulfonamide-inhibiting substances. By analogy it might be assumed that the pulmonary exudate called forth by the influenza virus would interfere with sulfonamide action. This possibility was tested in experiments in which bacterial infection was superimposed upon the inflammatory lesion produced by influenza virus but the viral lesion was found to have no effect on the results of chemotherapy.

Clinical observations during the pandemic (4) and studies on experimental infections in several species of animals (49–57, 43, 44) indicate increased severity of bacterial infection of the lung when combined with influenza viral infection. The experiments recorded in Table VI confirm this fact and have made it possible to determine whether the potentiating effect of the virus on the bacterial infection will interfere with chemotherapy. The results recorded in Table VII indicate no interference under such circumstances and are consistent with clinical experience in that pneumonia following influenza (58–60, 8),[9] measles (61, 62), and dog distemper (63) all appear to respond to sulfonamide drugs.

A possible explanation may be suggested for the fact that influenza viral infection of the lung apparently does not interfere with the chemotherapy of concomitant bacterial pneumonia. There is considerable experimental evidence that the virus acts on the mucosa of the respiratory tract, thereby making the host susceptible to secondary pulmonary infection. One of the chief mechanisms by which the bronchi rid themselves of inhaled foreign particles is by the adherence of the particles to the mucous covering of the epithelium and their propulsion toward the pharynx by ciliary action (64). At the same time a characteristic feature of influenza viral infection in man (4, 65) and most other animals (25–30, 66, 67) is the destruction of the epithelial cells that bear the cilia. The effect of the virus on respiratory epithelium may be its main action in lowering resistance to bacterial infection (5, 68). On the other hand, there is no evidence that influenza virus interferes with the activity of the phagocytic cells in the pneumonic exudate. Studies on the mechanism of recovery in bacterial pneumonia treated with chemotherapy have shown that most of the bacteria are ultimately destroyed by the phagocytes in the alveoli and bronchi (10, 69). It is not surprising, therefore, that influenza infection renders the host more susceptible to bacterial infection of the lung but at the same time fails to interfere with the action of chemotherapy upon the secondary bacterial pneumonia.

[9] Meikeljohn and Eaton have observed six cases of bacterial pneumonia following Type A influenza. The type of influenza was demonstrated by increase in antibodies in all cases and isolation of virus in five. The secondary pneumonia responded to therapy with sulfadiazine (personal communication).

SUMMARY AND CONCLUSIONS

1. Sulfonamide chemotherapy controls the bacterial component of combined infection with influenza virus and pneumococci in rats.

2. Reinstillation of fluid (broth, physiological salt solution) into the respiratory passages of mice several days after sublethal viral infection converts the viral infection into a lethal one.

3. Sulfonamide chemotherapy controls the bacterial component of combined bacterial and viral infection of mice, produced by intrabronchial inoculation of mixtures of bacteria and sublethal or lethal doses of virus.

4. Bacterial pneumonia may be superimposed upon sublethal viral infection in mice by inhalation of fine droplets of bacterial suspension several days after inoculation of virus. Normal mice inhaling fine droplets of bacterial suspension fail to develop obvious disease.

5. Sulfonamide chemotherapy controls bacterial pneumonia superimposed on sublethal viral infection by inhalation of fine droplets of bacterial culture.

6. The secondary bacterial penumonia does not convert the sublethal viral infection into a lethal one.

7. If another pandemic of influenza occurs, it is probable that sulfonamide chemotherapy will be valuable in the treatment of secondary bacterial pneumonia and will be effective in lowering the case fatality rate if the viral component of the infection is not severe enough by itself to cause death.

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