PULMONARY EDEMA IN INFLUENZAL PNEUMONIA OF THE MOUSE AND THE RELATION OF FLUID IN THE LUNG TO THE INCEPTION OF PNEUMOCOCCAL PNEUMONIA*,‡

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Plates 13 and 14

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In a preceding study (2) concerning the mechanism by which influenza viral infection of the mouse induces susceptibility to secondary pneumococcal pneumonia evidence was presented that this susceptibility is associated with the appearance of the lesion produced by the virus. The studies reported in the present paper indicate that pulmonary edema is a component of the influenza viral lesion in the mouse and that the presence of fluid in the lung is an important factor in the inception of pneumococcal pneumonia in this animal. In addition, evidence is presented that the chief action of fluid under these circumstances is to furnish a culture medium for growth of pneumococci.

Methods and Materials

Details of techniques have been described in earlier papers (2, 3). Mice were infected with the Weiss strain of influenza virus and the A5 strain of Type I pneumococcus.[1] The intrabronchial method of inoculation was used not only for the virus but also for introducing fluids or cultures into the lung. In addition, mice were allowed to inhale fine droplets containing pneumococci and were observed for survival or were killed and the pneumococcal content of the left lobe of the lung determined by grinding the tissue in a mortar and preparing poured blood agar plates.

Although pulmonary fluid could be seen in microscopic sections stained with hematoxylin and eosin, other staining procedures rendered fluid more easily and certainly visible. Iron hematoxylin (5) and phloxin with methylene blue (6) were used for this purpose and some undifferentiation of the latter was permitted. Pneumococci in the lung were visualized well with eosin and methylene blue (6) or Giemsa (6).

Reexpansion of lungs with fixative was carried out as before in order to see the structure of the lung adequately (7). However, because of the possibility that introduction of fixative into

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‡ A preliminary report was presented at the meeting of the Central Society for Clinical Research, October 31, 1947 (1).

[1] In one experiment, the 8 HCC strain of Type III pneumococcus was employed. This strain is the same as that utilized in other experiments in this laboratory (4).

the trachea might alter the locations of fluid or bacteria, control sections of unexpanded lungs were observed also. No significant difference due to reexpansion was detected.

For experimental induction of pulmonary fluid, sterile normal mouse serum was administered intrabronchially. In order to fill a large number of alveoli with fluid by this method, the amount of inoculum delivered from the cannula was doubled. Since the capacity of the cannula was 0.05 ml., this double amount was obtained by increasing the amount of serum injected into the cannula from 0.1 ml. to 0.15 ml.

Pulmonary edema in mice was induced by alpha naphthyl thiourea (ANTU).[2] This drug was ground finely in a mortar and suspensions in olive oil were administered intraperitoneally. Because of variation in the time of death after inoculation of the drug, groups of mice in each experiment were injected with doses varying from 0.5 mg. to 4.5 mg. Only animals dying within 4 to 6 hours after administration of the drug were selected for the experimental procedures.

In some experiments, a solution of 10,000 units of crystalline penicillin G in 0.1 ml. of saline was injected intramuscularly. For determination of the number of pneumococci in lungs of mice injected with penicillin, 10 units of penicillinase[3] was added to each poured blood agar plate.

EXPERIMENTAL PROCEDURES AND RESULTS

Pulmonary Edema in the Viral Lesion.—For microscopic study of the viral lesion, lethal or sublethal doses of influenza virus were injected intrabronchially into mice and sections of consolidated lungs were prepared after 5 days; that is, at the same time after inoculation that growth of inhaled pneumococci in the viral lesion had been demonstrated. Examination of these sections showed the lesion of viral pneumonia and it was noticed that a prominent component of the lesion consisted of pulmonary edema (Fig. 1).

It was found that fluid could be demonstrated in some of the alveoli of practically every section with viral pneumonia of 5 days duration. The fluid first appeared at about the same time that macroscopic lesions of the lung became visible and, in mice with sublethal infections, fluid persisted for at least 30 days after inoculation. Frequently the largest amounts of fluid were not in areas of greatest cellular infiltration of the tissue, and the impression was gained that the distribution of fluid in the lung was determined to a large extent by gravity.

Experimental Induction of Pulmonary Edema[4].—The presence of fluid in the lung as a part of the lesion of influenza viral pneumonia in the mouse suggested that inhaled pneumococci might become implanted in such fluid and find conditions favorable for rapid growth. It was thought that a way to test this possibility would be to induce pulmonary edema in the mouse without the other components of the influenza viral lesion and to test the susceptibility of such mice to inhaled pneumococci. However, inasmuch as the viral lesion is an inflammatory process, it was necessary to have a means for induction of pulmonary fluid that did not owe its effect to an irritation of the tissues.

[2] Kindly furnished by E. I. du Pont de Nemours and Co., Inc.

[3] Obtained from Shenley Laboratories, Inc., 350 Fifth Avenue, New York.

[4] In this paper, the term pulmonary edema is used to denote fluid in the lung regardless of whether the fluid is endogenous or injected into the air passages.

In order to meet these requirements, sterile normal mouse serum was administered intrabronchially to mice and the presence of serum in the lung was confirmed by microscopic sections. That this serum would persist long enough in the lung to allow tests of susceptibility to pneumococci was indicated by the work of others who have shown that protein-containing fluids are removed slowly from the lung chiefly by the lymphatics, in contrast with crystalloidal solutions which are absorbed rapidly into the blood stream (8–11). Fig. 2 shows the appearance of pulmonary edema due to intrabronchial injection of serum.

Rarely were more than half the alveoli filled with serum in a given section. The fluid was present not only in the alveoli but also in the lymphatics and interstitial tissues. Significant amounts of fluid were present in the alveoli 7 hours after injection and fluid was detected in the air sacs even 24 hours later.

Another method employed[5] to cause accumulation of fluid in the lung involved the use of alpha naphthyl thiourea (ANTU).

When this drug is administered to animals death from pulmonary edema results from a selective effect on the capillaries of the lung rendering them permeable to plasma proteins (12,13). In trials with ANTU in mice, efforts were made to adjust the dose so that animals would have a degree of pulmonary edema compatible with survival. It was found, however, that sublethal doses of ANTU failed to cause fluid to collect in the alveoli although fluid was present in the lymphatics and interstitial tissues. On the other hand, lethal doses were followed by pulmonary edema with involvement not only of interstitial tissues but also of considerable numbers of alveoli.

Fig. 3 shows the miscroscopic appearance of the lung of a mouse dying from a lethal dose of ANTU.

Although these two methods of inducing pulmonary edema appeared to be without an irritant effect on the lung, it was desirable to substantiate lack of inflammation by histologic methods. Sections of mouse lungs after injection of serum were examined for the presence of inflammatory cells but no significant numbers were found within the early hours after injection. However, 24 hours after inoculation there was an increase in alveolar macrophages and some sections showed the findings of spontaneous secondary bacterial pneumonia. Microscopic sections of the lungs of mice dying from ANTU showed pulmonary edema but no cellular evidence of inflammation.

Pneumococcal Infection of Mice with Injected Pulmonary Fluid.—In order to test the effect of pulmonary edema on susceptibility of mice to inhaled pneumococci, sterile normal mouse serum was injected intrabronchially into mice

[5] Unsuccessful attempts to produce histologically demonstrable pulmonary edema in the mouse included administration of lethal amounts of ether, chloral hydrate, pilocarpine, histamine, and a glucoside of digitalis. Asphyxia as well as compression of the chest, abdomen, or aorta also failed to produce this effect.

and immediately after inoculation the animals were allowed to inhale fine droplets containing pneumococci for 1 hour. As controls in each experiment normal mice, mice injected with saline, or mice subjected to cannulation without injection of fluid inhaled the bacteria in the chamber at the same time. The results shown in Table I indicate that mice with pulmonary fluid induced by this method have increased susceptibility to infection by inhaled pneumococci.

Growth of Pneumococci in Lungs with Injected Pulmonary Fluid.—It seemed likely that the increased susceptibility of mice with injected pulmonary fluid was due to growth of pneumococci in this fluid. In order to learn whether growth actually occurred in such lungs, mice were injected intrabronchially with serum, allowed to inhale pneumococci, and the bacterial content of the

TABLE I

Fatal Infection of Mice Inhaling Pneumococci after Intrabronchial Injection of Serum

Experiment No.	Intrabronchial injection	Died*	Survived‡	Total	Chi square
1	Serum	17	2	19	22.8
	None	1	17	18	
2	Serum	18	3	21	7.4
	Saline	9	13	22	
3	Serum	11	9	20	3.0
	None§	4	14	18	

* Shown to be fatal pneumococcal infection by recovery of the organism in every instance.

‡ Killed after 11 to 14 days and culture of each lung failed to show pneumococci.

§ Mice were anesthetized and the cannula was passed into the bronchus but no inoculum was injected.

lung was determined immediately and 6 hours later. The results presented in Table II show that pneumococci multiply in the lung under these circumstances.

In determining the number of pneumococci in the lungs of mice inhaling this organism after intrabronchial injection of sterile serum, it was found that contaminating organisms were more numerous and frequent than in similar determinations with normal mice or mice with influenza viral infection. Nearly every plate showed at least a few contaminating colonies and, when any doubt existed as to the identity of colonies, pneumococci were demonstrated by capsular swelling with Type I antiserum. In Table II, all plates are represented and those showing enough contaminants to render the counts of pneumococci doubtful are designated and excluded from the statistical calculations.

Pneumococci are known to be capable of growth in normal serum (14). In separate confirmatory tests, normal mouse serum was inoculated with small numbers of pneumococci and determinations made of the number of pneumococci present by means of poured blood agar plates. By this procedure, significant growth was demonstrated within 3 hours.

As in the previous work showing that pneumococci would grow in the viral lesion (2), there

TABLE II

Growth of Inhaled Pneumococci in Lungs with Pulmonary Fluid Due to Injection of Serum

Experiment No.	Time after inhalation	Plate counts																					Means	Statistical evaluation*
	hrs.																							
1	0	261	C‡	49	4	8	323	90	103	24	217	16	C	173	210	50	162	354	C	126	263		143.1	4.8
	6	863§	>1000	>1000	>1000	>1000	>1000	>1000	78	20	>1000	>1000	>1000	428	0	>1000	>1000	>1000	455	>1000	195	>1000	337.8	
2	0	96	C	48	C	15	C	99	C	C	C	C	88	0	40	91							59.6	4.0
	6	0	>1000	>1000	>500	C	0	>500	>1000	>1000	10	2	>1000	>1000	>500	3	>1000						267.7	
3	0	15	30	86	62	C	31	C	31	C	20	C	25	19	21	11	C	C	C	32	51		33.4	4.5
	6	C	C	198	266	112	438	487	67	>500	0	112	158	71	>1000	>1000	485	30	120	0	>1000	0	207.0	
4‖	0	2	5	C	C	C	17	4	5	1	28	C	0	C	C	C	C	C	23	4	0	20	0	8.3	3.7
	6	C	48	17	0	277	34	18	12	41	67	76	161	248	C	192	C	129	122	1	C	30	361	101.9	

* The difference of the means divided by the standard error of the difference.

‡ In the experiments shown in this table, all plates are recorded and those in which contamination interfered with count of pneumococcal colonies are designated with the letter C. Such contaminated plates were excluded from the statistical calculations.

§ In order to make a conservative evaluation of the data, all counts greater than 400 have been considered as 400 in the statistical calculations.

‖ In this experiment, 0.2 ml. of the suspension of pulmonary tissue was used to prepare the blood agar plates. This amount is one-fifth of that used in Experiments 1, 2, and 3.

TABLE III

*Growth of Inhaled Pneumococci in Lungs with Pulmonary Edema Due to ANTU**

Experiment No.	Drug treatment	Plate counts																			Means	Statistical evaluation‡
1	ANTU	105	10	130	11	368	29	150	52	141	71										106.7	2.9
	None	14	5	18	3	4	4	0	16	0	2	12									7.1	
2	ANTU	58	22	12	230	270	36	74	39	42	53										83.6	2.6
	None	4	7	19	37	10	0	1	3	9	1										9.1	
3	ANTU	48	3	5	48	9	6	4	6	62	0	1	6	2	13	2	2	10			13.4	2.7
	None	2	3	0	0	0	0	1	0	1	2	0	0	0	0	1	3	0			0.8	
4	ANTU	423	16	112	50	6	20	35	72	0	99	7	35	19	95	15	28	41	16	59	36	59.2	2.6
	None	0	1	1	1	1	0	16	11	0	0	60	0	9	1	5	1	0	6	13	0	6.3	
5§	ANTU	69	12	165	142	252	25	27	85	164	15	57	104	10	47							83.8	3.6
	None	86	3	8	0	4	0	2	4	9	11	1	4	1	10	3						9.8	

* Alpha naphthyl thiourea.

‡ As in Table II.

§ In this experiment the right lung of each mouse in the ANTU series was placed in fixative immediately after removal of the left lobe for determination of its pneumococcal content. Sections of the right lungs showed extensive interstitial edema in every instance. The amount of fluid in the alveoli varied in sections of different mice and was absent in only two animals. In seven instances alveolar edema was severe.

was need to exclude the possibility that pneumococci might grow elsewhere in the body and be transported to the lung by the blood. Therefore, mice were injected intrabronchially with serum, allowed to inhale pneumococci, and 6 hours later determinations were made of the pneumococcal content of the blood and left lobe of the lung of each mouse. The absence of bacteria from the blood in nearly all instances gave further evidence of primary pneumococcal growth in the lung.

Pneumococcal Content of Lungs with Pulmonary Edema Due to ANTU.—Mice with pulmonary fluid due to this drug were tested for susceptibility to inhaled pneumococci because it was thought that endogenous fluid arising from a drug administered systemically would have minimal irritating properties and would be more closely comparable than serum to the pulmonary fluid of the viral lesion. Also, contaminating bacteria from intrabronchial inoculation would not be present in endogenous pulmonary fluid. However, for ANTU to cause collection of fluid in the alveoli, it was necessary to administer lethal doses of the drug, as already stated, and test for susceptibility to pneumococci by determining the bacterial content of the lung. Therefore, lethal doses of ANTU were administered to mice and immediately thereafter the animals were allowed to inhale fine droplets containing pneumococci for 1 hour. Normal mice for control were allowed to inhale pneumococci in the chamber at the same time. Bacterial counts were made on the left lobes of mice dying of pulmonary edema 4 to 6 hours after injection of ANTU. Animals dying before 4 hours or after 6 hours were discarded. Whenever the bacterial content of the lung of a mouse with pulmonary edema was determined, a control mouse was killed at the same time and a similar count done for comparison. The results are shown in Table III where it may be seen that lungs of mice dying of pulmonary edema contained more pneumococci than those of control mice.

While it appeared probable that the greater number of pneumococci in lungs with pulmonary edema due to ANTU was caused by growth of these bacteria in edema fluid, the possibility remained that this difference was brought about by interference with mechanisms for elimination of inhaled bacteria. To obtain evidence bearing on this point, advantage was taken of the fact that penicillin is known to act only on growing bacteria (15–19).

Mice were injected with ANTU as already described and immediately thereafter half of them were treated with penicillin. All the mice were then allowed to inhale fine droplets of pneumococci for 1 hour in the chamber at the same time and the pneumococcal content of the left lobe of the lung was determined in mice dying between 4 and 6 hours later. Penicillinase was used in the poured blood agar plates of mice treated with penicillin. The results of these experiments are shown in Table IV.

It is apparent from the table that treatment with penicillin caused a diminution in the pneumococcal content of the lung with pulmonary edema. It is concluded, therefore, that the lung with pulmonary edema supports the growth of inhaled pneumococci in contrast with the normal lung in which growth does not occur.

That endogenous fluid induced by ANTU would support rapid growth of pneumococci was indicated by testing *in vitro* the pleural fluid of mice dying from this drug. The fluid, collected aseptically and prevented from clotting with heparin, was inoculated with small numbers of pneumococci and the bacterial content tested before and after incubation for 3 hours. Significant growth of pneumococci occurred under these conditions. Also, ANTU placed directly in fluid culture medium did not inhibit growth of pneumococci.

Further evidence suggested that ANTU does not inhibit mechanisms of the lung for elimination of bacteria. The effect of ANTU on movement of bronchial cilia was tested by a direct method for observation of these structures to be described in a subsequent paper. Visualization of cilia in lungs of mice dying from ANTU showed active movement. Furthermore, exposure *in vitro* of fresh slices of lung to the small amount of ANTU in a saturated solution in saline had no apparent effect on movement of bronchial cilia. In addition, it has

TABLE IV

Effect of Penicillin on the Growth of Inhaled Pneumococci in Lungs of Mice Dying from ANTU

Experiment No.	Drug treatment	Plate counts																		Means	Statistical evaluation‡
1	ANTU plus penicillin*	2	0	0	2	1	0	0	2	0	0	0	1	0						0.6	14.5
	ANTU	0	6	2	2	38	8	6	32	36	15	4	1	35	47	0	21	3		15.1	
2	ANTU plus penicillin*	1	0	0	70	0	0	0	0	0	0	0	0	0	0					5.1	3.1
	ANTU*	94	370	47	>500	44	21	42	26	31	1	20	12	15	54	443	233	144		114.9	

* Ten units of penicillinase were added to each plate.
‡ As in Table II.

already been reported that lymphatic flow is increased in animals injected with ANTU (11, 20) and this condition might be expected to result in fewer numbers of pneumococci remaining in the lung than normal.

Production of the Lesion of Bacterial Pneumonia by Serum and Pneumococci.— Having shown that the edematous lung will allow the growth of pneumococci, it was important to know whether the lesion of bacterial pneumonia would result from such growth. Therefore, pneumococci suspended in normal mouse serum were injected intrabronchially into mice and sections of the lungs prepared about 24 hours later. It was found that the lesion of bacterial pneumonia was produced in nearly every instance; masses of polymorphonuclear leucocytes were present in the alveoli and interstitial tissues. Many of these leucocytes were degenerated and showed karyorrhexis of nuclei. In some areas edema fluid was seen and pneumococci were often found there in large numbers. In other experiments, normal mouse serum was injected intrabronchially and the mice were allowed to inhale pneumococci for 1 hour. Sections were prepared at the time of death 2 to 4 days later and it was found that pneumococcal pneumonia

was present although less frequently and to a milder extent than when the pneumococci were suspended in the serum. A pneumococcal lesion thus produced is shown in Fig. 4.

In order to administer pneumococci without fluid as a control, mice were allowed to inhale the organism in the form of fine droplets. Type III pneumococci were used for this purpose because of the known greater pulmonary virulence of this type of pneumococcus (21). Type I pneumococci were used also and in this case inhalation was permitted to continue for 4 hours so as to overcome the pulmonary resistance previously shown for this strain. With both strains of pneumococci, some of the mice developed general infection 2 to 11 days after inhalation and sections of the lung were prepared when the mice were moribund or immediately after death. Microscopic examination of such lungs showed no lesions of pneumonia and the appearance under low power of the microscope was that of the normal lung (Fig. 5). Under the oil immersion objective, large numbers of pneumococci were found particularly in the pulmonary blood vessels (Fig. 6).

Paucity of Early Phagocytosis in the Lung.—Because it is known that phagocytosis is an important means of resistance to pneumococcal infections in the lung (22), a microscopic study was carried out to evaluate the phagocytic mechanism in the earliest stages of pneumonia caused by pneumococci suspended in serum. Cultures of pneumococci were subjected to angle centrifugation and the bacterial sediments were suspended in normal mouse serum. Such heavy suspensions of bacteria in serum were injected intrabronchially into mice and microscopic sections were prepared immediately thereafter and at intervals for 6 hours.

In the normal lung of the mouse, only occasional polymorphonuclear leucocytes are seen and these are chiefly in the capillaries of the alveolar walls or in the larger blood vessels. After injection of pneumococci in serum, fluid and bacteria were seen in the alveoli and interstitial tissues. Three hours later many more pneumococci were visible, apparently growing in the pulmonary fluid (Fig. 7), but not until this time did significant numbers of polymorphonuclear leucocytes begin to appear. At 3 hours, these phagocytes were present in larger numbers in the interstitial tissues and a few could be seen entering the alveoli. During the period from 3 to 6 hours a progressive increase in the numbers of polymorphonuclear leucocytes was seen. Essentially no phagocytosis of pneumococci by polymorphonuclear leucocytes was observed during the first 3 hours and only moderate phagocytosis from 3 to 6 hours.

In sections of normal mouse lungs, macrophages are found regularly but not all the alveoli contain them. Small numbers of these cells are present also in the interstitial tissues. After intrabronchial injections of heavy suspensions of pneumococci in serum, the number of macrophages present did not appear to increase significantly after the first 6 hours. In addition, during the first 3 hours, it was noted that remarkably little phagocytosis took place. Although some macrophages were found to contain a few pneumococci, most of the organisms were seen in the pulmonary fluid outside of macrophages. The degree of phagocytosis exhibited by macrophages is illustrated in Fig. 8. In this alveolus, pneumococci are demonstrable within a macrophage but many other pneumococci in the same alveolus are extracellular.

The possibility arose that the large numbers of pneumococci produced leucotoxic substances that caused a slow and inefficient phagocytosis by macrophages. For this reason, heat-killed pneumococci were washed in Locke's solution, suspended in serum, and injected as before. Even with dead pneumococci, no increase of phagocytosis by macrophages was detected.

Lungs with influenza viral lesions of 5 days duration have cellular infiltration with mononuclear cells including macrophages in the interstitial tissues and in addition many macrophages are present in the alveoli. The number of polymorphonuclear leucocytes in such lungs varies greatly. In some sections, polymorphonuclear leucocytes are rare and gradations in number occur so that, in some other sections, large numbers of these cells are found and the appearance is that of secondary bacterial pneumonia. However, aerobic and anaerobic cultures of such lungs usually do not reveal a secondarily infecting bacterium. Because of the large numbers of phagocytic cells already present in the lung with fully developed viral pneumonia, we have found it difficult to evaluate the phagocytic mechanism after injection of large numbers of pneumococci suspended in normal mouse serum. Nevertheless, it has been possible to show that some phagocytosis takes place both by polymorphonuclear leucocytes and by macrophages. However, we have gained the impression that migration of polymorphonuclear leucocytes and macrophages is no faster in the lung with a viral lesion than in the normal lung. Also, the rate of phagocytosis by macrophages in the lung with a viral lesion appears to be about the same as the rate in the normal lung.

The results of microscopic study of the earliest phases of the infectious process resulting from intrabronchial injection of pneumococci suspended in serum showed that there was a significant delay in the migration of polymorphonuclear leucocytes and macrophages into the infected lung. Furthermore, the macrophages already present in the normal lung engulfed pneumococci at a slow rate. Although evaluation of phagocytosis in the lung with a viral lesion was more difficult, a comparable insufficiency of the phagocytic mechanism appeared to exist.

DISCUSSION

The evidence presented indicates that the presence of pulmonary edema under the conditions studied is a decisive host factor in determining whether inhaled pneumococci will grow in the lung, produce the lesion of bacterial pneumonia, and cause death of the animal. Inhaled pneumococci were eliminated rapidly from the lung of the normal mouse, pneumonia did not occur, and most of the animals survived (2, 3). This influence of pulmonary fluid on the inception of pneumonia is to be distinguished from the transporting effect of edema fluid in lungs already the site of bacterial infection (23–35, 7), although spread of pneumonia by the latter mechanism may be considered inception of pneumonia in the new areas (29). The implications of the present experiments are that the presence of a nutrient fluid favors the initiation of pneumococcal infection of the lung and that the genesis of the fluid need not be due to reaction from the bacterium itself.

It is necessary to assume that inhaled pneumococci grow in pulmonary fluid just as bacteria grow in fluid culture media, since growth of the organism took place in the lung only in the presence of fluid. Furthermore, serum and pleural fluid of mice were shown to support rapid growth from small inocula of pneumococci *in vitro*. On the other hand, pulmonary fluid is not demonstrable histologically in the lungs of normal mice and any imperceptible fluid that

might be present would not be sufficient to support growth of pneumococci since growth has been shown not to occur.

Data recorded in the previous paper demonstrated that inhaled pneumococci grow in a mouse lung with influenzal pneumonia and the present observations show similar growth in the lung with pulmonary fluid without the other components of the viral lesion. In view of these facts, it appears that the pulmonary edema of the influenza viral lesion[6] accounts adequately for growth of inhaled pneumococci in the viral lesion. Furthermore, pulmonary fluid in a sublethal viral lesion would explain the greater than normal susceptibility of such mice to general pneumococcal infection after inhalation of these organisms. In either the lung with viral pneumonia or the lung with experimentally induced pulmonary fluid, the rate of growth of inhaled pneumococci must be rapid enough to make it possible for the bacterial content of the lung to increase in spite of factors that tend to eliminate bacteria such as ciliary action, phagocytosis, and lymphatic drainage. However, it is possible that these antibacterial mechanisms of the lung are also impaired by the viral lesion.

There is evidence that progress or arrest of pneumococcal pneumonia depends on whether or not the organisms can grow faster in the edema fluid than elimination can take place through phagocytosis (27, 31). In the present studies on inception of pneumonia, it has been shown not only that the presence of pulmonary fluid enables pneumococci to grow but also that there is a significant delay in appearance of polymorphonuclear leucocytes. Pneumococci grow in the body fluids of the mouse within 3 hours, but even with the maximal stimulus of many of these organisms polymorphonuclear leucocytes do not migrate into the lung during this time.[7] This delay in appearance of phagocytic cells may be due to a diluting effect of the edema fluid on the toxic products of pneumococci. However, regardless of its mechanism, delayed arrival of leucocytes under these circumstances is similar in its end result to the inhibition of migration of leucocytes due to anesthesia with ether or alcohol (44) in that time is permitted for growth of the organisms.

Macrophages are not abundant in the alveoli of the normal mouse lung and in addition were found to engulf pneumococci too slowly to have a significant retarding effect on bacterial growth in alveolar fluid. This slow action of the pulmonary macrophages indicates that these cells are not efficient as a first-line defense in the mouse lung and that polymorphonuclear leucocytes must act in

[6] In descriptions of the microscopic lesion in influenzal pneumonia of the mouse, the presence of pulmonary fluid has been noted before (36–43).

[7] In experimental pneumonia of monkeys, Loosli (33) observed the migration of polymorphonuclear leucocytes into the infectious focus in the lung as early as 1½ hours after injection of pneumococci suspended in a mixture of starch and broth. Probably, the more rapid migration of leucocytes under these circumstances was due to the irritant nature of the inoculum rendering the pulmonary capillaries more permeable to leucocytes.

this capacity. A similar slow action of macrophages has been found in experimental pneumococcal lymphadenitis of rats (45). However, in this situation, migration of polymorphonuclear leucocytes is more rapid than in the normal mouse lung and suggests some difference in capillary permeability in these two sites.

One might expect that a lung with fully developed influenza viral pneumonia would have a more efficient immediate phagocytic mechanism than the normal lung because polymorphonuclear leucocytes are often present already and macrophages are abundant. The fact that inhaled pneumococci grow in the lung with a viral lesion would not invalidate this hypothesis since rapid growth of pneumococci in pulmonary fluid could result in a net increase of bacteria in spite of the destruction of some organisms by phagocytosis.

The question arises as to what effect pulmonary fluid may have on the phagocytic properties of macrophages already present in the normal lung or macrophages and polymorphonuclear leucocytes in the viral lesion. It seems likely that fluid in the alveoli could interfere with phagocytosis because it has been shown that pneumococci floating freely in fluid away from alveolar walls are not ingested because the phagocytes are unable to trap the floating organisms against a suitable surface (46).

No histologic evidence of inflammation was detected within several hours after the induction of pulmonary edema so that the effect on susceptibility to bacteria cannot be attributed to irritation from the experimental procedures. It might be argued that only the fluid component of an inflammatory process (47, 48) was present and was not detected histologically but, if this were so, it would support our concept concerning the effect of pulmonary fluid. In addition, it seems probable that the mechanism by which injection of irritating substances facilitates initiation of pneumococcal pneumonia (25, 49, 33) is by causing the fluid component of inflammation to enter the alveoli. Furthermore, the production of pneumonia in animals by injection of pneumococci alone (50) may be due to a similar reaction from the organisms themselves.

Although it is recognized that conditions in mice may not be strictly comparable to those in human beings, it seems likely that the concept of pulmonary fluid as an important factor in susceptibility to pneumonia may be valid in human beings also since secondary pneumonia is especially frequent in diseases often complicated by pulmonary edema. In this connection it is noteworthy that pulmonary edema was a characteristic feature of pandemic influenza (51) and it has been suggested as a contributing cause of secondary bacterial pneumonia in this disease (52).[8] That edema fluid in the human lung may furnish

[8] The etiology of pandemic influenza is unknown and most reports of pulmonary lesions in influenza are complicated by the presence of bacterial pneumonia. However, a case of fatal influenza has been reported apparently without secondary bacterial infection and in which pulmonary fluid was a prominent feature of the lesions (53). In this instance, the virus was recovered from the lung and significant bacteria were absent.

a favorable medium for proliferation of bacteria has been suggested as a predisposing factor in the pneumonias that follow congestive heart failure (54)[9] and shock (52).[10] Finally, it should be mentioned that secondary bacterial infection is characteristic in conditions elsewhere than the lung in which abnormal amounts of fluid accumulate such as nephrotic ascites (57) and lymphedema (58). In such cases, it seems probable that growth of bacteria in the fluid is an important factor in lowered resistance to bacterial infection.

SUMMARY

Pulmonary edema is a component of the fully developed influenza viral lesion in the mouse.

Mice with experimental pulmonary fluid have an increased susceptibility to inhaled pneumococci and under these circumstances the organisms grow in the lung and produce the lesion of bacterial pneumonia.

The presence of pulmonary edema in the lesion due to the influenza virus in the lung of the mouse appears to account adequately for the previous observation that inhaled pneumococci grow in the influenza viral lesion.

Mice dying of pneumococcal septicemia after inhaling fine droplets containing this organism do not have pneumonia.

The delay in migration of polymorphonuclear leucocytes into the lung after injection of pneumococci suspended in serum is an important factor in susceptibility to infection since it allows ample time for pneumococci to grow in the pulmonary fluid.

The slow phagocytic action of pulmonary macrophages likewise permits growth of pneumococci.

Conditions in human beings that are known to be complicated by pulmonary edema are also known to be associated with increased susceptibility to secondary bacterial pneumonia.

BIBLIOGRAPHY

1. Harford, C. G., and Hara, M., *J. Lab. and Clin. Med.*, 1947, **32,** 1406.
2. Harford, C. G., Leidler, V., and Hara, M., *J. Exp. Med.*, 1949, **89,** 53.
3. Harford, C. G., Smith, M. R., and Wood, W. B., Jr., *J. Exp. Med.*, 1946, **83,** 505.
4. Wood, W. B., Jr., and Smith, M. R., *J. Exp. Med.*, 1949, **90,** 85.
5. Cowdry, E. V., Microscopic Technique in Biology and Medicine, Baltimore, The Williams & Wilkins Company, 1943, 100.
6. Mallory, F. B., Pathological Technique, Philadelphia and London, W. B. Saunders Company, 1938, 86, 195.

[9] In a series of 86 cases of secondary pneumococcal pneumonia excluding cases of alcoholism and preceding respiratory infection, the commonest antecedent disease was found to be congestive heart failure (55).

[10] Pulmonary edema has been shown to occur in dogs after severe hemorrhage without shock and has given rise to the suggestion that growth of bacteria in pulmonary edema fluid of human beings may be a cause of postoperative pneumonia (56).

7. Loosli, C. G., *Arch. Path.*, 1937, **24,** 743.
8. Drinker, C. K., and Yoffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue. Their Physiological and Clinical Significance, Cambridge, Harvard University Press, 1941, 85.
9. Cameron, G. R., and Courtice, F. C., *J. Physiol.*, 1946, **105,** 175.
10. Courtice, F. C., and Phipps, P. J., *J. Physiol.*, 1946, **105,** 186.
11. Drinker, C. K., and Hardenbergh, E., *J. Exp. Med.*, 1947, **86,** 7.
12. Richter, C. P., *J. Am. Med. Assn.*, 1945, **129,** 927.
13. Latta, H., *Bull. Johns Hopkins Hosp.*, 1947, **80,** 181.
14. White, B., The Biology of Pneumococcus, New York, The Commonwealth Fund, 1938, 42.
15. Hobby, G. L., Meyer, K., and Chaffee, E., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50,** 281.
16. Hobby, G. L., and Dawson, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56,** 181.
17. Miller, C. P., and Foster, A. Z., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56,** 205.
18. Chain, E., and Duthie, E. S., *Lancet*, 1945, **1,** 652.
19. Davis, B. D., *Proc. Nat. Acad. Sc.*, 1949, **35,** 1.
20. Drinker, C. K., and Hardenbergh, E., *Am. J. Physiol.*, 1949, **156,** 35.
21. Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1933, **58,** 465.
22. Wood, W. B., Jr., and Irons, E. N., *J. Exp. Med.*, 1946, **84,** 365.
23. Loeschcke, H., *Beitr. path. Anat. u. allg. Path.*, 1931, **86,** 201.
24. Heinrichs, H., *Virchows Arch. path. Anat.*, 1932, **284,** 187.
25. Robertson, O. H., Coggeshall, L. T., and Terrell, E. E., *J. Clin. Inv.*, 1933, **12,** 467.
26. Gunn, F. D., and Nungester, W. J., *Arch. Path.*, 1936, **21,** 813.
27. Robertson, O. H., *J. Am. Med. Assn.*, 1938, **111,** 1432.
28. Hamburger, M., and Robertson, O. H., *J. Exp. Med.*, 1940, **72,** 261.
29. Robertson, O. H., and Hamburger, M., *J. Exp. Med.*, 1940, **72,** 275.
30. Loosli, C. G., *J. Lancet*, 1940, **60,** 49.
31. Wood, W. B., Jr., *J. Exp. Med.*, 1941, **73,** 201.
32. Loosli, C. G., *J. Exp. Med.*, 1942, **75,** 657.
33. Loosli, C. G., *J. Exp. Med.*, 1942, **76,** 79.
34. Robertson, O. H., *Ann. Int. Med.*, 1943, **18,** 1.
35. Sale, L., Jr., and Wood, W. B., Jr., *J. Exp. Med.*, 1947, **86,** 239.
36. Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2,** 859.
37. Francis, T., Jr., *Science*, 1934, **80,** 457.
38. Francis, T., Jr., *J. Am. Med. Assn.*, 1935, **105,** 251.
39. Straub, M., *J. Path. and Bact.*, 1937, **45,** 75.
40. Dal, M. K., *Ark. biol. nauk*, 1938, **52,** 107 (English summary).
41. Nelson, A. A., and Oliphant, J. W., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 2044.
42. Dubin, I. N., *Am. J. Path.*, 1945, **21,** 1121.
43. Loosli, C. G., *J. Infect. Dis.*, 1949, **84,** 153.
44. Pickrell, K. L., *Bull. Johns Hopkins Hosp.*, 1938, **63,** 238.
45. Smith, R. O., and Wood, W. B., Jr., *J. Exp. Med.*, 1949, **90,** 555, 567.
46. Wood, W. B., Jr., Smith, M. R., and Watson, B., *J. Exp. Med.*, 1946, **84,** 387.

47. Moore, R. A., A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, 131.
48. Menkin, V., Dynamics of Inflammation, New York, The Macmillan Company, 1940, 16.
49. Terrell, E. E., Robertson, O. H., and Coggeshall, L. T., *J. Clin. Inv.*, 1933, **12**, 393.
50. Heffron, R., Pneumonia with Special Reference to Pneumococcal Lobar Pneumonia, New York, The Commonwealth Fund, 1939, 204.
51. Winternitz, M. C., Wason, I. M., and McNamara, F. P., The Pathology of Influenza, New Haven, Yale University Press, 1920, 16.
52. Moon, V. H., Shock and Related Capillary Phenomena, New York, Oxford University Press, 1938, 348.
53. Parker, F., Jr., Jolliffe, L. S., Barnes, M. W., and Finland, M., *Am. J. Path.*, 1946, **22**, 797.
54. Fishberg, A. M., Heart Failure, Philadelphia, Lea & Febiger, 1940, 244.
55. Finland, M., and Sutliff, W. D., *Arch. Int. Med.*, 1934, **53**, 481.
56. Eaton, R. M., *J. Thoracic Surg.*, 1947, **16**, 668.
57. MacLeod, C. M., and Farr, L. E., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 556.
58. Drinker, C. K., and Yoffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue, Cambridge, Harvard University Press, 1941, 296.

EXPLANATION OF PLATES

Sections were photographed by Mr. Cramer Lewis.

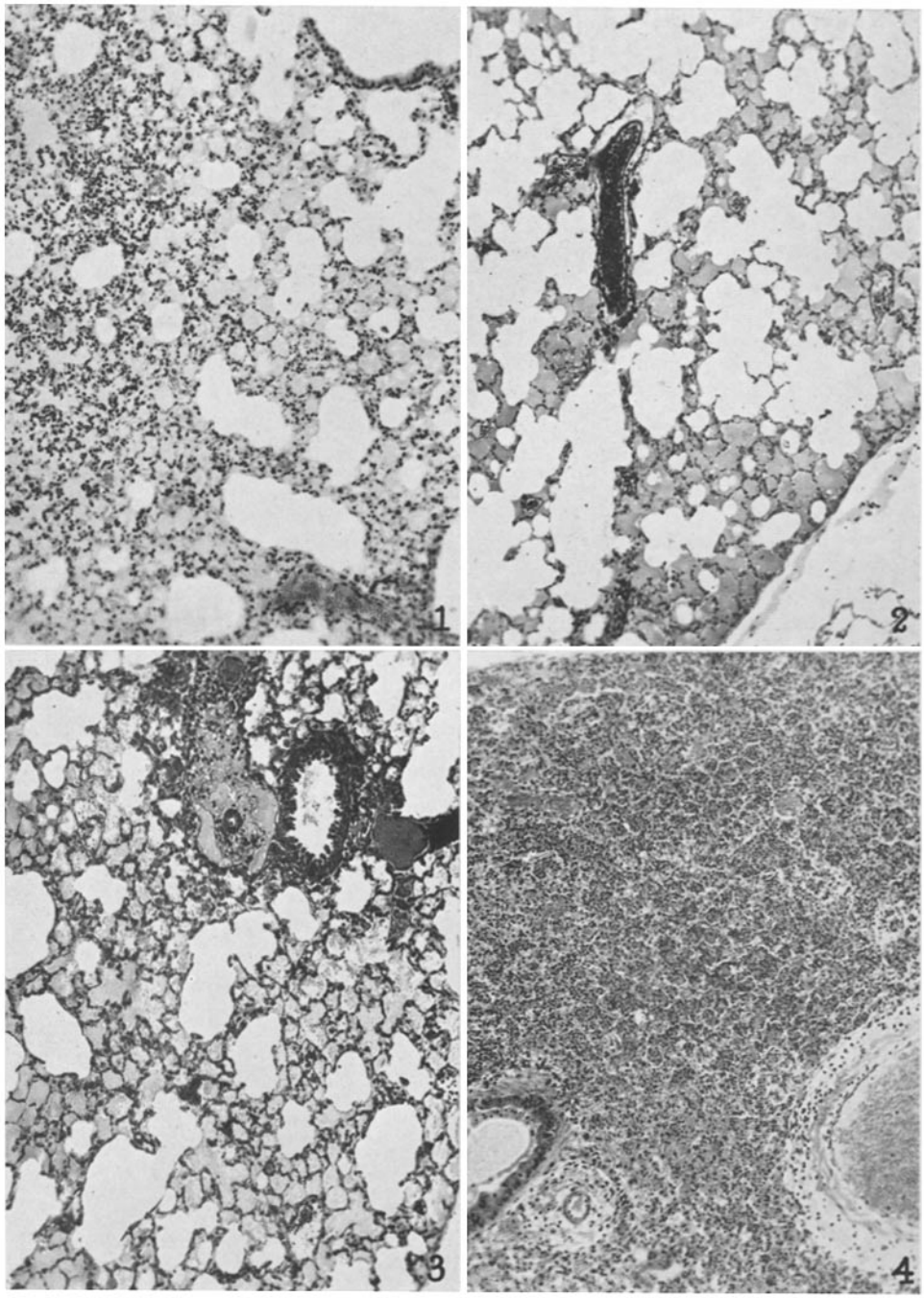
PLATE 13

FIG. 1. Pulmonary edema in a lesion due to influenza virus 5 days after inoculation. Hematoxylin and eosin. × 100.

FIG. 2. Pulmonary edema due to intrabronchial injection of serum. Phloxin and methylene blue. × 100.

FIG. 3. Pulmonary edema due to ANTU. Note dilated lymphatic vessels. Iron hematoxylin. × 100.

FIG. 4. Pneumococcal pneumonia in a mouse from intrabronchial injection of pneumococci suspended in normal mouse serum. Hematoxylin and eosin. × 100.



(Harford and Hara: Pulmonary edema and pneumonia)

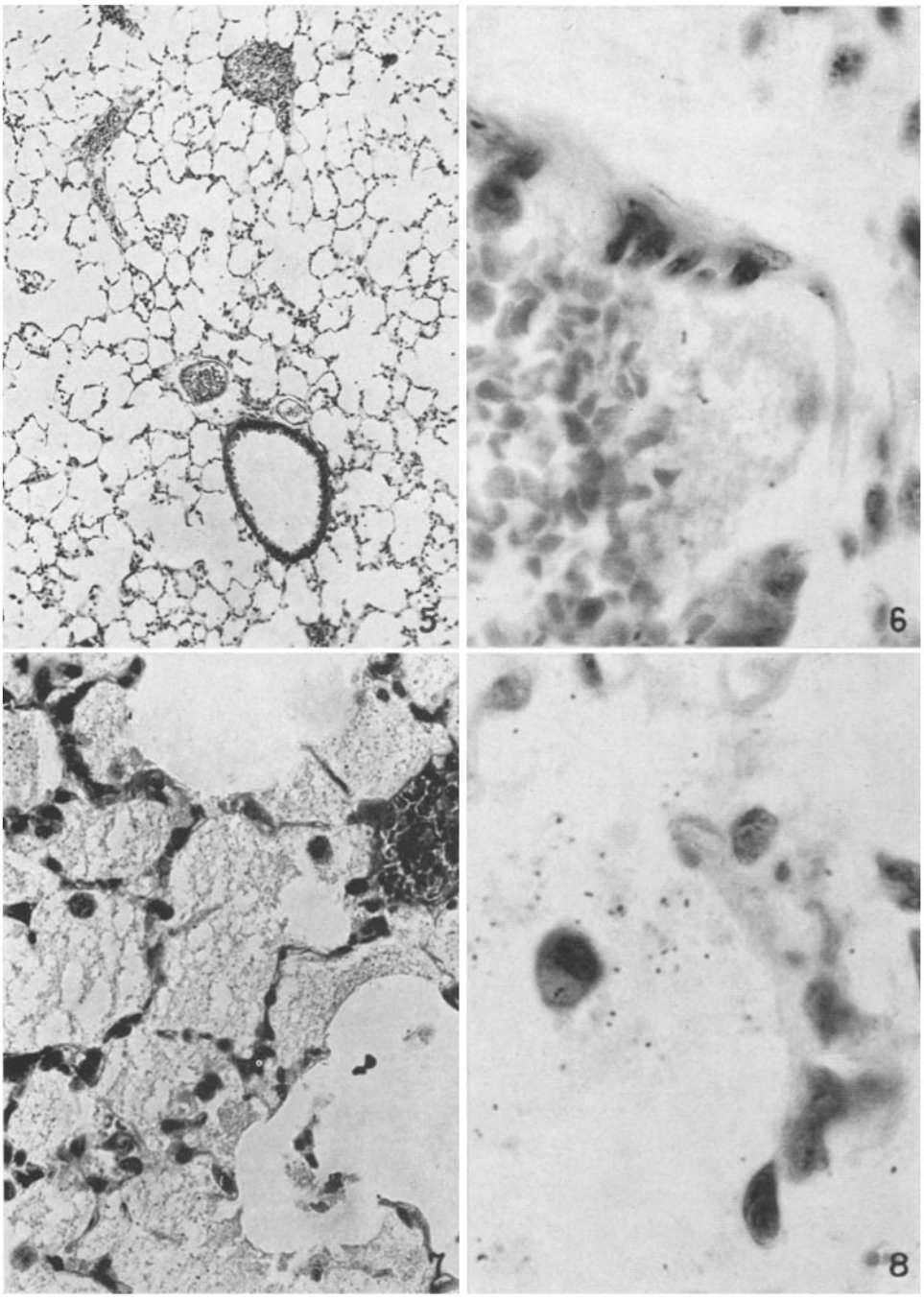
PLATE 14

FIG. 5. Lack of pneumonic exudate in a mouse moribund from septicemia 7 days after inhalation of Type I pneumococci. Eosin and methylene blue. × 100.

FIG. 6. Type III pneumococci in pulmonary blood vessel of mouse dying of septicemia 6 days after inhalation of this strain of bacteria. Giemsa. × 1200.

FIG. 7. Pneumococci apparently growing in alveolar fluid 4 hours after intrabronchial injection of many organisms suspended in normal mouse serum. Note the absence of polymorphonuclear leucocytes. Eosin and methylene blue. × 400.

FIG. 8. Phagocytosis of only one pair of pneumococci by an alveolar macrophage 4 hours after intrabronchial injection of the organism suspended in serum. Numerous unphagocyted pneumococci are present in the same alveolus and polymorphonuclear eucocytes are absent. Taken from the same section as Fig. 7. × 1200.



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