PATHOLOGY OF PNEUMOCOCCUS INFECTION IN MICE FOLLOWING INTRANASAL INSTILLATION

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PLATES 3 TO 5

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Webster and Clow (1) have shown that mice raised under controlled conditions present marked individual responses to pneumococcus infection; for example, a complete refractory state, a prolonged carrier condition, cervical adenitis with or without fatal termination, fatal pneumonia, or death from septicemia without any localization. Moreover, Webster had pointed out in an earlier paper (2) that individual breeds of mice behave in a predictable way and differently towards intranasal infection.

A study of the pathological changes in these mice has seemed warranted in order to determine not only the individual differences in mice belonging to one breed, but also the differences in the picture produced in the different breeds by the same strain of pneumococci and the reaction of identical groups of mice all of one breed to infection with pneumococci belonging to different types. The work to be presented shows that, contrary to previous reports, provided strains of pneumococci be selected on the basis of their intranasal as distinct from their intraperitoneal virulence, and the balance of virulence of organisms on the one hand and resistance of the mice on the other be so chosen that the mice succumb in not less than 4 days, a pneumonia can be evoked in mice which have received no preparatory treatment.

In 1924 Stillman and Branch reported the production of pneumococcus pneumonia in alcoholized mice (3) and gave a description of the lesions produced (4). The percentage of mice showing evidences of localization in the lung in the groups previously immunized with homologous or even heterologous pneumococci was far greater than in the non-immunized groups. Congestion of the interalveolar capillaries was the first lesion they noted, and this was often associated with a serofibrinous pleurisy without any other lesion in the lung. The congestion was

followed by interstitial inflammation of the alveolar walls and dilatation of the perivascular lymphatics which contained cell debris; later, the alveoli contained red cells and polymorphonuclears with a little fibrin. The most advanced stage showed a leucocytic exudate and extreme anemia of the alveolar walls. The initial lesion was in the alveoli and spread occurred both centripetally and centrifugally.

Stillman and Branch studied the inception of the lesion (5) and noted, 6 hours after spraying the animal with pneumococci, small areas of interstitial inflammation of the alveolar wall with a very slight amount of exudate.

Griffith (6) twice noted consolidation of the lungs and bilateral pleural effusion in mice dying with Type II pneumococcus infection after having been immunized beforehand with Type I serum, and also a grey consolidation of one lobe in a mouse succumbing to a Type I pneumococcus of low invasiveness. He gives no account of a histological study.

More recently, Neufeld and Kuhn (7) have described pneumonia in etherized mice infected intranasally with Type I and Type XIX strains of pneumococcus. Unless the mice were anesthetized when the inoculation was made, pneumonia did not develop, save in two cases in which the mice had been infected intranasally daily for 2 weeks with a Type XIX strain. The histological picture of the Type XIX pneumonia, briefly described, is said to be similar to the picture in experimental pneumonia in the monkey (8) and in human lobar pneumonia. Hoyle (9) also reports the production of all stages from mild congestion to severe pneumonia in etherized mice infected intranasally with pneumococci.

Little has been published on pathological changes in the other organs of mice following pneumococcus infections. As stated above, Webster and Clow (1) noted cervical adenitis, and Neufeld and Etinger-Tulczynska (10) also noted swelling of the whole face in mice infected with Type I organisms, a condition which, as will be pointed out below, is probably secondary to a cervical adenitis.

Material and Technique

For our experiments several inbred breeds of mice and many different strains and types of pneumococci were used. All strains of pneumococci used were freshly isolated from human cases of pneumonia. It is important to note at this point that, as Griffith (6) and Webster and Clow (1) have shown, not all strains are suitable for certain phases of the work, especially for those having to do with intranasal virulence and the production of pneumonia. A strain may be of maximum intraperitoneal virulence and yet fail to produce death or disease even when introduced into the noses of highly susceptible mice. The converse, high intranasal and low intraperitoneal virulence, is also found though the intraperitoneal virulence can never be very low. Thus, strains of moderate intraperitoneal virulence may have a high intranasal virulence, whereas strains of very low intraperitoneal virulence do not kill when introduced into the nose. The use of inbred strains of mice which will react in a predictable manner and of

carefully selected strains of pneumococci has proved of the utmost importance. Neglect of these two essentials will explain many of the inconsistencies and failures of the past. Observance of them provides the delicate balance between organism and host, which Wadsworth (11) deems essential to the consistent production of pneumonia. It is often necessary to test as many as 50 or 60 strains of pneumococci to obtain one or two with an appreciable intranasal virulence even for highly susceptible mice, and this is especially true when Type III strains, which are most frequently intranasally virulent, are excluded.

In the case of each breed it was possible, after a few preliminary experiments, to predict within certain limits the mortality rate and the incubation time following the intranasal inoculation of a given strain of pneumococci. The technique of inoculation has already been described (1). The inoculated mice were placed in separate cages and kept under observation for the next 2 or 3 weeks. Whenever possible, a postmortem examination was performed immediately on death and animals in a moribund condition were sacrificed for this purpose, though no mice were killed until there was a certainty that they would die shortly. In some cases, death occurred during the night and postmortem changes had set in when the autopsy was performed. A note was always made to this effect. Cultures of the heart's blood were taken at autopsy. All gross lesions were noted, and besides the lungs, the kidneys, liver, spleen, and cervical lymph nodes were taken for section and fixed in Zenker's fluid. A tube was passed down the trachea, the lungs filled with fixative under gentle pressure, the tube withdrawn, the trachea tied off, and the lungs fixed *in toto* before removal. Blocks of both were so prepared as to yield sections containing a portion of each lobe. In this way, a representative picture was obtained of both lungs. In a few cases serial sections were made. Sections were stained in eosin-methylene blue or hematoxylin and eosin.

While chief attention has been paid to the pneumonia and its development, the lesions in the other organs have been studied as well. The general pathological picture will be described first, both in relation to the different breeds of mice and to the different types of pneumococci.

Lesions in Different Breeds of Mice

Numerous scattered observations in this laboratory had shown that the lesions produced in different breeds of mice following the intranasal inoculation of one and the same strain of pneumococci differed from breed to breed. Experiments were undertaken to bring out this point.

Twenty albino resistant mice and twenty albino susceptible mice, both of Institute stocks, were each given an intranasal inoculation of 0.02 cc. of a 1/100

dilution of an 18 hour culture of Type III pneumococcus obtained from the heart's blood of a mouse dying of an intranasal inoculation with this organism.

The mortality was high in the susceptible group—65 per cent—, and low in the resistant group,—25 per cent. The susceptibles, moreover, were the first to succumb, and for those that died in this group the average survival time was only 72.5 hours compared with 120.8 hours in the resistants.

An examination of the sections prepared from the different organs revealed the fact that there was a difference in the manner in which the two breeds of mice reacted to the intranasal inoculation of the same strain of microorganism. The circumstances under which the test was made give assurance that the only variable factor lay in the breed of mice inoculated.

Lungs.—The lungs from the two groups showed quantitative differences. Macroscopically, the resistant mice showed no changes while three of the susceptible group showed consolidation and one showed serofibrinous pleurisy. In the resistant group, despite the fact that survival time was longer, all five mice showed very mild lesions microscopically. All showed congestion and early dilatation of the perivascular lymphatics, in four cases the channels containing only fluid and a few organisms. In the fifth, there were also polymorphonuclears and debris. One case showed very early infiltration of the alveolar walls in a small area and another a little fluid exudate into the alveoli.

In the susceptible group the changes were definitely more marked, but in eleven out of the thirteen cases could still be described as mild. There were congestion and perivascular lymphatic involvement, with mononuclear cells in the dilated lymph vessels. Three cases showed moderately large areas of exudate both interstitially in the alveolar walls where it consisted of leucocytes, and in the alveoli, consisting chiefly of fluid and red cells with a few polymorphonuclears. In one mouse which died in 75 hours the changes were similar in nature, but considerably more advanced.

Cervical Nodes.—The changes in the lymphatic system were next in importance after those in the lung. The lymph nodes from parts of the body other than the cervical region, except those at the hila of the lungs and occasionally those of the pre-aortic group, showed no changes. A pelvic node was frequently included in the section of the kidney, and was invariably normal. In the resistant group the changes in the cervical nodes were always conspicuous. The nodes,—which in the gross were large and opaque,—proved microscopically to be the site of large abscesses which completely or almost completely replaced the normal structure. This lesion was present in every mouse. In the susceptible group nine of the thirteen showed changes. Macroscopically, the nodes appeared

enlarged, but in only one case were they opaque. In four, the microscopic lesions were mild and consisted only of a dilatation of the peripheral sinuses which contained numbers of mononuclear cells and a few organisms. In five cases there were abscesses similar to those found in the resistant group.

Spleen.—The striking picture in the spleens of the resistant group is the necrosis or abscess formation in the follicles. In four of the five mice there was abscess formation in most of the follicles. In the susceptible group the lesion was less advanced than in the resistant group. In ten of the thirteen there were changes in the follicles, and in seven of these the lesion amounted to abscess formation. One spleen was normal and the other two showed an increased number of mononuclear cells in the pulp.

Kidney.—Normal in both groups.

Liver.—Few lesions in any of the mice. However, two resistant mice showed thromboses of the branches of the portal vein with infarcts of the liver.

An attempt was made to repeat this study of the lesions in different breeds of mice with other than Type III pneumococci. The difficulty encountered was to find a strain which would kill resistant albino mice when inoculated intranasally. One intranasally virulent strain was obtained, however.

An 18 hour culture of a Type XIX strain, which had proved highly virulent for Swiss mice when inoculated intranasally, was given intranasally to 20 resistant albinos and 20 susceptible albinos. The technique followed was that already given. The mortality rate and average survival time of the susceptible group was 55 per cent and 77.7 hours. In the resistant group only three died (mortality 15 per cent), and all of these died early (survival time 52.3 hours).

In the resistant group there was no evidence of localization in the lungs. One mouse showed a bilateral serofibrinous pleurisy, but there were no demonstrable lesions in the underlying lung. The other two showed only capillary congestion. In two of the three there were slight changes in the kidneys—granular debris in Bowman's capsule and convoluted tubules and some dilatation of the convoluted tubules. The other organs were normal.

In the susceptible group eight of the eleven mice showed some evidence of localization in the lungs. Of these, four had shown consolidation recognizable macroscopically. In three there was only capillary congestion. Four of the other eight showed only slight interstitial accumulation of polymorphonuclears and monocytes with dilatation of the perivascular lymphatics which contained fluid and a few cells. In the other four mice the lesions were advanced. They consisted of interstitial accumulation of cells and fluid in the alveolar septae, exudate of fluid with some red cells and polymorphonuclears into the alveoli, which was in some instances lobar in extent, some dilatation of the perivascular lymphatics which contained fluid and some cells, and in two cases a bilateral serofibrinous pleural exudate. In the other organs the changes were slight, save in

the kidney. Two of the eleven mice showed a frank diffuse nephritis with fibrin thrombi in the glomerular loops, changes in the cells of the convoluted tubules which contained colloid droplets or were frankly necrotic, dilatation of these tubules, and granular casts. In four others there were moderate tubular changes. In one case, the liver showed many areas of necrosis, and in two there were very small areas of necrosis in the splenic pulp.

In summary, of the lesions in the two breeds of mice, it can be said that there were definite quantitative differences in the two breeds (Table I). Thus, in the experiment with Type III organisms, the resistant mice showed very slight changes in the lungs, but marked lesions in the cervical nodes and follicles of the spleen. The susceptible mice showed more advanced lesions in the lungs, but less marked

TABLE I

Breed of mice	Type of pneumococcus	Lesion in organs		
		Lungs	Lymphatic system	Kidneys
Resistant	III	± (interstitial)	++	0
Susceptible	III	++ (interstitial)	+	0
Resistant	XIX	0	0	±
Susceptible	XIX	++ (interstitial)	0	+ (glomerular)
Unselected	I	++ (diffuse)	±	++ (glomerular)
Unselected	II	++ (confluent)	±	+ (tubular)
Unselected	III	++ (interstitial)	++	0

changes in the lymphoid tissue of the cervical nodes and spleen. In the experiments with Type XIX organisms the differences in the lungs were even more striking, although the small number of resistant mice which succumbed might raise the question as to the significance of the differences in this one particular case. Thus, none of the three resistant mice showed any local reaction in the lung, though one showed serofibrinous pleurisy. On the other hand, eight of the eleven susceptible mice showed local reaction in the lung, and in four of these the changes were advanced. It was impossible to be certain of differences in other organs, but it was possible that the susceptible group showed a higher incidence of nephritis and of significant changes in the liver.

Lesions Produced by Different Types of Pneumococci

Although different strains of the same type of pneumococcus differed in virulence when introduced intranasally into a standard group of mice, nevertheless the end result in those mice which died was found to be the same. Thus, it was found that, despite the varying mortality rate caused by different strains of the same type of pneumococcus, the lesions produced in those mice that died were closely similar for the one type of pneumococcus in a given breed of mice and were predictable. They differed, however, with the type of organism used. There had been few observations on this latter point.

The variation with the type of organism used is clearly brought out by the pathological changes found in those unselected albino mice which died as a result of the intranasal inoculation of different types of pneumococcus, as described elsewhere (1). Autopsies were performed on mice dying from the intranasal inoculation of Types I, II, III, and V strains. Three strains of Type I were used, four of Type II, two of Type III, and one of Type V. Twelve mice died from both Type I and Type II strains, eleven from Type III strains, and only one from the Type V strain. In every case in this experiment an autopsy was performed within 30 minutes of the time of death of the mouse, and no mice dying during the night were included. Since the lesions were very similar for the strains of one type but differed from type to type, the mice dying from infection with each type will be described together.

Type I and Type V.—The lesions in the single mouse dying from a Type V infection were so similar to those of the Type I group that they will be described with this group.

Macroscopically, the changes in the thoracic cavity were the most conspicuous. Pneumonic consolidation was infrequent, but the lungs were usually dark red and moist on section, unlike the normal pink, crepitant lung. Pleurisy was common and consisted usually of a thin layer of fibrin dulling the shiny surface of the lung. Effusion when present was scanty, thin, and watery. The bronchial and paratracheal nodes were normal. The cervical nodes were occasionally enlarged and opaque, but this was not a common finding. The spleen was also enlarged in several instances, but without macroscopic changes. In a certain number of cases petechiae could be seen on the surface of the kidneys.

Microscopically, one mouse out of the thirteen showed completely normal lungs, and in two others the lesions were very slight. The rest showed moderate or marked changes. In what appeared to be the earliest stages, the interalveolar

capillaries were dilated and filled with blood. There was often a slight pleural exudate of fibrin and polymorphonuclears, and the subjacent lymphatics would be filled with an exudate similar to that on the pleura. The blood vessels showed a dilatation of spaces in the region of, or more probably just outside, the adventitia which were presumably lymphatics. This dilatation increased in extent as the vessels ran centripetally, and the spaces contained serous fluid, fibrin, and pneumococci with but few cells. In this early stage there was very little exudate into the alveoli, and what was present consisted of fluid and pneumococci usually with a very few cells. The changes were scattered diffusely through both lungs. The blood vessels contained many pneumococci. In the later stages all changes became markedly increased. The vascular engorgement was extreme and the alveolar capillaries contained large numbers of leucocytes, both polymorphonuclears and monocytes. Pleural exudate when present was conspicuous and consisted of a layer, 1 mm. or more in thickness, of fibrin, polymorphonuclears, and organisms. The subpleural lymphatics were sharply outlined with their content of polymorphonuclears, fluid, and organisms, and this could be followed through the perivascular lymphatics to the hilum. The peribronchial lymphatics were very rarely involved. There was more exudate into the alveoli. In some places this was diffuse and consisted of serous fluid and pneumococci with relatively few cells, while in others it was found in small localized areas of a few alveoli and consisted of masses of leucocytes and a little fibrin tightly interwoven (Figs. 1, 2, 3). Pneumococci were not as plentiful in the blood stream as in the earlier stages.

The cervical nodes were enlarged, but rarely showed definite lesions. Occasionally there was an acute lymphangitis of the entering or leaving lymphatics which were dilated and filled with fluid, polymorphonuclears, and pneumococci. These nodes lie in close relation to the salivary glands and presumably drain the mucosa of the nasopharynx and throat. The spleen was also enlarged and occasionally showed necrosis of the Malpighian corpuscles. The normal structure was lost and the corpuscles were occupied by masses of polymorphonuclears, debris, and pneumococci. In one case there were several small infarcts in the liver, but otherwise this organ showed little but cloudy swelling or fat in the cells at the periphery of the lobule. The kidneys were the seat of a marked change which amounted in many cases to a diffuse nephritis. There was some proliferation of the endothelial cells of the glomerular capillaries, and in many cases the latter were occluded with fibrin thrombi (Fig. 12). The glomerular capsule spaces contained debris, and adhesions between tuft and capsule wall were found. In a few cases blood was found in the tubules that had come presumably from the glomerulus above. Besides blood, the dilated tubules contained loose casts of debris and, occasionally, pneumococci. The cells of the convoluted tubules frequently contained small hyaline droplets, described elsewhere (12) as colloid granules, and in a certain number of cases this lesion was striking. In all, ten out of the twelve mice showed changes which could be described as acute diffuse nephritis.

Type II.—Macroscopically, the most conspicuous changes were again in the thoracic cavity. Consolidation, which was often lobar in extent, was more commonly observed than in the other series, while the dark red, moist lung and the rather dry pleurisy were similar to those changes noted in the Type I group. It was very unusual to find any change in the lymph nodes whether tracheal, bronchial, or cervical, though in one case the latter were slightly enlarged and opaque. The spleen was not infrequently several times its normal size but showed no macroscopic lesions. The kidneys and other organs showed no changes.

Microscopically, in the ten of the twelve mice which showed lesions in the lung the peculiarity lay in the greater amount of exudate into the alveoli in the Type II infection than in those due to Type I or Type III infections. Even in the early stages the exudate was conspicuous, the alveoli containing large amounts of serous fluid and many pneumococci, together with a few cells of which the larger number were red cells. Other changes included capillary dilatation; early pleural effusion and exudate; dilatation of the subpleural lymphatics which were filled with fluid, cells, and organisms; and many organisms in the blood vessels. In the later stages, the alveolar exudate showed an increase in the number of leucocytes, and a slight deposition of fibrin (Figs. 4, 5). The organisms were present in the alveolar exudate but had decreased in the blood. The pleural exudate and involvement of the perivascular lymphatics were more marked.

No changes were noted in the cervical nodes. In the spleen the follicles occasionally showed areas of necrosis, at times so extensive as to obliterate the whole follicle. In one case, the portal veins of the liver contained thrombi and these had produced small cortical infarcts. The changes in the kidney were not as striking as with the Type I infection. The glomerular capsular spaces were dilated and contained albuminous fluid and pink hyaline debris. In two cases, the convoluted tubules contained small amounts of blood coming presumably from the glomerular tuft above. The most conspicuous changes were in the tubules. These were dilated, and the lining cells showed cloudy swelling and occasional necrosis with a loss of cytoplasm into the lumen of the tubule; special stains revealed colloid granules in the cytoplasm in many cases (Fig. 13). The tubules contained well formed casts of loose plugs of debris, the latter coming both from the glomerular transudate and from the cytoplasm of the cells of the tubules.

Type III.—Macroscopically, the picture of the infection due to Type III strains differed from those already described. The changes in the thoracic cavity were still very striking, but not in every case were they the most conspicuous lesion. Pleurisy with a copious gelatinous exudate was a very frequent finding. It was often associated with a mediastinitis and pericarditis, less frequently with a peritonitis. The change most commonly seen in the lungs was a large, moist, dark red lung; but consolidation, which tended to be lobular, was more common than in Type I cases. The other conspicuous change was in the lymphoid tissue. Bronchial and tracheal nodes were sometimes enlarged and opaque or even frankly

necrotic, but most striking were the changes in the cervical nodes which were usually enlarged and opaque, often necrotic and occasionally, the infection having spread to the surrounding tissues, were actually sloughing through the skin. In many cases the swollen lymph nodes obstructed the free return of lymph and the face region of the affected mice became swollen and edematous, especially on the side on which the nodes were principally affected (Fig. 15). The spleen was constantly enlarged, filled with blood, and showed opaque yellow nodules occupying the lymph follicles. In three cases, the enormously engorged spleen had ruptured and an extensive hemorrhage had occurred into the peritoneum. The other organs showed no macroscopic changes.

Microscopically, two of the mice showed no lesions in the lungs. In the other nine, the lesions differed from those due to either Type I or Type II strains. A most important feature was the tendency for the greater part of the lesion to be interstitial and localized in the alveolar septum. This, in the earliest stages, was shown by the very marked dilatation and engorgement of the alveolar capillaries (Fig. 6). At first, leucocytes were scanty, but later they increased in number. Diapedesis took place and large numbers of leucocytes together with fluid and some fibrin collected outside the vessels but within the septa (Figs. 7, 10). Later, there was a true interstitial inflammation progressing as far as necrosis of the septa. In two cases of the Type III infection, infiltration occurred round the main bronchi, a lesion never observed with the other types. The peribronchial lymph spaces were dilated and contained fluid, leucocytes, and a small amount of fibrin. Subpleural and perivascular lymphatic infiltration, similar to that described above, was also present. Alveolar exudate was similar to that found in Type I. In one case there was a plug of cells and debris in the bronchus, but in all cases the bronchial mucosa was intact.

Lesions were found in the lymph nodes at the hilum of the lung, in the cervical nodes, and in the follicles of the spleen. In the nodes, the change began as an enlargement of the node due to a swelling of the peripheral lymphatics which were filled with mononuclear cells and some organisms. Later, polymorphonuclears appeared and increased in number, while at the same time the pneumococci became more plentiful and areas of necrosis appeared. These increased in size until they filled the whole node, the normal structure of which was completely destroyed (Fig. 14). There could be found an acute lymphangitis of the entering or leaving lymphatics. The lesions in the cervical nodes were the most conspicuous and were often present when the nodes at the hilum of the lung were normal. In the follicles of the spleen, the lesion began in the center. Some of the lymphoid cells appeared necrotic and pneumococci could be seen in the neighborhood. Following this, there was a loss of most of the lymphoid cells, though whether chiefly from necrosis or from migration away from the site could not be determined, and only the framework of the follicle remained with polymorphonuclears lying at the periphery. Still later, these polymorphonuclears filled the follicle round a necrotic core, and a true abscess was formed (Fig. 16). In one case, small areas of necrosis were found in the liver, but otherwise there was nothing but cloudy swelling. The kidney showed nothing but cloudy swelling of the epithelium of the tubules.

In summarizing the lesions produced in a standard breed of mice by strains of pneumococci belonging to different types, the following points are most worthy of emphasis (Table I). As far as the lungs are concerned, the lesions differed considerably in the three types. In Type I, the changes were diffuse, without any particular point of localization and without lobar distribution. In the lungs from the Type II infections, the exudate into the alveoli and the lobar distribution were the most conspicuous features, while pleural exudate was infrequent. In the Type III cases, the localization of the lesion in the interstitial tissue of the alveolar septa was the most conspicuous difference, but there was also to be observed a very infrequent peribronchial infiltration. Copious gelatinous pleural exudate was also a feature of the Type III infections. In this group also were found the marked lesions in the lymphatic system, cervical nodes, hilar nodes, and lymph follicles of the spleen. Similar changes were found in the mice infected with the other types, but much more rarely and in lesser degree.

In the other organs the renal lesions were most striking. In ten out of twelve Type I cases there was an acute diffuse nephritis; in the Type II cases lesions were also found, but were predominantly tubular; while the kidneys of the Type III infections showed no lesions worthy of note. It is interesting that rabbits infected with Type I, Type II, and Type III strains of pneumococci showed renal lesions only with the Type I strains (12, 13), thus resembling the mice, but it must be stated that in the rabbits no attention was paid to the virulence of the strains of pneumococci used, and that most of them were stock strains.

The Development of the Pneumonia Following Intranasal Inoculation

In order to obtain information as to the origin and mode of spread of this pneumonia in mice following intranasal inoculation, serial sections of lungs were examined. It is realized, of course, that this method can merely suggest the point of origin and method of spread, and shows little or nothing as to the method by which the organisms reached the presumed point of origin. Serial sections were made only in Type III pneumonias.

As a result of the examination and a comparison of these findings with those obtained by the routine examination of several sections

from each lung, certain statements can be made. The earliest lesion in every case of Type III pneumonia, and the only one which is found by itself, is a dilatation and engorgement of the capillaries, especially those of the interalveolar septa (Fig. 6).

It is difficult to say which, if any, of the three changes next to be described precedes the others. These are: the collection of cells and fluid in the alveolar septa but outside the capillaries—an interstitial lesion (Figs. 7, 10); the exudate of albuminous fluid, rich in organisms but poor in cells, into the alveoli (Figs. 8, 11); and the dilatation of the subpleural and perivascular (but not the peribronchial) lymphatics with fluid, pneumococci, and monocytic cells.

The first two changes—the early exudate and the interalveolar inflammation—occur apparently independently in different parts of the lung or different parts of the same lobe. In a certain number of cases the interstitial lesion seems to precede the exudate and is well marked in the walls of alveoli in which exudate is just appearing. Yet, this is not always so, and often the walls of alveoli filled with exudate appear merely congested. It may be that in such cases the interstitial inflammation has been of ephemeral character and has already disappeared. The lymphatic involvement seems to follow a little later, and this is probably always the case with the subpleural lymphatics. It may appear in some microscopical preparations that lesions are present in the perivascular lymphatics in lobes with no other lesion save engorgement, but it has never proved possible to demonstrate this in serial section.

One can state then that in the mouse the pulmonary changes seem to progress in the following stages: (*a*) engorgement; (*b*) interalveolar interstitial exudate; (*c*) an albuminous fluid exudate into the alveoli and into the perivascular and subpleural lymphatics draining the affected region.

From now onwards, each separate process develops almost independently. The alveolar exudate becomes more cellular and eventually strands of fibrin, always less in amount than in pneumonia in man, are laid down. The interstitial inflammation may subside, but occasionally becomes so intense that the whole septum is destroyed and its structure lost. The subpleural and perivascular lymphatics become still more dilated and the contents become largely cellular

with polymorphonuclears predominating (Fig. 9). The pleura overlying the affected lymphatics is the site of an inflammatory exudate—an acute pleurisy—and this may be almost as early a lesion as the exudate into the alveoli.

The spread along the lymphatics can be followed to the nodes at the hilum. In these, the earliest change is one of dilated sinusoids which contain mononuclear cells and a few organisms. Later, these nodes become the seat of an intense inflammation so that the whole organ is converted into an abscess which ruptures and the organisms escape out into the mediastinum to produce an acute mediastinitis. The paratracheal nodes, lying higher up along the trachea, are always affected much later.

The cervical adenitis appears to have no direct connection with the pulmonary lesions as outlined here. In many cases—perhaps the majority—the most severe cervical adenitis is found in association with lungs that are almost normal or show little else than congestion.

SUMMARY

Pneumonia can be produced in mice, which have not been previously prepared, by intranasal inoculation of broth cultures of certain strains of pneumococci.

Lesions which are quantitatively different can be produced in different breeds of mice by inoculation of the same type of pneumococcus. Similar inoculation of different types of pneumococci into one breed of mice results in lesions which are qualitatively different.

In general, these lesions are as follows: a diffuse pneumonia and an acute glomerular nephritis in unselected mice receiving Type I strains; a confluent pneumonia and a tubular nephritis in the case of Type II strains; and as result of Type III strains, an interstitial pneumonia with extensive gelatinous pleurisy, together with necrosis and abscess formation in the spleen and cervical lymph nodes. Resistant strains of mice with Type III pneumococci show slight changes in the lungs, but marked lesions in the spleen and cervical nodes, while susceptible mice with the same type of pneumococcus show marked changes in the lung and moderate lesions in the spleen and cervical nodes.

The method of development of Type III pneumonia, studied by means of serial sections of nasally infected mice, appears to proceed

in the stages of vascular engorgement, interalveolar interstitial exudate, albuminous fluid exudate into the alveoli and the perivascular lymphatics draining the affected site, and finally, a frank pneumonia with a cellular exudate in the alveoli but without much fibrin.

BIBLIOGRAPHY

1. Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1933, **58**, 465.
2. Webster, L. T., *J. Exp. Med.*, 1933, **57**, 819.
3. Stillman, E. G., and Branch, A., *J. Exp. Med.*, 1924, **40**, 733.
4. Branch, A., and Stillman, E. G., *J. Exp. Med.*, 1924, **40**, 743.
5. Stillman, E. G., and Branch, A., *J. Exp. Med.*, 1930, **51**, 275.
6. Griffith, F., *J. Hyg.*, 1926, **25**, 1.
7. Neufeld, F., and Kuhn, H., *Z. Hyg. u. Infektionskrankh.*, 1935, **116**, 697.
8. Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, **31**, 445.
9. Hoyle, L., *J. Path. and Bact.*, 1935, **41**, 163.
10. Neufeld, F., and Etinger-Tulczynska, R., *Z. Hyg. u. Infektionskrankh.*, 1931, **112**, 492.
11. Wadsworth, A., *Am. J. Med. Sc.*, 1904, **127**, 851.
12. Blackman, S. S., Brown, J. H., and Rake, G., *Bull. Johns Hopkins Hosp.*, 1931, **48**, 74.
13. Rake, G., *Guy's Hosp. Rep.*, 1933, **83**, 430.

EXPLANATION OF PLATES

PLATE 3

FIG. 1. Type I pneumonia. Localized area of leucocytic exudate into alveoli just below pleura. There is a thin film of pleural exudate on the surface. Eosin-methylene blue. ×100.

FIG. 2. Type I pneumonia. Similar localized pneumonic area in the substance of the lung. At the lower right corner a portion of a blood vessel appears with a dilated lymphatic channel surrounding it, which is filled with leucocytes. Eosin-methylene blue. ×100.

FIG. 3. Type I pneumonia. Higher magnification showing the type of cells forming the alveolar exudate. Eosin-methylene blue. ×650.

FIG. 4. Type II pneumonia. Confluent area of pneumonia. The alveoli contain masses of leucocytes and necrotic debris. The lymphatic channels around the two larger vessels are greatly dilated and filled with leucocytes and debris. Eosin-methylene blue. ×100.

FIG. 5. Type II pneumonia. Higher magnification showing the alveolar exudate of leucocytes and debris. Eosin-methylene blue. ×650.

PLATE 4

FIG. 6. Type III pneumonia. Showing the general vascular engorgement. Eosin-methylene blue. ×100.

FIG. 7. Type III pneumonia. Great thickening of the alveolar walls with the collection of leucocytes inside and outside the alveolar capillaries. Eosin-methylene blue. ×100.

FIG. 8. Type III pneumonia. Showing many alveoli filled with albuminous fluid. Eosin-methylene blue. ×100.

FIG. 9. Type III pneumonia. Most of the alveoli are filled with a mass of leucocytes, debris, and fibrin. Many alveolar walls are necrotic. In the lower part of the field is a blood vessel with an enormously distended lymphatic channel filled with fibrin and leucocytes. Hematoxylin-eosin. ×100.

FIG. 10. Type III pneumonia. High magnification showing the interstitial infiltration of the alveolar walls. Eosin-methylene blue. ×650.

FIG. 11. Type III pneumonia. High magnification showing the alveoli filled with albuminous fluid and a few cells. There is interstitial infiltration of the walls. Eosin-methylene blue. ×650.

PLATE 5

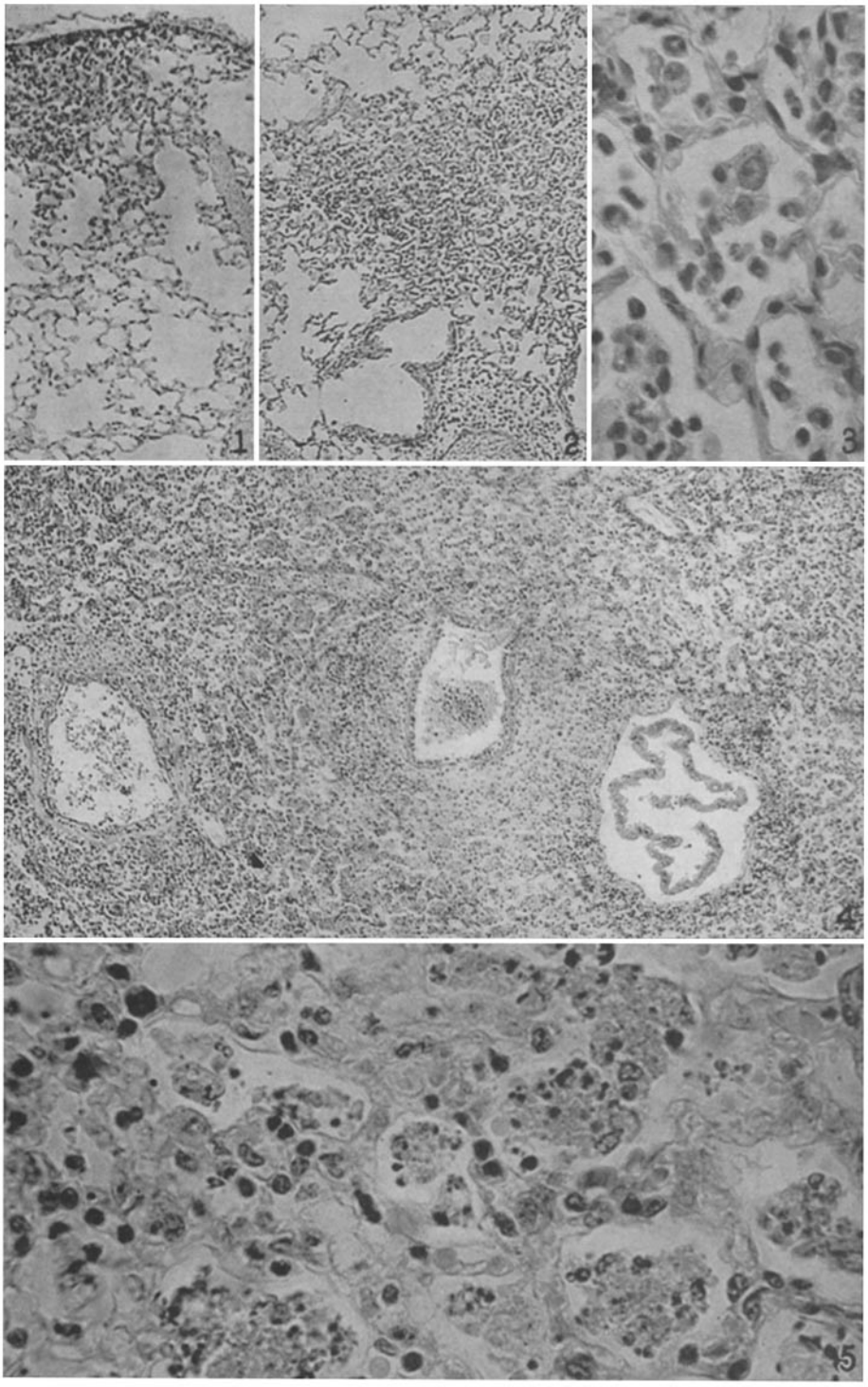
FIG. 12. Type I infection. Renal glomerulus with capillary loops occluded with fibrin thrombi. Weigert's fibrin stain. ×650.

FIG. 13. Type II infection. Deeply staining "colloid" granules in the cells of the convoluted tubules. Weigert's fibrin stain. ×650.

FIG. 14. Type III infection. Cervical lymph node the center of which is occupied by an abscess. The main lymph vessel to the right also shows inflammation. Portions of the salivary glands may be seen. Eosin-methylene blue. ×100.

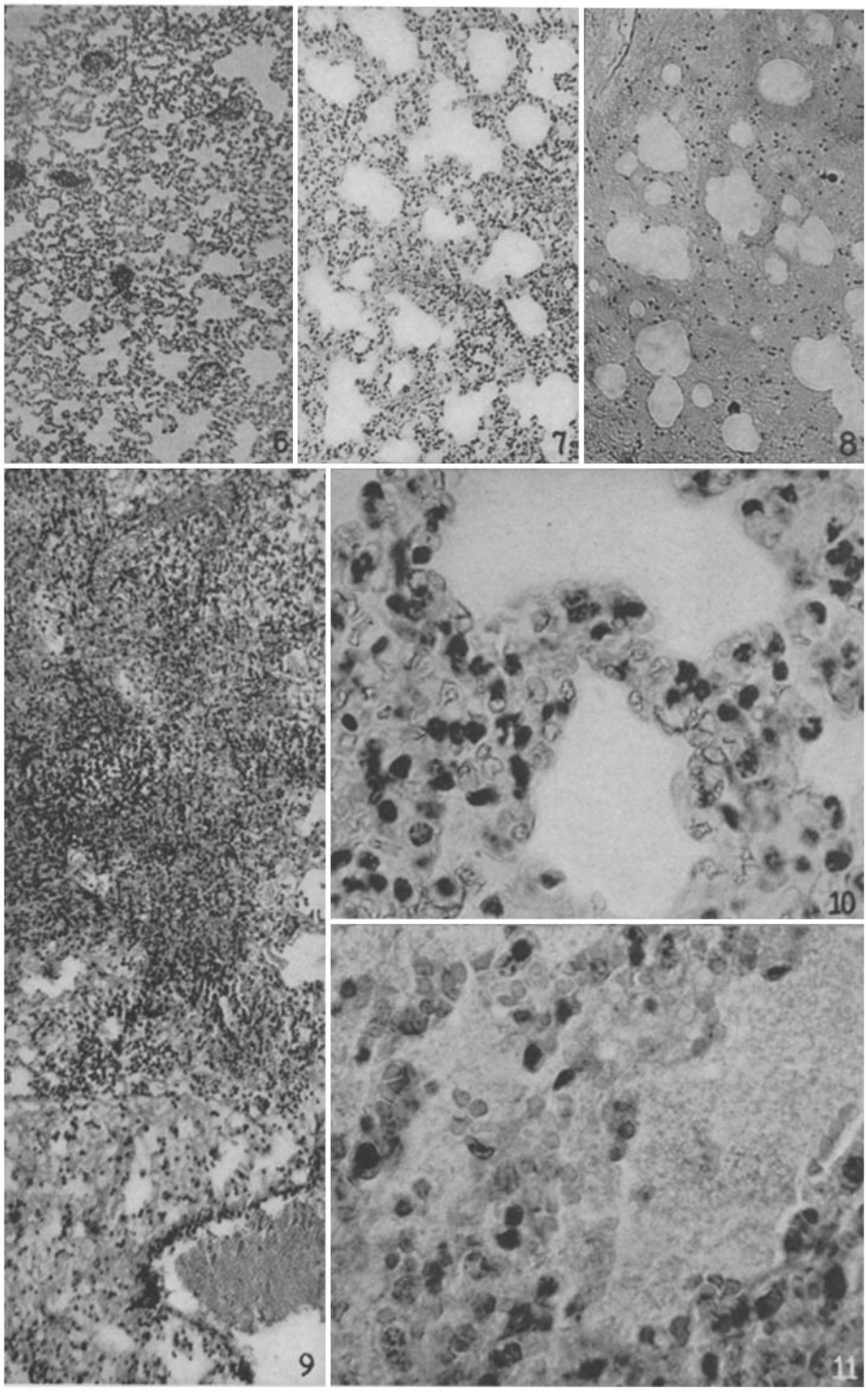
FIG. 15. Type III infection. Albino mouse showing ulceration through the skin below the left mandible (three dark areas through the hair), and great swelling of the whole head and face from edema. The eye is closed by the edema. Natural size.

FIG. 16. Type III infection. Spleen showing outline of Malpighian corpuscle the center of which is occupied by an abscess. Eosin-methylene blue. ×100.



Photographed by Louis Schmidt

(Rake: Pneumococcus infection in mice)



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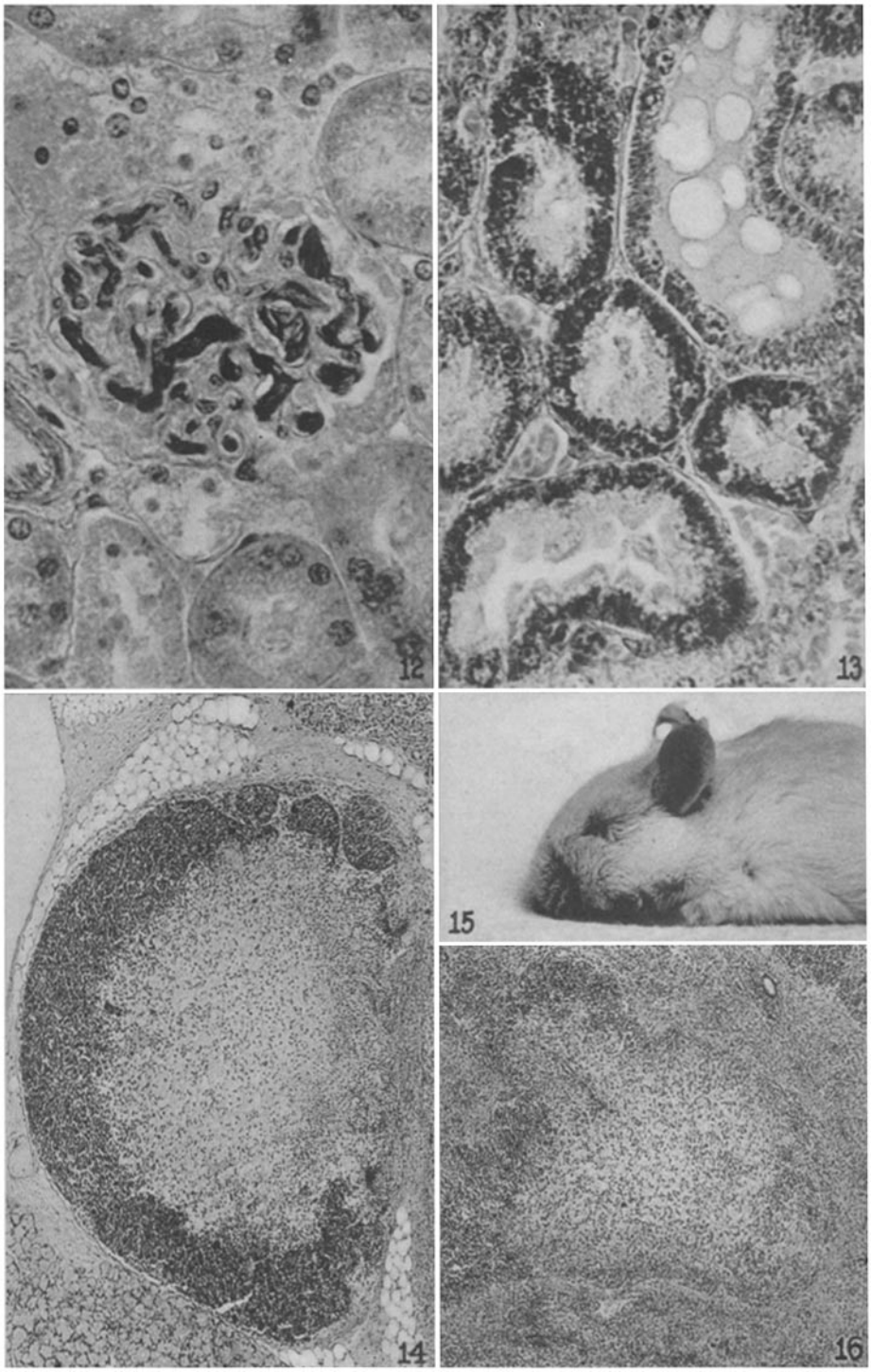
(Rake: Pneumococcus infection in mice)



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(Rake: Pneumococcus infection in mice)