SOME BACTERIOLOGICAL AND ENVIRONMENTAL FACTORS IN THE PNEUMONIAS OF LOWER ANIMALS WITH SPECIAL REFERENCE TO THE GUINEA-PIG.*

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Many of the domesticated animals are afflicted with pneumonias and each species seems to have a type of its own. We encounter primary pneumonias, pneumonias that are the local expression of general infections, and those that accompany or follow other infectious diseases. The relation of the bacillus of septicemia hemorrhagica to the pneumonias of domestic animals is now well known. This species with its many varieties is found in the air passages of healthy animals, and bears the same relation to the lung infections of these animals that is borne by the pneumococcus to human pneumonia.

The utilization of these animal diseases for the study of the general underlying conditions of infection of the respiratory tract is possible only when they are of freqeunt occurrence in a given neighborhood, and even then proper facilities for study may be lacking. In 1899 the writer became interested in an epidemic pneumonia of guinea-pigs. It occurred to him that a general study of the pneumonias of small laboratory animals might perhaps help to elucidate some of the accessory conditions without which the specific microorganism is unable to multiply in the lungs. This pneumonia reappeared from season to season and more or less attention was given to it from time to time. Though the work has not progressed beyond the bacteriological stage, it has seemed best to publish a few notes on it at this time.

The importance of the guinea-pig as a laboratory reagent to toxins and living bacteria is very great to-day, and the time has come to call attention to the necessity for ridding

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the species of those infections which tend to interfere with investigations and which place a serious financial burden on the laboratory. It is now a difficult matter to obtain smaller animals free from latent infections. An acquaintance with these infections is essential in avoiding serious misinterpretations in the study of induced diseases, which extend over longer periods of time, as is the case in the study of tuberculosis and in the immunization of animals for agglutinins and other antibodies.

During these observations two different species of bacteria were encountered as agents. This has complicated the study very much, since it became necessary to make a fairly exhaustive bacteriological examination of each case. One of these organisms is closely related to the motile bacillus recently described by M'Gowan,[1] Ferry,[2] and Torrey,[3] as the agent of distemper in dogs. The other is the pneumococcus (Diplococcus lanceolatus). The motile bacillus was found at the very start in 1899, and obtained in pure culture from hundreds of lungs since. It was also isolated from another entirely separate collection of breeding guinea-pigs. The pneumococcus was first definitely cultivated in 1908 and every season since then. It has seemed best to briefly describe at this time these two organisms as they occur in the pneumonic lesions of the guinea-pig, and to leave any general discussion based on these and earlier studies for a later paper.

BACILLUS BRONCHISEPTICUS (M'GOWAN) IN PNEUMONIA OF THE GUINEA-PIG. — In the small population of guinea-pigs accessible to the writer there were from thirty to fifty breeding males and females and their offspring. The breeders were kept in separate, adjoining pens, three or four females and one male in each pen. The young were kept with the mothers for four weeks and then herded together in a large enclosure in the same room. The building was kept at about 50° F. in winter, and a certain limited ventilation provided for. The food in winter consisted of hay,

oats, and carrots; in summer, of fresh grass and oats. The building is in a rural location, free from dust.

The pneumonia studied occurs only in the winter season (December to May). Very rarely a case was found in the warm season which, however, as a rule, dated back to the previous winter. Besides the seasonal fluctuation there was an annual fluctuation, with some years of little or no disease, and others in which it assumed epidemic proportions. Every year presented some slight or more pronounced departure of the characters of the disease from those observed before. In the population studied we had to make accessions from other sources to maintain a supply of young. It was therefore impossible to determine whether the disease could wear itself out completely if fresh virus were kept away.

The clinical manifestations of lung disease are variable and uncertain. Many animals below adult age pass through the disease without any noticeable disturbances. The remnants of the disease may be discovered months later. The adult

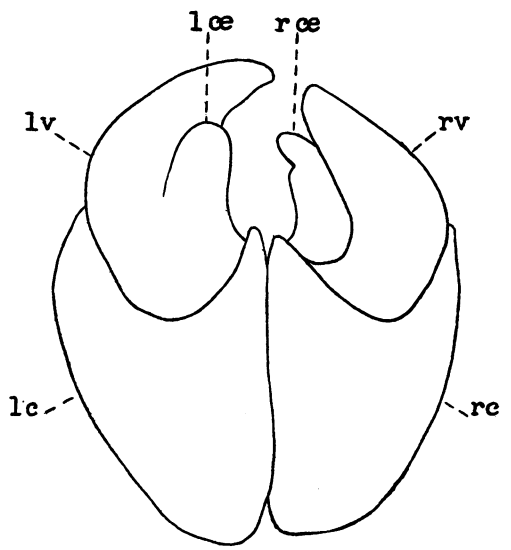

FIGURE I.

Lungs of guinea-pig. Dorsal aspect: ce, cephalic lobes; v, ventral lobes; c, caudal (or principal) lobes.

may pass through a period of evident illness and recover after several weeks. A certain number die after a very short illness of perhaps one or two days. In general, it may be stated that the uncomplicated attack is rarely fatal. The high mortality observed among breeding females has a definite underlying factor to be discussed later, not found among the young and immature.

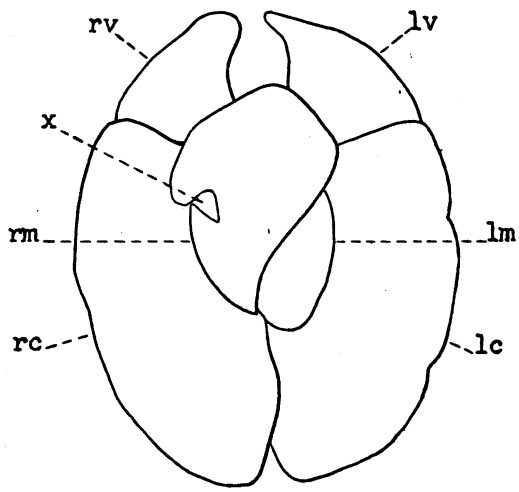

FIGURE II.

Lungs of guinea-pig. Diaphragmatic aspect: v, ventral lobes; c, caudal lobes; m, median lobes; x, notch in right median lobe for vena cava.

The inflated lungs of the guinea-pig are shown in Text-figures 1 and 2. The nomenclature of the lobes adopted by the writer many years ago for the mammalian lung will be used here. In Figure 1, giving the dorsal view, the principal or caudal lobes make up about two-thirds of the bulk of the organ. The cephalic portion consists of a larger lobe adjoining the caudal, and called ventral. A smaller lobe (right) or merely an adjunct of the ventral (left) is denominated cephalic lobe.

On the ventro-caudal or diaphragmatic aspect, there are two small median lobes, which occupy the place of the

unpaired azygos lobe in many other mammals. The right median is more than twice the size of the left and has a roundish notch on its right margin for passage of the posterior or caudal vena cava. It will be seen that the lobes designated cephalic are not visible from the diaphragmatic side, and that the lobes designated median are not seen from the dorsal aspect. The space between the ventrals and above the right median lobe is occupied by the apex of the heart.

In the type of pneumonia under consideration the lobes most frequently involved are the median, next, and in addition, the cephalic, the ventral, and, in very severe cases, the adjacent portion of the caudal lobes. Rarely more than one-half the total bulk of the lungs is found consolidated. In general, it may be said that the hepatized portions correspond to the lowest regions of the lungs in the standing animal. The consolidation is usually, but not regularly, symmetrical. The involved tissue varies from a uniform flesh-red or dark red to a variegated grayish red in which the gray is more or less focalized. The grayer the tissue the firmer it is. The firmer and grayer lobes are usually the median or cephalic or both. The inference, from these gross appearances and from sections, is that the disease is oldest and farthest advanced in the small lobes mentioned. Occasionally yellowish focal necroses are observed. The pleura is not involved in the uncomplicated disease.

The examination of sections from a large number of lungs in various stages showed that the disease is essentially a focal pneumonia in its fully developed state. Bronchioles plugged with dense necrotic cell masses are surrounded by a circle of alveoli and groups of the same, plugged with dense cell masses of the same character (Plate XIX., Fig. 1). These do not, as a rule, permit one to decide upon their nature as the nuclear débris is very dense. In foci less advanced there can be seen polymorphonuclear leucocytes, alveolar epithelium and endothelial cells mixed together with rare traces of fibrin. These multiple foci are not confluent and the parenchyma immediately surrounding each focus may be filled with a loose granular exudate, red cells, fibrin fibrils, shed

alveolar cells and leucocytes, in small numbers. The epithelium of the associated bronchioles which are usually filled with cell débris may be intact. The cell masses in them evidently come from the affected alveoli.

The earliest stages of the disease are not definitely known in spite of the many cases examined. Evidence, however, points to a congestion involving either entire small lobes or large portions of larger lobes. Upon these congested regions the focal pneumonia is probably grafted. In a certain number of cases, up to a year after the active epidemic, the affected lobes have been found very firm, grayish, and shrunken to quite small dimensions. Usually, one or both median lobes are found small, grayish, and firm. When such lungs are artificially inflated all but these lobes respond. They finally become very small, almost unrecognizable appendages of the roots of the lungs. In sections of such lobes the alveoli have largely disappeared, owing to the formation of dense fibrous tissue. Necrotic remnants of the broncho-pneumonic foci may still be present, suggesting the possibility of a survival of the infection in such cases from season to season.

The accumulation of dense necrotic cell masses may lead to a necrosis and softening of the whole pneumonic focus. When situated under the pleura adhesions may occur. This is especially common between diaphragm and diseased median lobes. There remains, however, the doubt whether the necrosis or, at any rate, the adhesive inflammation is not due to an associated or secondary invader, the pneumococcus. This necrosis may lead to fatal intrathoracic hemorrhage.

THE BACILLUS ASSOCIATED WITH ONE TYPE OF PNEUMONIA IN GUINEA-PIGS (B. BRONCHISEPTICUS M'GOWAN). — Most of the following notes on the bacillus were written in 1902 from observations gathered in 1899. They were not published at that time, partly because there seemed no urgent reason, partly because the bacillus had evidently been seen and more or less satisfactorily described by earlier

observers. The oldest communication I could find was by Tartakowsky.[5] Though he failed to notice the motility of the bacillus, his recognition of the peculiar, glanders-like growth on potato, as well as other data, led me to assume that he had the same bacillus under cultivation.

In 1900 Strada and Traina[6] investigated this disease in Italy where it prevailed as an epizootic of considerable severity. Cognizant of Tartakowsky's work, they, however, do not accept the identity of his and their bacillus which they name *Bacterium pneumoniæ caviarum*. In general, their descriptions and results of experiments agree with mine.

A paper published without cognizance of Tartakowsky's in 1900 by Martini,[7] consists of a description of a bacillus evidently identical with Tartakowsky's and that of the writer. Martini studied only two guinea-pigs. He names his organism *B. pulmonum glutinosus*.

In 1906 Selter,[8] working experimentally on some problems in pneumonia, described *Bacillus cavisepticus mobilis*, which is probably the same organism as the one first described by Tartakowsky. In 1910 M'Gowan[1] observed this organism in his stock of healthy and inoculated guinea-pigs. He was able to obtain pure cultures from the trachea of guinea-pigs whose lungs were healthy. He obtained the same or a similar strain from the respiratory passages of rabbits, and of dogs affected with distemper. Soon after, Ferry[2] and Torrey[3] studied this same organism in its relation to canine distemper.

The bacillus of guinea-pig pneumonia is a minute rod with rounded ends. From agar slants, it measures about .7 μ long and .5 μ broad. Longer rods are frequently seen, but they are evidently in process of division. From bouillon, the rods are somewhat longer (1 to 1.5 μ). Thread formation and clumping (in presence of immune fluids or of bits of tissue in cultures) is now and then observed. The bacillus is readily stained with the ordinary aniline dyes. Strong carbolfuchsin followed by .1 per cent acetic acid gives the sharpest and most distinct outlines. The rods stain solidly. They are decolorized according to Gram. The bacilli are motile; their movements very rapid.

The flagella, in carefully prepared films, are present in varying numbers

from none to six per bacillus. When parallel preparations of typhoid bacilli are compared with these films, the flagella of the pneumonia bacilli appear much shorter, somewhat thicker, and with undulations of lower amplitude.

The bacillus grows moderately well on gelatine at the ordinary room temperature. The colonies in gelatine rolls or plates do not present any very characteristic features. The deep colonies appear, after thirty-six to forty-eight hours, as minute spheres, with homogeneous disc which later shows one or more concentric marginal rings. Coarse granulations are not seen. They do not become over .5 millimeter in diameter.

The surface colonies vary somewhat with the gelatine. They may expand into discs 5 to 6 millimeters in diameter, or remain limited to 2 millimeters. The larger ones have a more or less irregular, lobed border. The center is thickest, and ridges radiate from it in all directions to the marginal zone, which is very delicate and translucent. In this stage, they slightly suggest *B. coli*. The smaller colonies are somewhat more compact and lack the delicate marginal zone. Liquefaction was not observed.

In gelatine tubes the growth in the wire track is very feeble, while the surface growth expands more or less in a manner similar to a surface colony.

On ordinary agar the smooth, grayish, somewhat glistening expansion presents nothing noteworthy. When bits of lung, or material from the air tubes are rubbed on agar, the colonies may not appear during the first twenty-four hours.

On potato in tubes the growth varies with the potato, but in nearly all cultures made a rich yellowish brown layer appeared within a week. Comparative trials with a freshly isolated glanders bacillus showed that it is impossible to distinguish the layer produced by it and that of the pneumonia bacillus. All writers agree upon the rich growth on potato and its peculiar color. (The bacillus of cattle abortion (Bang) produces a similar growth on potato but it appears more slowly.)

On Loeffler's blood serum a fairly rich, pale yellowish layer appears after one or two days.

In bouillon there is cloudiness during the first twenty-four hours. When the tube is shaken, the clouds show agitation, and very delicate iridescent patches of a surface membrane cling to the glass, as with the typhoid and the colon bacillus. This membrane becomes like delicate tissue paper in forty-eight hours. The bouillon becomes quite turbid, and a copious deposit appears, which becomes very ropy in two or three weeks.

In milk cultures no visible changes take place. In litmus milk there is no immediate reddening. After some weeks the upper half of the milk assumes a distinctly blue color; the lower portion is decolorized by reduction.

Indol is not produced, nor are there any odors from the cultures.

The bacillus is strictly aërobic, for in the presence of sugars it multiplies only in the bulb of the fermentation tube and leaves the branch

clear. Gases and acids are not set free. The fluid in the bulb and in ordinary test-tube cultures becomes slowly alkaline, finally reaching and passing beyond the phenolphthalein neutral point.

A curious phenomenon was observed a number of times when turbid bouillon cultures several weeks old were boiled. The fluid became distinctly viscid so that air bubbles imprisoned in it failed to rise. A few drops of acetic acid induced a separation of the viscid material, often in form of a twisted strand or of minute flakes, which were composed of bacilli. Evidently a mucin-like substance is formed, probably as capsular substance more or less soluble in the increasingly alkaline culture fluid. Occasionally, when the old, alkaline, quite turbid bouillon cultures are boiled, the bacilli are aggregated into very minute flakes which rise to the surface as a scum and adhere to the sides of vessel.

In its bio-chemical character, the bacillus approaches the pyocyaneous group, if we assume that in the course of special adaptation the former has lost the power of liquefying gelatine and producing pigment. Like the latter, it is also strictly aërobic and without action on any sugars. It is interesting to note that there are very few pathogenic bacteria which fail to act on dextrose as indicated by acid or gas production. (The bacillus of cattle abortion is also without action on sugars.)

If the consolidated lung tissue is pierced with a platinum wire and this rubbed on slanted agar, a variable crop of colonies of the motile bacillus appears within forty-eight hours. As a rule, there is little or no visible growth during the first sixteen to twenty-four hours. The bacilli are most numerous in the early grayish red stage of the pneumonia, scarce in the old sclerotic lesions, and in the very fresh, dark red stage. It is surprising how rarely cultures from bits of hepatized lung tissue or from mere stabs contain more than one kind of organism. Such cultures were in nine-tenths of the cases pure, unless the pneumococcus was present. In smears from the affected lung tissue the delicate bacilli are in some cases easily detected, and then chiefly within cells or else they are very scarce. The culture tube is, in many

cases, the only demonstration of their presence. They were uniformly absent from spleen, liver, and kidney, but occasionally found in the uterine horns, and then chiefly in association with dead embryos.

The interesting observation of Mallory[4] on the presence of bacteria on the ciliated epithelium of the air tubes in fatal cases of pertussis, drew my attention to the ciliated epithelium in smears from diseased lungs. In a certain number of cases, the minute bacilli could be seen attached to the cilia sometimes in large numbers (Plate XX., Figs. 3 and 4).

The bacilli are readily obtained in cultures from the bronchi of animals in which the lungs are diseased. The best method is to expose the trachea of the dead animal and sear its surface. A little flap is cut out with sterile scissors and the wire loop inserted into each bronchus. When the animal was killed with chloroform, or had been placed in the refrigerator immediately after death, the cultures thus obtained on slanted agar contained many colonies of the motile bacillus and rarely colonies of adventitious organisms. The chloroforming does not seem to influence adversely the success of the culture.

Latterly, the bronchi of many guinea-pigs, chloroformed in the laboratory, have been examined bacteriologically, and in a small per cent of apparently healthy pigs with normal lungs, taken from the infected stock, pure cultures of the motile bacillus were obtained. These findings bring us back to the work of M'Gowan who stated that B. bronchisepticus was present in trachea of his healthy guinea-pigs. It is highly probable that M'Gowan's animals had been through a pneumonia epidemic, for in a group of guinea-pigs from an outside source we did not obtain cultures of this organism. In guinea-pigs which show traces of former disease, to be seen most frequently in the consolidated shrunken median lobes, the motile bacilli are quite regularly obtained from the bronchi.

The pathogenic action of this bacillus is not pronounced unless large doses are administered. Subcutaneous injection usually leads to a large painful swelling which may subside

or ulcerate. In several cases a large element of the swelling was the necrosis of subcutaneous fat. The animal loses some weight but is otherwise not affected. The local swelling in another case fixed seven days after inoculation showed extreme edema of the subcutaneous tissue, with focal collection of polynuclear leucocytes.

Intra-abdominal injection of bouillon cultures leads to death in twenty-four to forty-eight hours. The size of the dose and the age of the bouillon culture (in days) are important factors. One-half cubic centimeter of a forty-eight-hour bouillon culture has usually proved fatal when strains were used which had not been cultivated long. The inoculated animal shows signs of great prostration within twelve hours, with staring coat, accentuated breathing and drowsiness, and convulsive movements before death.

The lesions are confined, as a rule, to the peritoneal cavity. The serous membrane is spotted with small hemorrhages and a viscid translucent exudate, consisting, in part, of polymorphonuclear leucocytes makes it appearance, becomes more abundant with prolongation of life, and form pseudo-membranes on liver, spleen, and the less mobile parts of the intestines. The injected bacteria are easily recovered, though not abundantly, from the peritoneum. They are very scarce in the blood, liver, and lungs.

Intrathoracic injection leads to a similar train of occurrences. The fatal dose may be very small (.005 cubic centimeter bouillon culture). Death occurs usually within forty-eight hours. The lungs are very deeply congested and a very thin grayish exudate covers the visceral pleura. Sections show great distension of the pulmonary network of capillaries with red cells, and occasional intra-alveolar hemorrhage. The delicate exudate is composed of polynuclear cells, often within a network of proliferated cells from the pleural covering. Among the cell débris are dense groups of bacilli.

In the pneumonic foci of the natural disease as well as in the exudates produced by inoculation into serous cavities, the cells (alveolar, endothelial, polynuclear) appear to undergo

a rapid necrosis. The nuclei become fragmented, pycnotic, or else assume bizarre shapes; they are usually crowded to one side in the cytoplasm which has an attenuated, edematous appearance. The picture presented suggests an action analogous to that of leucocidins. Experiments are now under way to analyze in more detail the action of this organism on cell-life.

Cultures have been fed, rubbed into the nares, and inserted into the vagina of guinea-pigs without causing any disease or abnormal pregnancies. To eliminate the possible factor of a filterable virus, the lungs of advanced cases were ground, suspended in salt solution, shaken, and filtered through Berkefeld filters. The sterile filtrate injected into thorax and abdomen of guinea-pigs had no injurious effect.

In 1899 a number of intraperitoneal injections were made with filtrates of bouillon cultures passed through Pasteur-Chamberland filters. Guinea-pigs stood doses up to five cubic centimeters with only a slight drop in weight. Toxic effects were not noticed. From one of the guinea-pigs the same pneumonia bacillus was isolated from both uterine horns. It was thought that perhaps the Pasteur filter had allowed some bacilli to escape into the filtrate, and this led to the experiments of Dr. William B. Wherry[9] in this laboratory on the permeability of certain filters. Wherry found the Chamberland filter impervious to this minute organism, and I am inclined now to believe that the motile bacillus may have been present in the guinea-pigs before inoculation. This assumption is based upon the finding of this bacillus in the uterine horns of several guinea-pigs in later years. The small Berkefeld filter (No. .5), however, cannot be relied upon to hold this bacillus back.

A rabbit and several mice were not affected, the former after an intravenous injection of .5 cubic centimeter, the latter after a subcutaneous dose of .1 and .03 cubic centimeter of a bouillon culture.

Experiments to test the pathogenic action of this bacillus should not be made on guinea-pigs from infected populations since their immunity may be raised. If actual pneumonic foci

are absent, cultures should be made from the lower trachea, and bronchi of some animals to determine the presence or absence of this bacillus.

During the period of 1899–1913, diseased lungs of guinea-pigs used in other experiments have been examined bacteriologically from time to time. In these, as well as in spontaneous cases, a number of other bacteria have been encountered, and usually in pure culture. Among these were B. coli, B. lactis aerogenes, a very minute Gram-positive bacillus, a very minute streptococcus, and others. The colon bacillus was most frequently present, the rest encountered but once.

THE RELATION OF THE DISTEMPER BACILLUS TO THE PNEUMONIA BACILLUS. — The writer's attention was first called to the close resemblance of these two strains of bacilli while reading Torrey's article. Dr. Torrey kindly sent a strain for comparison and the two agreed in every particular so far as morphological and cultural characters are concerned.

Two rabbits were immunized with the two strains respectively, by three subcutaneous injections of the living bacilli. The local lesions were either absent, or transient swellings resulted. It should be stated that the rabbits were born and raised in a building entirely separate from the guinea-pig population affected with pneumonia. The result of the test is given in Table I. The tubes were kept in the incubator two hours, then at 45°–50° F. for twenty hours. The agglutination relationship is seen to be very close.

TABLE I.

Action on B. bronchisepticus 97.

(After 22 hours.)

Serum of rabbit immunized towards	Serum Dilution.									
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
B. bronchisepticus 97 (Torrey)	C*	C	C	C	C	C	++	+	O	O
B. guinea-pig pneumonia XIV.	C	C	C	C	C	+++	++	+	O	O

Action on bacillus of guinea-pig pneumonia.

(After 22 hours.)

B. Bronchisepticus 97 (Torrey)	C	C	C	C	++	+	O	O	O	O
B. guinea-pig pneumonia XIV.	C	C	C	C	C	++	+	+	O	O

*C = Complete clumping.

Somewhat later, a culture came into my hands sent out from some commercial laboratory as a pertussis culture. A cursory examination showed that it was culturally, at least, identical with the guinea-pig bacillus. When the two sera used in Table I. were applied to it, the agglutination was as high as with the distemper organism.

This species is of interest in that the minute bacilli are found attached to the cilia of the bronchial epithelium of guinea-pigs in an infected population. These may be found during or long after the active pneumonia or in animals showing no traces of a former pneumonia. In a recent paper, Mallory[4a] quotes Rhea as having found the variety of this bacillus which is associated with canine distemper attached to the ciliated epithelium of the respiratory tract in dogs. This and other evidence makes it highly probable that the bacilli found by Mallory[4] in the respiratory tract of animals treated with B. pertussis of Bordet were B. bronchisepticus. In view of Mallory's findings in whooping cough, it would be of interest to determine whether these two

species, B. pertussis in man and B. bronchisepticus in certain animals, are in any way related, or whether the method of attachment to the cilia is a device capable of being used by all respiratory parasites. Rhea in the same paper refers to a similar organism as the cause of a disease in rabbits associated with much sneezing and known as snuffles. To prevent any further confusion about these organisms it should be stated that the organism associated with snuffles or, at any rate, with the fatal pectoral lesions of this disease, is a wholly different species belonging to the group of rabbit septicemia bacilli. M'Gowan first pointed out the presence of the minute motile bacilli (B. bronchisepticus) in the lower respiratory passages of rabbits and guinea-pigs, but his animals were not ill. The rabbit is thus liable to harbor two wholly different species of pathogenic bacteria at the same time in the respiratory organs.

It is of interest to note, furthermore, that the attachment of the B. bronchisepticus to the cilia was first seen by Tartakowsky in 1896 and clearly described by him[5] in 1898. He strangely enough found no healthy carriers among guinea-pigs. Rabbits exposed to the infection did not acquire a parasitism of the epithelium. These discrepancies are most likely due to the higher virulence of his strain which was fatal to rabbits after intra-abdominal inoculation.

It seems, then, that this organism is represented by a number of strains either identical or modified slightly in their respective hosts, which have the peculiarity of adhering to the cilia in the respiratory tract. Their relation to one another should be more carefully studied, and more particularly their relation to the non-motile Bordet bacillus. The capacity to stick to the cilia may be due to the mucin-like substance first noticed by the writer in 1899 and referred to in the preceding pages.

THE PNEUMOCOCCUS IN PNEUMONIA AND INFLAMMATION OF SEROUS MEMBRANES IN THE GUINEA-PIG. — There is always some practical interest in those pathogenic microorganisms which are shared by man and the lower animals.

The diseases produced in the latter by such organisms offer additional means for study, since they represent types of parasitism which, through the exaggeration of certain features, may greatly aid in the elucidation of the human disease. We may also assume that microörganisms affecting a variety of hosts, unless highly specialized into distinct races, may, at times, be of greater virulence for other hosts than are the varieties regularly present in the latter. It may also be that they are of no significance whatever to other hosts. Before proceeding to a brief summary of the relation of the diplococcus to pneumonic and other lesions in guinea-pigs, some reference to the literature of this microörganism as affecting the lower animals may be of interest.

In 1897 Dürck,[10] in studying the bacteriology of healthy and pneumonic lungs, examined the healthy lungs of some slaughtered animals. In the lungs of ten swine he found, among other organisms, the pneumococcus twice; he also found it in a horse. Boni,[11] pursuing the subject farther in the same institute, found the pneumococcus five times in fourteen pigs' lungs. Quensel[12] examined the lungs of sixteen calves and found the pneumococcus once, "not pathogenic for mice." In fifteen lungs of sheep he found it once; in six pigs' lungs, twice. In the lungs of five horses none were found. Krautstrunk[13] found a diplococcus in nine out of seventy-three calves dying of diarrheal and septic diseases. This organism has a capsule, is Gram-positive, and grows only in serum bouillon and blood agar. It did not multiply in bouillon even when sugars were added. Rabbits and guinea-pigs were highly refractory to large doses; white mice quite susceptible to small doses. Krautstrunk was able to produce a fatal infection in new-born calves by feeding the diplococci in milk and by intravenous inoculation. The cultures in serum bouillon maintained their virulence for new-born calves for nine months. Balzer[14] found the pneumococcus in three calves affected with septicemia. The capsulated, lancet-shaped diplococci were demonstrated in the tissues according to Gram-Weigert, and isolated in cultures. A strain isolated from the sputum of a case of pneumonia in man was found not to differ in its agglutination and complement-fixing characters from the calf organism.

Beco[15] examined, bacteriologically, the respiratory organs of a number of mice, guinea-pigs, dogs, cats, and rabbits. These were sterile from the middle of the trachea downward to the alveoli. He also examined the respiratory organs of several horses, cows, sheep, and swine obtained from the abattoir. In cultures from the lungs of a horse and a cow Gram-positive pneumococci were isolated. The remaining lungs were sterile.

After the completion of this paper Christiansen[23] published a thorough study of capsulated Gram-positive diplococci encountered in diseased

calves. The lesions were not characteristic. Calves, new-born and older, succumbed to the feeding of small doses of cultures. Christiansen made a careful comparative study of calf and human strains of pneumococci. Morphologically, the calf strains were a trifle smaller. Variations towards carbohydrates were encountered in both groups. Both were dissolved in bile salts. The calf strains were less virulent for rabbits. One of four human strains was pathogenic for calves. Mice treated with several pneumococcus sera were not protected against the calf strains. Even among the latter there were atypical strains similar to those found by Neufeld among human cultures.

Concerning the diagnosis of pneumococci in lower animals caution must be exercised, inasmuch as a somewhat similar bacterium (known as the bacillus of septicemia hemorrhagica, and including such races as the rabbit septicemia, swine plague, and fowl cholera bacilli) is very frequently present in the upper respiratory passages of many of the domesticated mammals.[16] This organism is not infrequently found encapsulated. It is not stainable according to Gram. It is not improbable that most of Dürck's, Boni's, and Quensel's positive findings in pigs' lungs, belonged to this group, for neither Dürck nor Boni mention Gram-positive characters in their diagnosis. With these authors the emphasis was evidently placed, not so much on the character of the bacteria encountered, as upon the presence of bacteria at all in normal lungs.

Turning now to the guinea-pig we find more positive evidence of the occurrence of pneumococci. Binaghi[17] found capsulated diplococci in the pneumonic lungs of a young guinea-pig. In cultures, they appeared in chains like streptococci. The culture died out on agar.

Stefanski[17a] studied a winter epizootic in Odessa. Chiefly adult and old guinea-pigs died. The "upper" lobes of the lungs were involved and in some cases there was peritonitis. Fatty degeneration of liver and heart frequent (lungs not mentioned). The sick did not infect the well. The writer thinks the low winter temperature responsible. The capsulated diplococci found killed rabbits, guinea-pigs, and mice after subcutaneous inoculation. Evidently this strain was much more virulent than the writer's.

H. Weber[18] describes a plague among guinea-pigs which he associates with an earlier rabbit plague without, however, bringing forth any evidence of such relation. He found in hepatized lungs a Gram-positive diplococcus without capsule, which grew well on potato, and was viable in cultures. The disease in guinea-pigs suggests our own, in that chiefly females were affected and died immediately before or after parturition. It is evident from the above that this is not the pneumococcus, unless Weber was working with impure cultures. The pneumococcus does not grow well on potato, nor is it viable in cultures more than a few days. Selter[8] found genuine pneumococci in guinea-pigs, which died with congested (pneumonic?) lungs, and enlarged spleen. One died of suppurative peritonitis. Selter found both pneumococci and B. cavisepticus mobilis (B. bronchisepticus?) in the normal lungs of a guinea-pig, and this led him to make

a special study of the healthy organs of rabbits and guinea-pigs.[19] He was able to isolate from the lungs of a certain number of guinea-pigs, either one or the other or both of the bacteria mentioned above. It is of importance to note that he was unable to find these organisms in thirteen guinea-pigs obtained from three outside sources. The presence of pneumococci in guinea-pigs is indirectly shown in a paper by Ungermann,[20] who uses in his experiments, as one of his strains, a pneumococcus isolated from a spontaneous peritonitis of the guinea-pig. This form of pneumococcus disease in guinea-pigs I have seen repeatedly.

Christiansen[23] describes a recent diplococcus epizootic among guinea-pigs. The lesions were localized chiefly in the lungs although peritonitis and metritis were also encountered. Agglutinative and serological relationships to the diplococcus strains from calves (see above) were so close that the author does not hesitate to assume that the calf disease gave rise to the guinea-pig outbreak.

Richters[24] reported a guinea-pig outbreak in which the disease was localized in the female sexual organs. The mucous membrane of vagina and uterus was intensely inflamed and beset with hemorrhages and ulcers. The lancet-shaped, capsulated diplococci found in the exudates and ulcers were pathogenic for mice, rabbits, and guinea-pigs.

My attention was first called to the pneumococcus as a cause of various affections in guinea-pigs in 1908. It may have been present in our animals before that date, since so-called streptococci occasionally appeared in cultures, some of which may have been genuine diplococci. In a spontaneous case examined in the winter of 1903 there were scattered through all sections of the highly congested lungs, plugs of bacteria in the capillaries, and in some glomeruli of the kidneys. A reëxamination of the sections recently suggests streptococci. The cultures made at the autopsy are described in my notes as pure cultures of streptococci. It is, therefore, quite probable that the pneumococcus infection was introduced in 1907–08. It has been found every year since and it was frequently encountered in the epidemic of 1913. It was found as an active agent of disease in a large neighboring collection of breeding guinea-pigs, and it was isolated from a guinea-pig belonging to a third source. The pneumococcus may, therefore, be regarded as a widely disseminated pathogenic organism among guinea-pigs.

During 1908–09, a relatively large percentage of breeding females died as a result of intra-thoracic hemorrhage closely

following parturition in most cases. An examination showed the pleural cavities more or less distended with blood-stained fluid of which as much as fifty cubic centimeters were withdrawn in one case. Of course the extra space for such fluid was gained by crowding the lungs into a small space and pushing backward the diaphragm when this was not adherent. The serous membranes were in most cases covered with thin, fibrinous coagula. A closer examination usually revealed some lobe of the lung, either the small median lobes, or one of the cephalic or ventral lobes, solid, gray, shrunken and adherent at some one point, or wholly to the diaphragm or ribs. The point of adhesion was represented by necrotic, at times, pultaceous lung tissue which was probably the source of the hemorrhage. The small median lobes were frequently entirely concealed by exudation attaching the diaphragm to them. When adhesions were pulled away, these lobes could no longer be recognized, because of shrinkage and sclerosis or necrosis. The severer hemorrhages may have started in some cases where the inferior vena cava is enveloped by one of these lobes. In general, it may be said that these small lobes were most frequently and often exclusively involved. The diagnosis in these cases was old pneumonia due to B. bronchisepticus, with secondary invasion of the pneumococcus. In some cases, in which pneumococci were found, there was no macroscopic evidence of lung involvement beyond a slightly denser condition, but the lobes were glued to one another and to the chest-wall, either lightly or so firmly that they had to be torn out. In such cases the pericardium was much thickened, opaque, and occasionally adherent to the epicardium. The diaphragm was usually adherent in these cases. Occasionally a type of pneumonia was encountered in which one of the large caudal lobes was involved. The hepatization extended over most of the lobe. Other lobes were also involved, but, as a rule, where the caudal lobes were diseased, the pneumococcus was on hand. On the broncho-pneumonia of the motile bacillus was grafted a diffuse fibrinous infiltration of the alveoli in which there were

immense numbers of capsule cocci. This fresh pneumonia was found only several times.

Another type of pneumococcus infection seen since 1908–09, and perhaps earlier but overlooked, is the fibrino-cellular peritonitis, referred to by Ungermann,[20] and sometimes associated with a similar affection of pleura and pericardium. The exudate which contains many endothelial fatty cells and polymorphonuclear leucocytes may be loaded with capsule cocci.

In one case an abscess containing capsule cocci was found in the internal ear. The guinea-pig turned his head to one side and was reported as moving in a circle when caught. In this case the lungs, in places, were firmly adherent to the ribs.

The presence of the pneumococcus was thus associated most frequently with a plastic inflammation of the thoracic serous membranes, a fibrinous exudation into the pulmonary alveoli and a fibrino-purulent inflammation of the peritoneum.

If we classify etiologically the cases examined in 1913, we find that of thirty-six cases, twenty-nine were associated with the motile bacillus alone, seven with the pneumococcus alone, and eleven with both organisms. This classification is based on the results of cultures or microscopic examination of smears and sections, or of both. It cannot, however, be regarded as decisive. In general, it may be stated that when both bacteria are present, the pneumococcus is more likely to be overlooked in cultures and the motile bacillus in smears. The former is very characteristic in smears and sections, the latter grows very well in cultures, but is not easily demonstrated with the microscope.

The diplococcus isolated from the various types of lesions described above appeared in smears as an oval or lancet-shaped coccus usually in pairs, more rarely in short chains. It varied somewhat in size according to unknown conditions even in the same preparation. This variation cannot be due to a partial staining of the capsule for the latter was usually plainly recognizable as a distinct, uncolored, outer envelope when the smear, after staining, was examined in water. After

mounting in balsam, the details of the capsule were largely lost. The average length of a pair of cocci was about 2 μ, the width .6–.7 μ. When stained according to Gram (which is always positive) or Rosenow's capsule stain, the cocci appear somewhat broader, perhaps .8 to 1 μ. The lancet form appears more pronounced in some strains or some preparations (always from the tissues or exudates direct, however) than in others. Whenever found in smears these cocci were within capsules which were, in some cases, very large. Measurements of stained capsules in water were not made. In balsam there is considerable shrinkage and the very sharp halo is more or less lost. Attempts to stain the capsule were made with Rosenow's stain. These brought out the outline of the capsule very well (Plate XXI., Fig. 7). The size of stained capsules enclosing a pair is approximately 3 μ x 2 μ. When chains of three or four were seen the capsule enclosed the entire group.

Capsules were readily distinguished in preparations stained with the usual dyes, such as Loeffler's methylene blue, provided the specimens were examined in water before drying. The capsule was frequently unusually large, especially in abdominal exudates. When the diplococci were very numerous the capsules could be seen merging into unstained, irregular bands, and occupying a large proportion of the microscopic field. The capsules were, in some cases, visible as oval white bodies on a stained background, the contained bacteria remaining unstained. Leucocytes were found, at times, packed with these oval, unstained bodies. In cultures containing blood the capsules were still visible after several transfers, but disappeared later. In cultures this pneumococcus, as a rule, maintained the diplococcus grouping and the oval form. Chains of four were, however, present. Under certain conditions, especially in mixed cultures, the longer chain form may predominate and falsely give the impression of a genuine streptococcus. Pure cultures maintained for months were still predominantly diplococci.

Capsules have been demonstrated in sections of lung tissue stained with eosin-methylene blue. In certain sections the

capsules were very well brought out (Plate XXI., Fig. 8). A repetition of the same procedure frequently failed. It is evident that very slight variations in the staining procedure tip the scales in favor of or against the staining of capsules. Pneumococci and their capsules were deeply but irregularly stained with Delafield's hematoxylin in sections and smears (Plate XXI., Fig. 6). They are so conspicuous in tissues after this method that it is to be preferred to eosin-methylene blue when they are scarce or confined to certain localized areas in the section.

The cultivation of this organism is attended with the same difficulty encountered with the human strains. It grows very feebly upon the usual media and dies out within a few days. Media containing red corpuscles, serum or both, were chiefly used, but the cultures do not last any longer. A large number of strains were lost because of neglect to renew every second or third day.

The colonies on agar to which about ten per cent horse blood and dextrose up to .1 to .2 per cent had been added before sloping, are clear, watery, point-like bodies, when very numerous. They may become one to 1.5 millimeters in diameter when well separated. They are then flattish, disc-shaped, and centrally somewhat depressed. Equally good growth is obtainable by adding a single drop of horse corpuscles to sloped agar.

In bouillon the growth is feeble; the fluid becomes faintly clouded. On plain agar the colonies, when well separated, may attain, in the first sub-cultures from the animal, a diameter of one to two millimeters. In transmitted light, they are paraffin-like, opaque. The disc is round, fairly thick, margins abrupt, and in the center is a minute depression which may be taken for a knob of elevation unless examined with a hand-lens.

On blood agar made with blood laked at 60° C. for twenty minutes, colonies, when well separated, form smooth, slightly conical discs from mere points to 1.5 millimeters in diameter, according to numbers. Viewed by transmitted light they appear as deep brownish discs on a translucent reddish

brown background. When grown on unheated horse blood agar the growth appears greenish in transmitted light. Hemolysis does not occur.

The pneumococcus is quite regularly described as failing to produce any changes in milk cultures in spite of the fact that it is known to produce acids in lactose media. It is true that after incubation of a week the medium is unchanged, but if the tubes are placed in boiling water, speedy coagulation into a solid or loose flocculent curd takes place.

The pneumococcus from the guinea-pig conforms also in its fermentative characters to the human types. When a given sugar is attacked, the acidity of the fluid usually rises from about one to four per cent of a normal solution. As is customary in this laboratory, the fermentation tests were made in fermented sugar-free bouillon, to which the particular sugar to be tested was added from a sterile (ten to twenty per cent) solution to a concentration of one per cent. Such tubes after an incubation of one or more days are then inoculated. After a week's growth, the culture fluid is titrated against N/20 NaHO with phenolphthalein.

The presence of a fermentescible sugar stimulates the pneumococcus into active multiplication so that at the end of three or four days the culture fluid, when shaken, is quite turbid. Dextrose, saccharose, lactose, maltose, and raffinose were tried and all were attacked with production of acids. When ten per cent blood serum were added to sugar free bouillon the presence of inulin led to a rise in acidity. When only sugar free bouillon plus inulin was employed no acid appeared subsequently. Mannite was not attacked in bouillon with and without serum.

Three different strains were tested as to their behavior in the presence of taurocholate of soda. Twenty-four-hour bouillon cultures were used together with similar cultures of two different types of streptococci and a paratyphoid strain. When the taurocholate was added from a ten per cent watery solution until a concentration of about one per cent of the salt was reached, the pneumococci were speedily dissolved; the other cultures remained clouded.

The pathogenic effects of pure cultures of the pneumococcus upon the natural host, the guinea-pig, are feeble. Exudative peritonitis follows intra-abdominal injection in some cases; in others, no effect is produced. Doses of one cubic centimeter of fresh bouillon cultures of diplococci some three or four weeks after isolation and cultivated meanwhile on horse blood agar, failed to affect rabbits either by way of the abdomen or ear vein. Mice, however, succumb to intraperitoneal injection.

FATTY DEGENERATION OF THE LIVER AND LUNGS OF BREEDING GUINEA-PIGS IN THE WINTER SEASON. — During the entire course of these observations there was encountered a peculiar set of pathological phenomena in female guinea-pigs which will require special study. They were so interwoven with the pneumonias that they were at first regarded as necessary concomitants of the latter. The observations of a number of years made it possible, however, to disentangle them. The condition was at first found only with female pigs about to give birth or shortly after parturition. The animal became suddenly quiet with slightly ruffled hair and died within one or two days. Death occurred most frequently within one or more days after the birth of young, more rarely mature fetuses were found at autopsy. Very rarely the condition was seen in early pregnancies.

At autopsy there was found extreme fatty degeneration of the liver, more rarely of the kidneys. The normal brownish red color of the liver was replaced by a uniform grayish yellow, in some instances almost white color. The tissue was exceedingly friable, very readily crushed with the fingers. Microscopical examination showed only a mass of fat globules in place of liver cells. The lungs were edematous, somewhat larger than when collapsed normally and either with or without pneumonia of the anterior (cephalic) half of the lungs. Microscopic examination of teased bits of lung tissue showed here also extreme fat infiltration of the alveolar cells (Plate XIX., Fig. 2). The fat appears in one

large mass in the cell which may measure up to 25 μ in diameter. The fat blackens in osmic acid.

In sections of tissue hardened in Zenker's fluid, the large vacuolated cells are very conspicuous, project into the alveolar lumen and practically fill it up so that the normal lung picture is lost. The other constant condition is an empty stomach. Some watery or mucous fluid is usually present. Gentle scraping of the mucosa shows only fine refringent granules probably fat, as they darken in osmic vapor. The spleen is small, perhaps smaller than normal. The organs are free from bacteria.

Many healthy adult animals, chiefly old males and females, have been examined, but only traces of fat found in the lungs and liver.

To determine approximately the amount of fatty substances in lungs, tissue, which had been dried over sulphuric acid for some time, was extracted with ether. From one lung 29.4 per cent, from another, 23.3 per cent ether extract was obtained. From the lungs of a normal guinea-pig treated in the same way, 13.55 per cent of the dried lung tissue were separated as ether extract.

It was at first thought that the fatty condition of the liver and lungs was directly due to the pneumonic infection, until animals died without any traces of pneumonia, but with the same extremely fatty condition of lungs and liver.

For a number of seasons only adult breeding females succumbed with the pneumonia and the fatty degeneration, or the latter alone, although there were a number of adult breeding males exposed to the same conditions. The males and females were present in the ratio of one to three or four and kept in groups of four or five in compartments. The inference was drawn tentatively that the fatty changes associated with the late pregnant or parturient state, were in some way related to the beginning lactation. Quite unexpectedly during 1908 a number of breeding males died with hepatization of one-third to one-half of both lungs, and marked fatty infiltration of these organs and the liver. This disposed of the lactation hypothesis. This peculiar

pathological condition has been prevailing in our pens up to the present. Young have never been affected by it; males only during one year. Since evidence is quite strong that the pneumonia is not responsible for this abnormality in the fat metabolism, for it is found without pneumonic lesions and pneumonic lesions are found in its absence, it may be of interest to seek for other conditions underlying it.

It should be borne in mind that this disease is essentially a cold-weather phenomenon. Adult male and female breeders dying in the summer season do not show this extreme fat infiltration of lungs and liver. A small amount of fat may, however, always be found with the microscope in adults.

Among the predisposing conditions, the writer places first of all inadequate respiratory activity. The animals were supplied with less fresh air in winter. The encroachment upon the thoracic space by the growing fetuses in the pregnant female is very great during the last few days of pregnancy. The increased metabolism during this period may be halted until the accumulation of fat in the lungs makes them to all appearances nearly useless. In the male the contributory conditions are probably due to the pneumonia itself. Nearly one-half of the lungs was hepatized. To this mechanical insufficiency must be added the toxins due to both bacteria and accumulated cell-débris in the diseased lungs. This point of view is strengthened by the fact that no male died during the period of observation (1899–1913) with fatty infiltration of lungs and without pneumonia. In the pregnant female, on the other hand, perhaps the majority which died were without pneumonic lesions.

Another hypothesis to account for the fat metabolism disturbance is the presence of some concealed focus of infection. The uterus has been taken into consideration as a possible source of toxins. In several instances the pneumonia bacillus was isolated from the cavity of the uterine horns in case of dead fetuses. In other instances this same cavity was sterile. As stated above, the spleen, liver, kidneys, etc., are uniformly free from cultivable bacteria, and, therefore, the theory that fatty changes are due to some bacterial

toxin is not very promising, although it remains as a possibility to be reckoned with. Latterly, the pneumonia bacillus has been isolated from among a variety of bacteria found in the bronchi after death in cases of fatty degeneration. Since the same infection of the air tubes is found in healthy guinea-pigs, any significance cannot at present be attached to it.

A third hypothesis may be put forth that the fatty changes are, in some way, associated with the winter food. The guinea-pig appears to be more susceptible to a restricted diet than the mouse and the rabbit. The effect of a diet from which grass or succulent vegetables are eliminated was first noticed by the writer[21] in 1894, and briefly discussed in a report on mouse septicemia bacilli in 1895–96. Guinea-pigs thus deprived became ill and died within four to eight weeks. Certain joints were swollen and the muscles around them infiltrated with blood. There were also found subcutaneous and submucous hemorrhages. Control guinea-pigs, properly fed, remained well. This peculiar scurvy-like disease has since been exhaustively studied by Axel Holst.[22] During the winter of 1913 the writer repeated these old experiments on guinea-pigs to see if any fatty changes occurred when the diet was restricted to oats, hay, and water, but only the knee-joint lesions and intermuscular hemorrhages were produced.

These experiments do not, however, dispose of the food as a possible cause of the fatty changes. To demonstrate any relation existing between them it would be necessary to feed pregnant guinea-pigs on oats, carrots, and hay in summer and on oats, hay, and grass in winter.

That the winter diet has a depressing effect on the growth of young guinea-pigs has frequently been noticed in the population under investigation. The young languish towards spring; their normal increase in weight does not take place. In fact, this remains nearly stationary, until fresh grass is obtainable when the weight increases very rapidly.

EPIDEMIOLOGY OF GUINEA-PIG PNEUMONIAS. — Summarizing the foregoing, we have two types of pneumonic

lesions produced by two bacteria belonging to widely separated groups. The pneumonia produced by the motile bacillus is a self-perpetuating, independent disease, invading no other organs with the exception of the uterine horns. The disease produced by the pneumococcus is not limited to the lungs, but may attack various organs with a predilection for serous membranes and occasionally causing septicemia. The pneumococcus pneumonia in guinea-pigs needs further study, where the motile bacillus is absent. Added to these etiological factors, there is a third condition found in winter which manifests itself, in female breeders chiefly, as an extreme fatty degeneration or infiltration of lungs and liver, more rarely kidneys and other organs. This condition may or may not be associated with pneumonic lesions. The conditions presenting themselves were thus too complicated to permit any solution of the original question why the pneumonias occur almost exclusively in the winter months. I have already dwelt upon the food and oxygen supply with reference to the fatty changes occurring in winter. The low, more or less fluctuating temperature of the air may, in some way, be responsible for the incidence of pneumonic lesions. The condition of food and oxygen supply may contribute their share in the production of lung disease. Just how these may operate cannot be analyzed until the internal factors and conditions favoring pneumonia have been laid bare. Among those factors which play a part in the epidemics the virulence of the infecting microörganisms is probably the most important. To accurately gauge the virulence is a difficult matter and special methods must be devised to standardize the pathogenic activity of different strains from year to year, since they cannot be compared directly with one another, owing to the depressing influence of cultivation over long periods.

In the population of guinea-pigs under observation, the variation in the intensity of the disease due to B. bronchisepticus was quite marked from year to year. Following the first observed epidemic of 1898–99 there was very little disease in 1900–01. In 1902–03 there were some deaths

among breeding females associated with fatty degeneration of lungs and liver, but pneumonia was absent. In 1903–04 the mortality was moderate. Some females died with, others without, pneumonic lesions. Fatty changes were regularly present. In 1904–05 and 1905–06 there were very few deaths. In 1907–08 there were many deaths associated with fatty degeneration and pneumonia. In this season many breeding males died. In 1908–09 the pneumococcus appeared active. Females died as a result of intrathoracic hemorrhage. The hemorrhage was evidently caused by an invasion of old pneumonic foci by the pneumococcus. In 1909–10 the mortality was low. A few cases were associated with the pneumococcus. In 1910–11 there was considerable pneumonia with both organisms active. In 1911–12 only a few cases occurred. In 1912–13 the disease assumed its most virulent aspect. Not only adult breeding pigs but many young ones became affected for the first time. Both bacillus and pneumococcus were involved.

Though deaths among the older animals did occur in summer, they were usually traceable to causes other than those operating in the winter months. With rare exceptions the disease disappeared as a fatality early in May and reappeared in December.

Assuming that the bacillus is maintained from year to year in the air tubes of certain guinea-pigs which thereby become genuine carriers, one might suppose that the disease would be as virulent one year as another. This, however, is not the case. The epidemic of 1899 was followed by years of relative quiescence. The other severe epidemics occurred in 1908 and 1913.

In 1899 practically no young and no adult males were affected. In 1913 many young became diseased. A few died. In the rest the remnants of the disease were discovered later. The invasion of the young must be regarded either as a result of increased virulence or meteorological conditions.

The absence of disease in the years immediately following 1899 was, I believe, due to the fact that no guinea-pigs from

outside were introduced among the breeding stock. This was done primarily to check the disease. Here again we have two possible factors to consider, the gradual immunization of the entire stock and the reduction in virulence of the original strain, either of which may account for the subsidence of the disease. Subsequently, breeding animals were introduced from other sources and with them probably came new strains of B. bronchisepticus.

The whole problem of bacteria carriers is becoming of such overshadowing practical importance that an accurate determination of the fate of strains in a community to which no new strains are admitted from outside is urgently demanded. Do bacteria carried by healthy individuals maintain their virulence for long periods? Do they undergo modifications? Do they eventually die out? It is obvious that if we wish to solve any of the fundamental questions raised by such studies as these, it will be necessary to keep animals under observations under well-defined conditions and subject to no other interests than those of the problem in hand. We must consecrate the entire population to one subject such as is done, for example, in the studies on heredity under the Carnegie Institution.

The epidemiology of the pneumococcus disease and its relation to carriers is not in any way cleared up by the foregoing observations. In our cases the invasion of the pneumococcus was to all appearances secondary to that of B. bronchisepticus. It is, therefore, desirable that outbreaks be studied with a view to determine to what extent the invasion of the pneumococcus is dependent on existing lesions, and also whether it is ever carried by healthy animals as is B. bronchisepticus. A survey of the literature strongly suggests that the various guinea-pig epidemics reported from different countries were due to pneumococci of widely different degrees of virulence and that these epidemics cannot be directly compared with one another therefore.

The relation of the pneumococcus obtained from guinea-pigs to strains associated with human pneumonia has not

been touched upon in the foregoing studies. Fortunately Christiansen [28] has partly filled this gap.

SUMMARY.

1. The guinea-pig is the host of two pneumonia-producing bacteria: (*a*) A minute motile bacillus, originally (but imperfectly) described by Tartakowsky, rediscovered and independently named by three workers subsequently, and redescribed in 1910 by M'Gowan as the cause of canine distemper (B. bronchisepticus). It was encountered by Mallory in his studies on pertussis. It has been under observation by the writer since 1899; and (*b*) Diplococcus pneumonia or pneumococcus which also produces adhesive pleuritis, pericarditis, suppurative peritonitis, and general septicemia.

2. In the author's studies, the pneumococcus infection was usually grafted on the pneumonia due to the motile bacillus.

3. A study of the work of earlier observers indicates that both organisms have manifested gross differences in virulence in different epidemics.

4. Independent of and frequently associated with pneumonic lesions due to the motile bacillus is an extreme fatty degeneration of the liver, lungs, and other organs of chiefly female guinea-pigs leading to death just before or after parturition.

5. The fatty degeneration as well as the pneumonia is almost wholly limited to the winter season (December–May).

6. The motile bacillus lives over from winter to winter in old pneumonic foci or in the air tubes, attached to cilia, as described by Tartakowsky. It does not occur as a parasite of the air tubes in all guinea-pig populations and attempts should be made to breed from non-infected stock.

7. The reason for the seasonal incidence of pneumonia is not demonstrated. It evidently depends on a variety of interlocking, external as well as internal, factors, without the help of which the microörganisms cannot multiply in the parenchyma of the lungs.

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DESCRIPTION OF PLATES.

PLATE XIX., FIG. 1.— Section of lung of guinea-pig born September, 1896, and kept in breeding pens until death, January, 1899. Extensive pneumonia. Only the right caudal lobe air-containing. Note the dense cell masses with lobulated outline defining limits of individual alveoli. Eosin-methylene blue. x 100.

FIG. 2. — Section of lung of breeding female showing focal pneumonic lesions and extensive fatty infiltration of the alveolar epithelium. The fat appears in form of round vacuoles. Zenker's fluid. Hematoxylin and eosin. x 100.

PLATE XX., FIG. 3. — Smear from lungs of a breeding female whose uterus contained three large fetuses. Fatty degeneration of lungs, liver,

etc. Hepatization of all small and right large caudal lobe. Mixed infection with pneumococci and motile bacilli. The minute bacilli are shown in groups attached to the cilia of epithelial cells. Alkaline methylene blue.

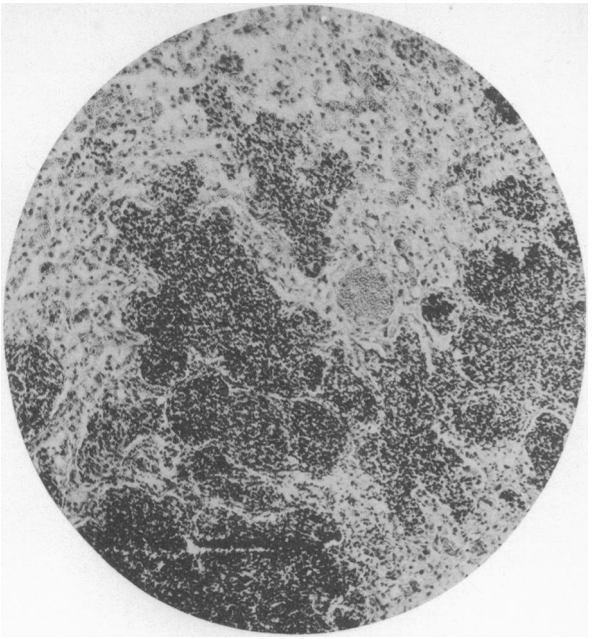
FIG. 4. — Section of small bronchiole of another female breeder showing masses of minute bacilli attached to the ciliated border of the epithelial cells. Eosin-methylene blue. The lesions of the smaller lobes in this case were largely indurative, the result of former pneumonia. Those of the larger caudal lobe, recent. Immense numbers of pneumococci embedded in a loose mesh of fibrin fill the alveoli of the latter lobe.

PLATE XXI., FIG. 5. — Smear showing pneumococci from purulent peritoneal exudate. Methylene blue. The pneumococci entered through necrotic median lobes (B. bronchisepticus) causing an adhesive pleuritis and extensive pseudo-membranous and purulent peritonitis.

FIG. 6. — Same exudation as in Fig. 5, but the smeared cover-glass was carried through hematoxylin-eosin as if a section. The irregular deeply-stained bodies are pneumococci of which more or less of the capsule is stained making the cocci appear large.

FIG. 7. — Smear from lung tissue showing capsulated diplococci (Rosenow's capsule stain). Case of female breeder having apparently a primary pneumococcus pneumonia, but cultures contained in part also the motile bacillus, some cultures only pneumococci. There were also a few necrotic foci in the parenchyma pointing to a primary bronchisepticus lesion.

FIG. 8. — Section of lung of guinea-pig from which smear of Fig. 7 was obtained. The capsules are well brought out with the eosin-methylene blue stain. Similar effects were obtained with hematoxylin-eosin.



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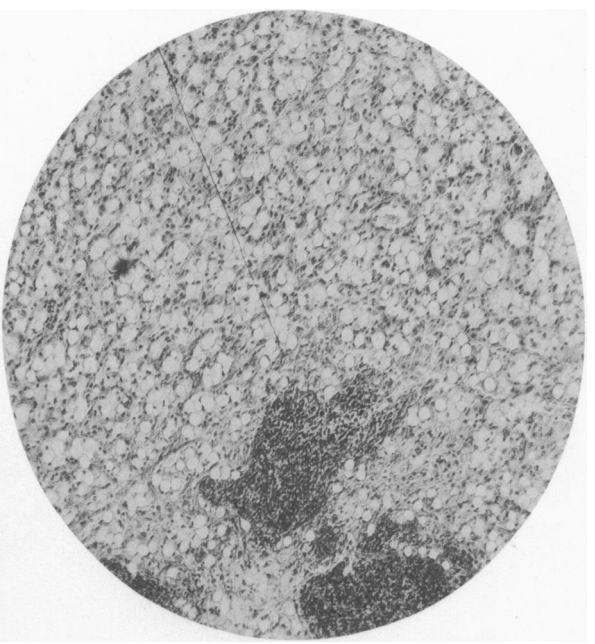

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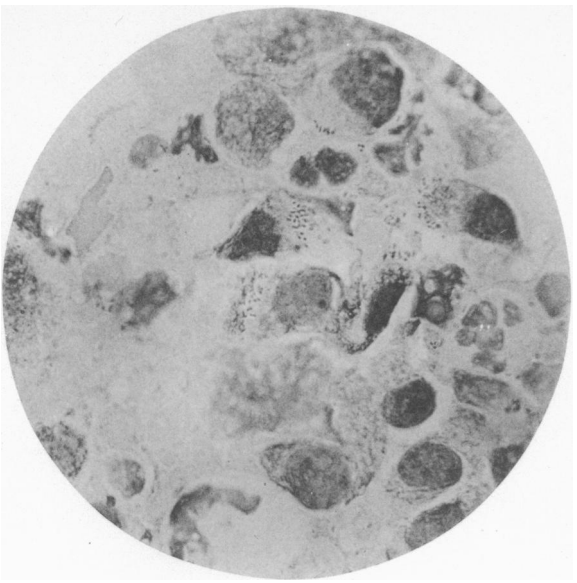

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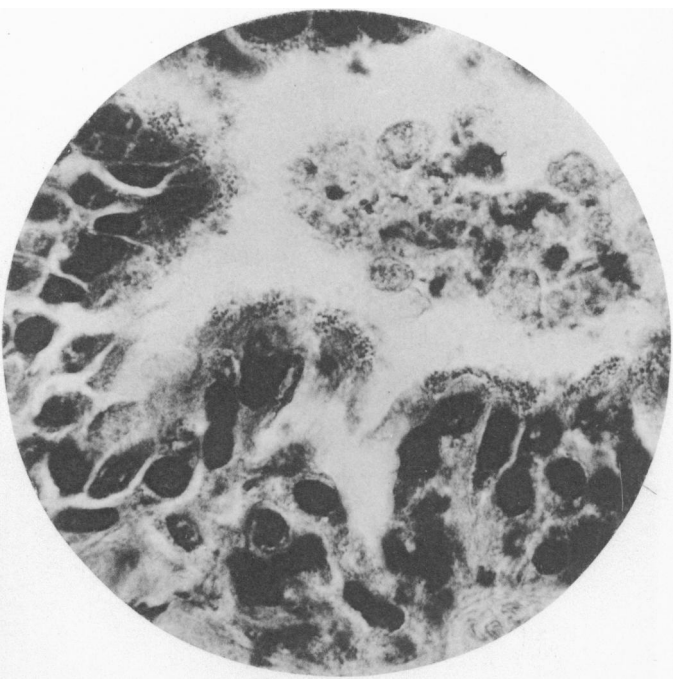

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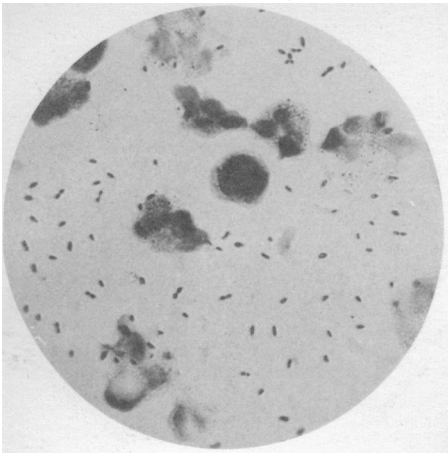

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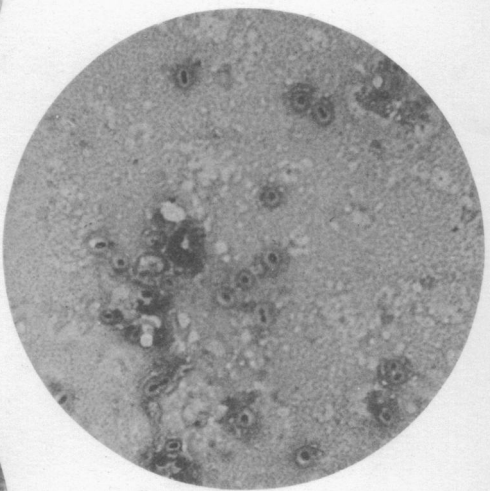

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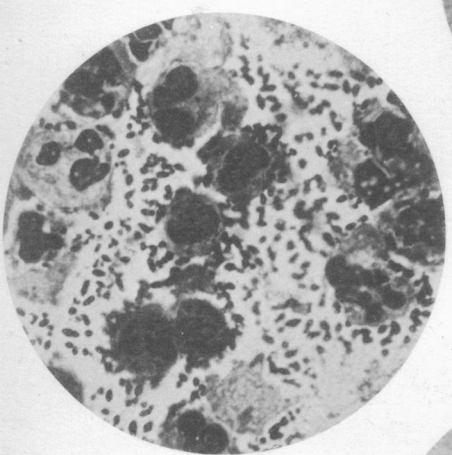

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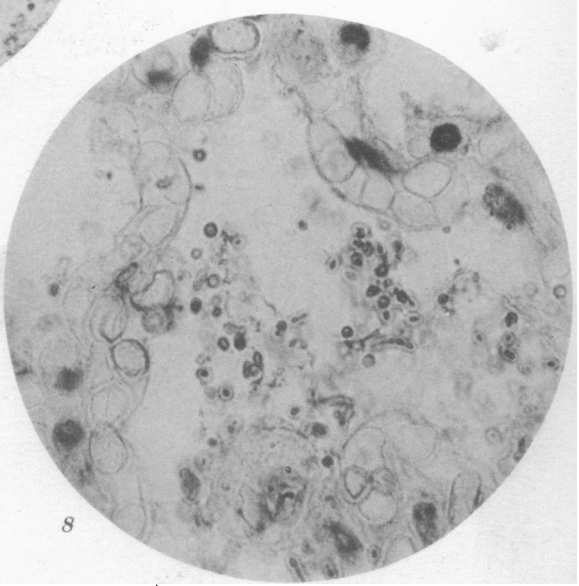

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