

## STUDIES IN INFLUENZA AND PNEUMONIA

### IX. CHANGES IN THE GREEN-PRODUCING STREPTOCOCCUS INDUCED BY SUCCESSIVE ANIMAL PASSAGE AND THEIR SIGNIFICANCE IN EPIDEMIC INFLUENZA

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In a previous paper<sup>3</sup> it has been shown that the green-producing streptococcus isolated quite constantly in influenza and early in influenzal pneumonia has peculiar and high invasive powers not possessed by the *Streptococcus viridans* or pneumococci normally present in the upper respiratory tract. By the intratracheal injection of this organism the findings which have come to be regarded as more or less characteristic of influenza have been reproduced. The severity of reaction, the degree of leukopenia, and the mortality in the animals were roughly proportional to the severity of the symptoms, the degree of leukopenia, and the mortality in the patients from whom the strains were isolated. In this paper I shall report the results obtained in the animals following successive animal passage of this strain, the changes induced in the bacteria, and correlate the findings with those noted in patients at different stages of epidemic waves of influenza.

The changes wrought in the green-producing streptococci by successive intratracheal injections as measured by the leukocyte count and mortality are summarized in table 1. It was found that the leukocyte count made twenty-four hours after injection was quite representative of the total reduction in leukocytes, and hence it is used as a standard for comparison. The average count before injection and twenty-four hours after injection, the percentage of reduction in leukocytes, the percentage of animals developing leukopenia, leukocytosis, or no change in the number of leukocytes, respectively, and the mortality percentage were determined for each series of animals. The reduction in leukocytes, and the percentage of animals showing leukopenia run roughly parallel with the mortality rate; the greater the former two, the greater the latter. The average percentage reduction in leukocytes in the first animal passage was 51, in the second 66, in the third 50, and in the fourth 38. The mortality percentage was

57, 100, 57, and 38, respectively. There were a progressive diminution in the percentage of animals showing leukopenia from 92 to 47, an increase in the animals showing leukocytosis, and no change in leukocytes in from 4 to 25 from the first to the fourth animal passage.

I shall now consider the changes wrought in these strains as evidenced by the character of pulmonary and other lesions in guinea-pigs and by the mortality rate following successive intratracheal application.

The technic employed throughout these experiments was uniform. The dose was 0.5 c c of a twenty-four hour dextrose-brain broth, or

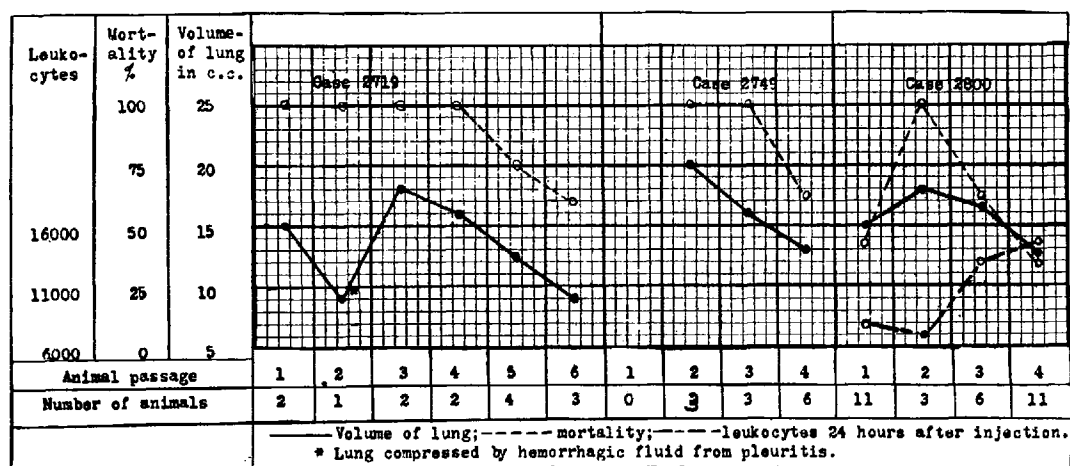


Chart 1.—Volume of lung, mortality and leukocyte count in experimental influenza pneumonia in guinea-pigs following successive intratracheal injections.

dextrose-blood-broth culture. In order to prove viability and identity of the bacteria injected a plating was made of all cultures which were injected.

The results summarized in chart 1 were obtained from a study of the cases of typical influenza or influenzal pneumonia, and owing to the importance of the findings it is best to consider them separately in some detail.

CASE 2719.—A woman, aged 41, was admitted to the Isolation Hospital Dec. 12, 1918, with a temperature of 103.3, pulse 96, and respiration 31. The patient had been taken sick one week before with headache, backache, aching of the limbs, and fever; this lasted for three or four days, then was less severe until the day before her admission. Examination showed decided cyanosis, moderate dyspnea, scattered

râles and areas of dullness, and bronchovesicular breathing over the right lung. Two days later fine râles were heard over both lower lobes and distinct dullness over most of the right lung. December 16, the patient was worse and the right lower lobe was found to be completely consolidated. December 18 the patient became delirious and constantly tried to get out of bed, and at intervals she was markedly cyanotic. In the evening cyanosis grew worse as the respirations became labored and very rapid, and death occurred four hours later.

The white blood count the day after admission was 7,400; no counts were made after that. The sputum December 19 was bloody and

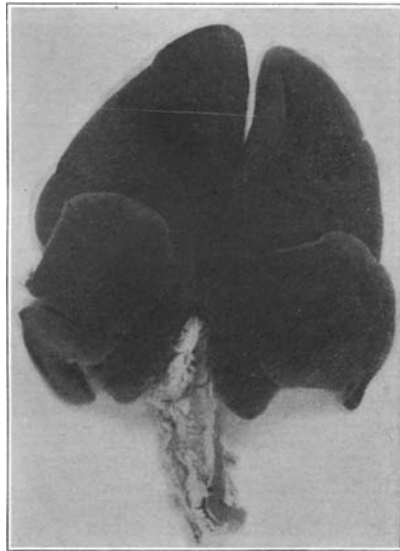


Fig. 1.—Photograph of lung of guinea-pig 853 twenty-four hours after intratracheal injection of green-producing streptococcus from case 2719 in the third animal passage;  $\times 1$ .

frothy, and the culture showed a large number of characteristic green-producing streptococci and many staphylococci.

The main findings at necropsy were: Lobar pneumonia of the right upper lobe in the gray hepatization stage; bronchopneumonia of the right lower lobe; hemorrhagic edema and edema of the left lower lobe; hypostatic congestion; and emphysema of the left upper lobe. Sections of the lung showed marked congestion, a filling of the alveoli with degenerating red cells, giant cells, degenerating necrotic alveolar epithelial cells in large numbers, and a relatively small number of leukocytes.

The culture from the lung exudate after death contained almost pure growth of the green-producing streptococcus. The washing from a small part of a blood-agar plate was injected intraperitoneally into a guinea-pig; it died in twenty-four hours from peritonitis. The moderately emphysematous lung contained localized hemorrhages and edema. Large numbers of the characteristic streptococcus were found in cultures from the blood and peritoneal fluid. The strain from this animal was used in the successive injection of 20 guinea-pigs; 14 intratracheally and 6 intraperitoneally. The average volume of the lungs and the mortality on successive intratracheal injections are summarized in chart 1. In this series the peritoneal exudate of the first animal was injected directly into the trachea of one guinea-pig (second passage). The culture in dextrose-brain broth from a single colony on a blood-agar plate from the lung of this animal was injected into 2 guinea-pigs (third passage). In the fourth, fifth and sixth passages the primary culture in dextrose-brain broth of the lung of the preceding animal was injected intratracheally.

In order to make sure that the diminution in virulence was not due to cultivation on artificial mediums the emulsion of the lung of one of the sixth passage series was injected directly into the trachea of one guinea-pig. The duration of the successive experiments in animal passages (chart 1) in the animals that furnished the strains for succeeding injections, was one, three, one, one, one, and four days, respectively, or eleven days; the duration of cultivation on artificial mediums between the animal passages was six days, the total seventeen days. Blood-agar plate cultures were made of the exudates of the lung and pleura, the blood, and of the material injected to control the results obtained. No marked change in morphology occurred, but the colonies which at first were quite moist and spreading became smaller and less moist after successive animal passages. None of the strains fermented inulin, nor were they bile soluble. The agglutinating power of the various immune serums, including the monovalent serum, was tested over this strain on isolation, and after one, two, three and four animal passages. It was agglutinated specifically by the monovalent serum for the influenza streptococcus in each of these, but in the fourth passage the strain was less highly differentiated for it was partly agglutinated by type I and type II pneumococcus serums and by anti-hemolytic streptococcus serum, but to a lesser extent than in the influenza serum. The best measure of the changes which the micro-

organism had undergone was found to be its effect on the animals. During the seventeen days of growth differences were noted in the character of the lesions of the lung, and in the mortality.

During the first three animal passages the effects were striking. Intraperitoneal injections were rapidly fatal and the tendency to localize and produce lesions in the lung was marked. The intratracheal injections in the second and third passages were followed by extreme lesions of the lung, consisting of marked exudation of dark hemorrhagic fluid into the alveoli or pleura, and increase in the size of the lung (fig. 1),



Fig. 2.—Lung of guinea-pig 869 thirty hours after intratracheal injection of the same strain in the fifth animal passage. Note the consolidation of the right diaphragmatic lobe and part of the left diaphragmatic lobe;  $\times 1$ .

associated with marked degeneration of alveolar epithelium and endothelium (figs. 4 a, 5 a, and 8 a) with relatively slight cellular infiltration and aggregation of large numbers of streptococci along the alveolar lining (figs. 4 b and 5 b). In the fourth passage both guinea-pigs showed relatively less hemorrhagic edema and more leukocytic infiltration. In the fifth passage the difference was striking. In all 4 guinea-pigs well marked areas of consolidation, mostly of lobar distribution, occurred. In 2 this was extremely marked twenty-four (fig. 1) and thirty hours after injection (fig. 2). The consistency of the involved

areas was quite firm, the cut surface quite dry, and grayish red, instead of hemorrhagic and edematous as noted in the earlier passages; sections showed marked leukocytic infiltration and relatively slight hemorrhagic edema, and the bacteria were no longer found chiefly along the alveolar lining, but more diffusely distributed throughout the exudate (figs. 6 a and b). In the sixth passage the picture of true pneumonia with no hemorrhagic edema was noted in all of the guinea-pigs injected (fig. 3). Sections showed marked leukocytic infiltration, little hemorrhagic edema, little necrosis of alveolar lining (fig. 8 b) and micro-organisms

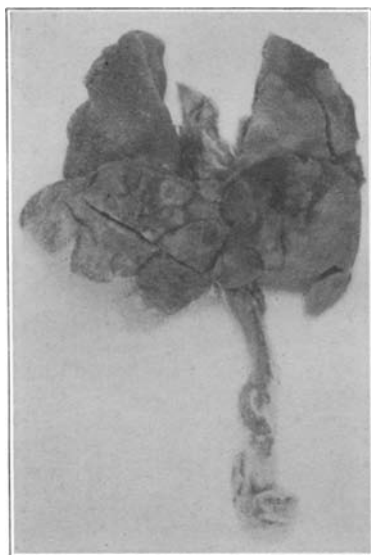


Fig. 3.—Lung of guinea-pig 886 four days after intratracheal injection of the strain in the sixth animal passage. Note the complete grayish consolidation of the left and part of the right diaphragmatic lobe;  $\times 1$ .

diffusely distributed throughout the exudate (fig. 7). The guinea-pig injected with the lung emulsion from the sixth animal passage developed slightly increased respirations, fever for a few days, and moderate leukocytosis; it then recovered. No pulmonary or other lesions were found when it was chloroformed on the twelfth day. As the gross and microscopic picture of the lung changed from that of a violent destructive reaction with little evidence of response on the part of the host to a less violent reaction in which marked exudation of leukocytes occurred, the volume of the lung and mortality rate decreased. The following experiments are illustrative:

Guinea-pig 820, weighing 370 gm., was injected intratracheally Dec. 23, 1918, with 0.15 cc of a suspension of the peritoneal exudate of a guinea-pig injected intraperitoneally with the sputum from case 2719. December 24 the animal appeared ill. It was short of breath, uncomfortable, made repeated violent efforts at respiration, and coughed violently at intervals. December 26 it was found dead. The pleural cavity contained a moderate amount of bloody turbid fluid. The lungs were only moderately distended (9 cc), and covered with a thin fibrinous film. The left diaphragmatic lobe was large and almost completely consolidated. The areas of consolidation were irregular, and mottled red and gray. Areas of irregular size showing hemorrhagic edema and partial consolidation were found in all the other lobes. The peribronchial lymph glands were edematous and hemorrhagic. The pleural fluid showed many green-producing streptococci; the blood, five colonies of green-producing streptococci; and the pneumonic lung, a large number of green-producing streptococci and a moderate number of staphylococci. In sections of the lung patchy areas of marked leukocytic infiltration were surrounded by areas in which the terminal bronchi were enlarged, the epithelium was desquamated, and the alveoli were greatly distended and completely filled with coagulated serum in which a variable number of red blood corpuscles and relatively few leukocytes were found (fig. 4a). Exudate in sections stained by the Gram stain showed a large number of diplococci and streptococci which usually were peripherally placed in the alveoli (fig. 4b). The subpleural and the perivascular lymph channels in areas were completely plugged and distended with gram-positive diplococci and streptococci. In the areas of marked leukocytic infiltration a rather large number of staphylococci were found, whereas in the areas showing hemorrhagic edema, few or none could be demonstrated. Under high power marked nuclear fragmentation and degeneration of epithelial cells and marked necrosis of endothelial cells of the capillaries in the alveoli were observed (fig. 8a).

Guinea-pig 853, weighing 440 gm., was injected intratracheally Dec. 28, 1918, with 0.5 cc of the dextrose-brain broth culture of the strain isolated from the blood of guinea-pig 820. The animal coughed up immediately a large part of the material injected. December 29 it was found dead. The trachea, larynx, and bronchi were filled with a bloody, frothy fluid. Approximately 5 cc of turbid chocolate colored fluid were found in the pleural cavity. The lungs were voluminous (18 cc), hemorrhagic throughout, and very heavy (fig. 1). The stomach was partially digested and a number of circumscribed areas in the horns of the uterus were congested and swollen. The uterus and vagina contained bloody mucus and the uterine horns a number of submucous hemorrhages. Cultures from the lung, pleural fluid, tracheal mucus, blood, and mucus from the left horn of the uterus showed a large number of the green-producing streptococci. Sections of the lung showed marked dilatation of alveoli and terminal bronchi, extreme congestion of the capillaries and veins, and marked constriction of the larger bronchi which were filled with coagulated serum and blood. There was marked desquamation of the alveolar epithelium and necrosis of the endothelium of the interalveolar capillaries (fig. 5a). Some areas were slightly infiltrated with leukocytes. The Gram stain showed enormous numbers of diplococci and streptococci. These were especially numerous along the alveolar walls surrounding the bronchi and blood vessels (fig. 5b). The number of gram-positive diplococci was so large that the outline of the alveoli could be made out readily under the low power of the microscope.

Guinea-pig 869, weighing 330 gm., was injected intratracheally Dec. 31, 1918, with 1 cc of the dextrose-brain broth culture of strain 2719 in the fifth animal passage. January 1 at 1 p. m. the animal was extremely short of breath, it had an expiratory grunt, its hair was ruffled, it was just able to walk, it sat humped up, breathing with all its might, the thorax appeared distended, and the respirations were chiefly abdominal. At 9 p. m. the animal was found dead, the body still warm. The lungs were moderately distended (15 cc); the right lung was almost completely consolidated; the areas of consolidation were quite uniform in consistency and grayish-red. The pleural cavity contained 2 cc of turbid, bloody fluid; the mediastinal lymph glands were edematous and surrounded by bubbles of gas in the mediastinal tissue. One fetus was aborted into the vagina. The area showing its attachment in the left horn of the uterus was hemorrhagic and edematous, and in the right horn was a loosely attached hemorrhagic fetal mass. Other parts of the mucous membrane of the uterus were markedly congested and showed small punctate hemorrhages. Cultures from the blood, liver, spleen, and adrenal were negative; those from the pleural fluid, lung, and the mucous membrane of the uterus and hemorrhagic placenta, showed a large number of green-producing streptococci in pure form. Cultures from the mucous membrane of the nose showed green-producing streptococci and some staphylococci; from the kidney, a few green-producing streptococci. Sections of the consolidated right diaphragmatic lobe presented a very different picture from those in the preceding animals. The alveoli were moderately distended; the epithelial lining only slightly desquamated; the nuclei of these cells and endothelial cells of the capillaries stained normally. The alveoli were filled with a highly cellular exudate consisting largely of polymorphonuclear leukocytes and a relatively small amount of coagulated serum and red blood corpuscles (fig. 6a). Gram-positive diplococci and streptococci were found in large numbers distributed throughout the alveolar exudate (fig. 6b).

Guinea-pig 886, weighing 320 gm., was injected intratracheally Jan. 2, 1919, with 1.5 cc of the dextrose-brain-broth culture from the lung of guinea-pig 869. The nostrils, before injection, were dry and clean; cultures from the right nostril showed a large number of indifferent colonies resembling staphylococci. January 3 at 7:30 a. m. the animal appeared quite well, although respirations were definitely increased. At 11 a. m. it appeared well; the respirations were still increased, and cultures from the nose showed a large number of green-producing streptococci and a moderate number of staphylococci. January 4 the nose was wet with a mucopurulent discharge; the weight loss was 50 gm., the respirations were somewhat rapid, and coughing occurred at intervals. January 6, 10 a. m., there was marked crusting about the nostrils almost to the point of causing obstruction, and on removal of the crust, several drops of mucopurulent secretion escaped from the nostril. At 6 p. m. the animal was found dead. The lungs were only slightly distended (9 cc). The left diaphragmatic lobe was completely consolidated and mottled grayish-red; the right diaphragmatic lobe and irregular areas in the right cardiac and apical lobes showed grayish consolidations surrounding the bronchi (fig. 3). The left nostril was plugged with a bloody, mucopurulent material. The right maxillary sinus was filled with bloody pus; the mucous membrane of the nose, trachea, and bronchi was extremely hyperemic. Cultures from the blood were negative. Cultures from the pleura, consolidated areas of the lung, and pus from the nose showed a large number of green-producing streptococci and some staphylococci. Sections of the lung showed slight dilatation of the alveoli, marked cellular leukocytic exudate of quite uniform distribution



filling the alveoli completely, with little or no admixture of coagulated serum and blood (fig. 7a). The epithelial cells lining the terminal bronchi and alveoli, and the endothelial cells of the inter-alveolar capillaries stained quite normally (fig. 8b). The bacteria were diffusely distributed in large numbers in the alveolar exudate (fig. 7b).

CASE 2749.—A man, aged 29, a nurse, developed headache, sore throat, severe aching all over, dry cough, and temperature of 100.2, Dec. 16, 1918. The following day his temperature ranged from 102.6 to 103. The headache continued, there were marked backache, soreness through the chest, and a severe cough. December 18 the patient felt weak, perspired, and was chilly at intervals; the ache in the back, soreness in the chest, and the cough were worse. The temperature ranged from 100.6 to 101.8. The cough and soreness in the

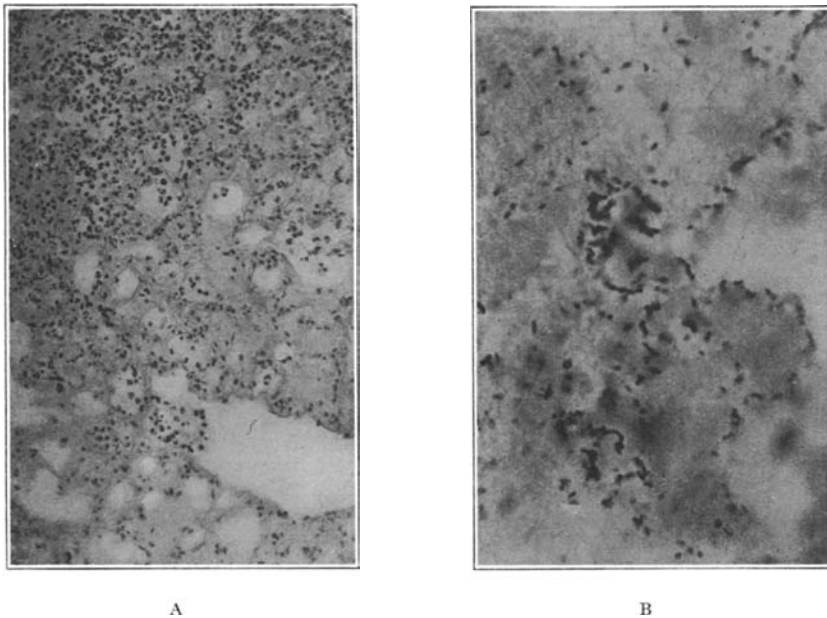


Fig. 4.—Photomicrograph of sections of lung of guinea-pig injected with the green-producing streptococcus from case 2719 after one animal passage. Note the marked edema, hemorrhage, dilatation of terminal bronchi and alveoli, necrosis of cells in alveolar walls and the relatively slight leukocytic infiltration in a, and the distribution of the bacteria along the alveolar wall in b. a. Hematoxylin and eosin;  $\times 100$ . b. Gram-Weigert;  $\times 500$ .

chest continued for a week; the nose bled December 22, and a large amount of mucopurulent blood-tinged sputum was raised December 23. This sputum was cultured and injected into animals. The temperature became normal December 23 and the patient made a good but slow recovery. Blood-agar plate cultures from the sputum showed almost pure culture of moist, spreading, green-producing streptococci, a few staphylococci, no hemolytic streptococci, nor influenza bacilli. The sputum, 0.2 c.c. was injected subcutaneously into a guinea-pig; it died three days later of subcutaneous cellulitis, beginning bronchopneumonia, and hemorrhagic endometritis. Cultures from the

blood, lung, and bloody mucus from the uterus, contained a large number of the characteristic green-producing streptococcus colonies. The culture from the uterus was used to inject 7 guinea-pigs, 4 intravenously, and 3 intratracheally, and 2 rats, one subcutaneously, the other intratracheally. All died as a result of the injection. All 4 animals injected intravenously showed moderate emphysema and evidence of localization in the lung, indicated by localized areas of hemorrhage and edema with or without beginning consolidation. The 2 females showed lesions of the mucous membrane of the uterus and both aborted. Two showed, in addition, localization in muscles and myocardium, one acute peritonitis, and one marked hemorrhagic pancreatitis. Cultures from all yielded the organism injected, together with a few staphylococci, and in one a few colonies of hemolytic streptococci developed from the lung and uterus. All 3 guinea-pigs and the rat injected intratracheally died from emphysema of the lungs filled with hemorrhagic bloody fluid, or with bronchopneumonia in various stages of development. The average volume of the lung in the guinea-pigs was 20 cc (chart 1). The rat and 2 of the guinea-pigs showed decided involvement of the pleura in addition to the lung involvement. The only female injected aborted; one of the others showed peritonitis and one hemorrhage and edema of the head of the pancreas. The characteristic streptococcus was isolated from all. The rat injected subcutaneously died in three days from subcutaneous cellulitis, emphysema, and slight hemorrhages of the lung. The green-producing streptococcus was found in the edema fluid and in the bloody mucus in the uterine horns.

The primary culture in dextrose broth from the pancreas, which showed marked swelling and inflammation, was injected intravenously into 2 guinea-pigs and 2 dogs; intratracheally into a guinea-pig and a rat; intraperitoneally into a mouse, and subcutaneously into a rat. All the animals except the dogs died. Both guinea-pigs injected intravenously developed well marked areas of localized bronchopneumonia, and one developed acute hemorrhagic pancreatitis and myocardial degeneration. The dogs were etherized on the fifth day. Lung lesions were absent, but there were lesions in the mucous membrane of the uterus; the one animal that had aborted showed pancreatitis. The green-producing streptococcus was isolated from the mucous membrane of the uterus in both dogs, from the blood of both guinea-pigs, and from the pancreas of the dog showing pancreatitis. The guinea-pig and rat that were injected intratracheally developed marked rhinitis and tracheitis, emphysema of the lung with hemorrhagic edema, and bronchopneumonia. The guinea-pig had endometritis and aborted. The rat injected subcutaneously and the mouse intraperitoneally developed, beside cellulitis and peritonitis, respectively, definite lesions of lung and pleura, from which the organism was isolated.

The primary culture from the hemorrhagic lung of one of the guinea-pigs injected intratracheally in the second animal passage was injected intratracheally into 2 guinea-pigs. Both developed massive bronchopneumonia and purulent bronchitis, and both yielded the organisms in pure cultures. The filtrate from this lung was injected directly intratracheally into 2 guinea-pigs, and after incubation in dextrose-brain broth into 4 guinea-pigs. One of the latter remained well, all the others developed well marked lesions of the lung, quite similar to those in the animals injected with the corresponding culture. The 2 females had endometritis, 3 had rhinitis, sinusitis, tracheitis, and bronchitis, and 1 each had myositis and mediastinitis. Cultures from the lesions and the blood in these yielded the characteristic organism. The details of these and other filtrate experiments have been given elsewhere.

Cultures from 3 of the animals injected with this strain in the third passage were injected intratracheally into 5 guinea-pigs. All developed moderate emphysema and bronchopneumonia of lobar type, 2 developed high grade myocardial degeneration, and 2 marked rhinitis and bronchitis. Two of the females had endometritis. The primary culture in dextrose-brain broth from the pneumonic lung of 2 of the guinea-pigs, third animal passage series, was injected intratracheally into 4 guinea-pigs, and cultures from the uterine horns of the dog that had aborted were injected into 2 guinea-pigs. One of the former and one of the latter recovered after several days of illness. The others died from two to four days after injection. All showed well marked exudative pneumonia, 2 definitely lobar in type with relatively slight hem-

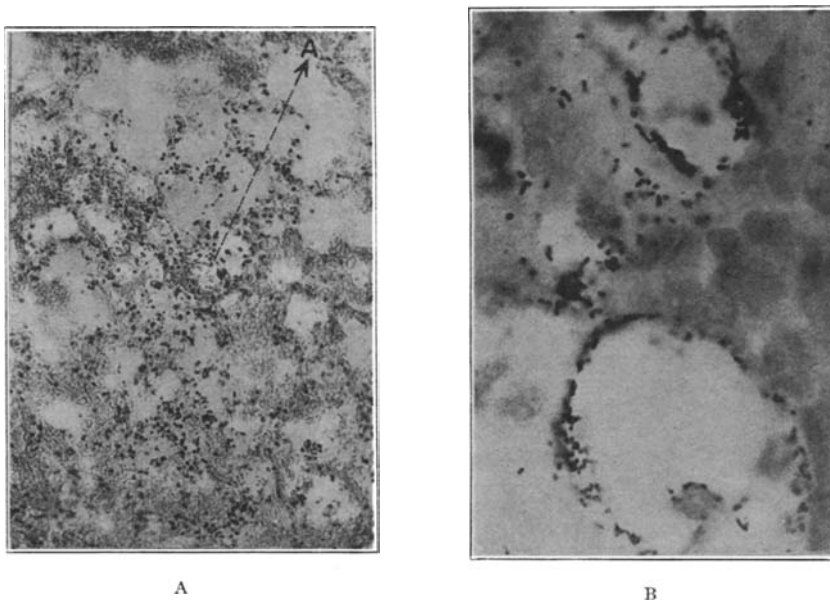


Fig. 5.—Sections of lung of guinea-pig shown in figure 1. Note the marked hemorrhage and edema, the absence of leukocytic infiltration and the marked necrosis of alveolar walls in a, and the peripherally placed streptococci in b. a, Hematoxylin and eosin,  $\times 100$ . b, Gram-Weigert,  $\times 500$ .

orrhagic edema, and only moderate emphysema (chart 1). Two of the 3 females had endometritis and myocardial degeneration and 2 had well marked rhinitis, and tracheitis; 1 had hemorrhages in the rectus muscle. The green-producing streptococcus was isolated from all. The filtrate from the lung of 2 of the guinea-pigs (third animal passage series) was injected into the trachea of 12 guinea-pigs. The 2 that were injected with the heated filtrate recovered after severe immediate symptoms of anaphylactic shock, 1 injected with the unheated filtrate died in ten minutes from anaphylactic shock, 6 of the others recovered after severe immediate symptoms, and 3 died. All showed bronchopneumonia; 2 showed marked lesions of the pleura, and 1 aborted. The green-producing streptococcus was isolated from the lesions, and from the uterus of the one that aborted. The curves giving the volume of the

lung and the mortality rate (chart 1) represent roughly the effects from the successive injections of this strain. The difference in the symptoms and types of lesions of the lung in the early and in the later animal passages was striking. In the former respiratory embarrassment, hemorrhage, and edema with relatively slight exudation of leukocytes in the lung dominated the picture; in the latter, respiratory embarrassment was less marked; exudative pneumonia, and a relatively slight edema with a greater tendency to involve the upper respiratory tract as well as the pleura, dominated the picture. The greater tendency of leukocytic infiltration was noted even in animals that lived the same length of time. The results on intravenous injection of the organism in the second and third passages, besides showing a tendency to localize in the lung and uterus, showed a marked affinity for the muscle, myocardium, and pancreas; pancreatitis occurred in three species of animals (guinea-pig, dog and rabbit). The lesions in the muscles were focal and hemorrhagic, often occurring in clusters, and often surrounded by edema and hemorrhage. Streptococci were found in large numbers in these lesions and in the pancreas, showing pancreatitis. Altogether 19 animals (guinea-pigs, rats, and mice) were injected with this strain intratracheally, intravenously, intraperitoneally, and subcutaneously, in the first, second, and third animal passages. All succumbed to the effects of the injection. Two of the 6 injected in the fourth passage recovered.

CASE 2800.—A woman, aged 24, was admitted to the isolation hospital Jan. 9, 1919, in a very weak condition with a temperature of 104, pulse 120, and respiration 34. The patient had been taken sick six days before with aching of limbs, headache, backache, chills and fever. She complained of pain over the entire chest, and coughed a great deal. The day after admission, her respirations were shallow and labored, she was pale and cyanotic, and the pulse was extremely rapid. A diffuse bronchopneumonia of the right base and bronchial breathing in the area opposite the angle of the scapula on the left side were found. January 12 her condition was very much the same; the chest was in full expansion and respirations were almost wholly diaphragmatic. The symptoms persisted, she grew worse as cyanosis increased and died January 14. The leukocyte count was persistently low, ranging between 1,900 and 3,700.

The chief findings at necropsy were: Bilateral pseudolobar pneumonia; hemorrhagic edema; left hemo-hydro-thorax, 500 c c; and mild acute nephritis. Sections of the lung showed dilatation of alveoli, marked congestion, and alveolar exudate rich in red blood corpuscles, edema fluid, little fibrin, and only a moderate number of leukocytes.

A culture from a throat swab January 12 showed a large number of moist, spreading colonies of green-producing streptococci, many colonies of *Staphylococcus aureus*, and moist hemolyzing streptococci. Cultures from the blood after death contained green-producing streptococci, hemolyzing streptococci, and staphylococci; and from cultures from the pleural fluid hemolyzing streptococci and staphylococci. The history and findings in this case are clearly those of influenza in which well marked lung lesions developed as the symptoms persisted.

The bacteria isolated from the sputum, blood, and lung exudate were a mixture of the organisms most constantly present in influenza. Aside from hemolysis the morphology and type of colony of the green-producing streptococci and hemolyzing streptococci were identical. It was thought worth while to study the effects, including the leukocyte counts, of the injection of mass cultures containing a mixture of these strains, and of pure cultures in

a large series of animals in order to note the changes which might occur in the lesions produced following successive animal passage. The effect of intratracheal injections of cultures of the green-producing and hemolytic streptococci or mixtures that occurred in the primary dextrose-brain broth from the blood, and sputum, throat, and lung exudates were very similar. Emphysema of marked grade and hemorrhagic edema with localized areas of peribronchial consolidation of varying size and age dominated the picture. Leukopenia was equally marked regardless of whether the strain was hemolytic or green-producing. The results obtained following successive intratracheal injections of the green-producing streptococci are summarized in

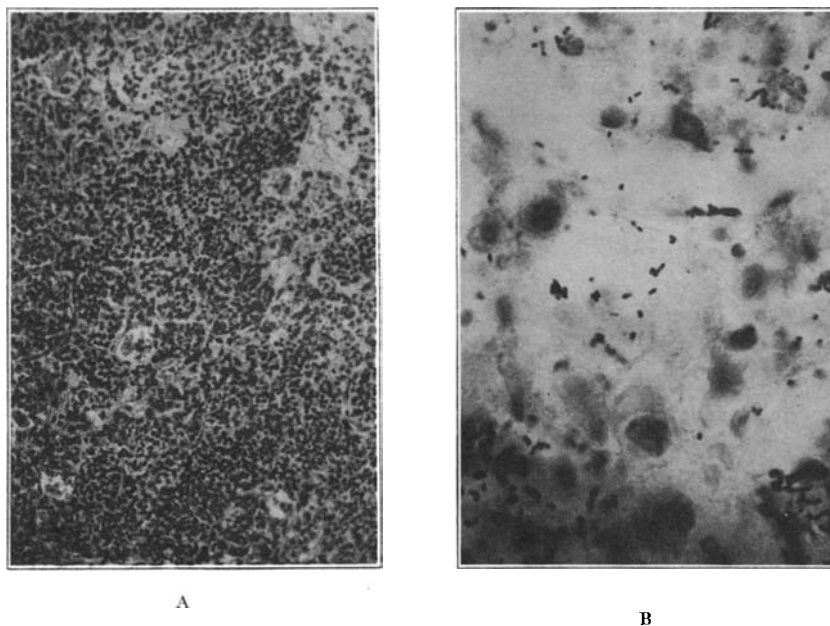


Fig. 6.—Sections of lung shown in figure 2. Note the marked leukocytic infiltration and the slight edema and hemorrhage, and the diffuse distribution of the streptococci in the exudate. a. Hematoxylin and eosin,  $\times 100$ . b. Gram-Weigert,  $\times 500$ .

chart 1. In this series of animals the strains used for subsequent injections were first plated on blood-agar. From this subcultures of green-producing streptococci were made in dextrose-brain broth and injected.

It will be noted that during the first and second animal passages, as the volume of lung (severity of reaction) increased, the mortality increased, the drop in leukocytes occurred, and as the volume of lung diminished during the third and fourth passages the mortality and the drop in leukocytes became less marked. The difference in the character of lesions in the lungs was similar to the difference noted between cases 2719 and 2749.

It has been pointed out elsewhere that the green-producing streptococci may acquire typical hemolyzing power (beta type hemolysis), and that the hemolyzing streptococci may acquire the power of producing typical green colonies (alpha type hemolysis). Moreover, a green-producing flora in patients is often displaced by a hemolytic flora and this in turn by a green-producing flora, especially in patients who recover. It was thought worth while to pass the hemolytic streptococcus through a series of guinea-pigs in the same manner as the green-producing streptococcus had been passed, and to observe whether or not the type of lesion produced changed, and whether the streptococcus changed. The primary culture in dextrose-brain broth of the throat swab was injected into the trachea of 6 guinea-pigs and a rat. Two of the guinea-pigs and the rat recovered. Four of the guinea-pigs died from typical lung lesions with or without pleural involvement, and all showed hemolytic streptococci in pure culture, or together with a few staphylococci.

Guinea-pig 947, weighing 400 gm., was injected intratracheally Jan. 12, 1919, 11 a.m., with 1.5 cc dextrose-brain broth culture of the throat swab. A blood-agar plate of the culture injected showed green-producing, hemolytic streptococci, and a few staphylococci. At 6 p.m. the respirations were rapid, the voice was weak, and the animal appeared ill. January 13, 7:30 a.m., the animal was found dead. A large amount of hemolyzed, dark chocolate colored fluid was found in the pleural cavity. The right diaphragmatic and intermediate lobes were large, extremely hemorrhagic and edematous on the cut surface. Both uterine horns contained a moderate amount of blood tinged mucus, and the mucous membrane was hemorrhagic in areas. Cultures from the blood and from the lung and pleural fluid showed a large number of hemolytic streptococci, a smaller number of staphylococci, but no green-producing streptococci; those from the mucus in the left horn of the uterus showed 150 colonies of staphylococci, 9 colonies of hemolytic streptococci, and 21 colonies of green-producing streptococci. The primary culture of the pleural fluid of this animal which yielded hemolytic streptococci and a few staphylococci was then injected into the trachea of another guinea-pig; it died within six hours. The leukocyte count dropped from 15,000 before injection to 3,100 after death. The lungs were huge in size (18 cc) hemorrhagic and edematous throughout, and the pleura contained about 1 cc of hemorrhagic fluid. Cultures from the blood, pleural fluid, and lung exudate showed many moist, spreading colonies of hemolytic streptococci, while those from the liver and kidney showed a few. The dextrose-broth culture from the blood of this guinea-pig was injected into the trachea of 5 guinea-pigs. One recovered and 4 died of hemorrhagic edema and bronchopneumonia, from one to four days after injection; two died with hemorrhagic pleuritis and 2 without. All showed predominating or pure cultures of green-producing streptococci. The findings in the animal whose strain was passed to the next series were similar to those in the others.



Guinea-pig 1043, weighing 480 gm., was injected intratracheally Jan. 23, 1919, with 2 cc of the glucose-broth culture from the blood of the guinea-pig that died six hours after injection. The leukocyte count was 16,800. January 24, 10 a.m., the respiration was extremely rapid, the leukocyte count was 2,200; at 9 p.m. respiration was extremely rapid, the animal was weaker, it made repeated violent efforts at breathing, resembling anaphylactic shock, and had an expiratory grunt. January 25 it was found dead. The pleural cavity contained a moderate amount of bloody chocolate colored fluid. The left diaphragmatic lobe was covered with a fibrinous film. The lung was greatly distended (18 cc) and heavy (17 gm.). A large part of the left lung was consolidated, consisting of coalescing areas of bronchopneumonia, which in places on the cut surface had become grayish and quite dry. Several

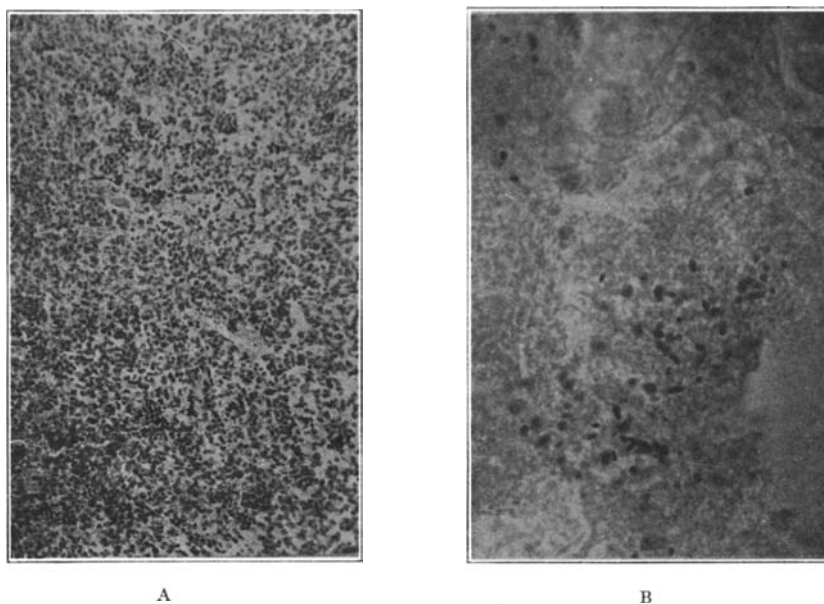


Fig. 7.—Section of lung shown in figure 3. Findings similar to those in figure 6. a. Hematoxylin and eosin,  $\times 100$ . b. Gram-Weigert,  $\times 500$ .

similar but smaller areas were found in the right diaphragmatic lobe. Generally the mucous membranes of the nose and trachea were extremely hyperemic and the trachea was filled with a hemorrhagic frothy fluid. A large area (2 by 1.5 cm.) of hemorrhage and edema was found in the right rectus muscle of the abdominal wall. The involved muscle was friable and ruptured on slight stretching. The leukocyte count of the blood from the heart was 4,400. The myocardium was yellowish gray, the ventricles were in firm contraction, and the auricles dilated. Cultures from the blood, pleural fluid, and lung showed a large number of green-producing streptococci in pure culture, and the liver, adrenal, kidney, brain, and spleen, showed a few. The culture in dextrose broth from the blood after one plating was injected into the trachea of 3 guinea-pigs; 1 of these died of bronchopneumonia in forty-eight hours,

1 recovered, and 1 was chloroformed three days after injection. The third had a moderate amount of bloody fluid in the right pleural cavity, a large wedge-shaped area of grayish consolidation of the right diaphragmatic lobe, and slight emphysema of the lung. The uterus was opaque and contained a moderate amount of mucus. The leukocyte count before injection was 9,400; twenty-four hours after injection 10,400; forty-eight hours after injection 7,200; and after death 8,000. Cultures from the blood were negative; those from the consolidated lung showed large numbers of staphylococci and green-producing streptococci, and the pleural fluid and uterus showed a few green-producing streptococci and staphylococci. This, then, is an example in which a hemolytic streptococcus remained as such throughout two animal passages, but in 4 animals in the third passage it appeared to lose the hemolytic power and to produce green colonies, a property which it retained through the next animal passage. Fifty-three guinea-pigs were injected with cultures of green-producing and hemolytic streptococci isolated from this case and after animal passage. Twenty-three were injected in the first passage, 14 (60%) died; 9 were injected in the second passage, 8 (90%) died; 9 were injected in the third passage, 7 (78%) died; and 14 were injected in the fourth passage, 6 (43%) died.

The average mortality resulting from the animal passage after intratracheal injection of the strains from the 3 cases reported herewith was 57 per cent. of 22 animals injected in the first animal passage, 90 per cent. of 10 animals in the second, 87 per cent. of 16 animals in the third; and 55 per cent. of 22 animals in the fourth. The total mortality, irrespective of the place of injection, ranged as follows: 60 per cent. of the 30 animals injected in the first animal passage, 94 per cent. of the 17 animals in the second, 90 per cent. of the 21 animals in the third, and 52 per cent. of the 19 animals in the fourth passage.

A study of the 3 cases shows clearly that the lesions in the lung in the first few passages resemble very closely those noted in the lungs of the 2 patients who died. They are characterized by marked emphysema, extreme hemorrhage and edema of the lungs (fig. 1), marked evidence of destruction and desquamation of epithelial lining (figs. 3 and 4 a), absence of staining or fragmentation of nuclei of endothelial cells, of capillaries of alveoli (Fig. 8 a), and aggregation of streptococci along the alveolar lining (figs. 3 b and 4 b). Moreover, the relative lack of response, on the part of the host, is evidenced by the slight leukocytic exudation in the lung and the marked reduction of leukocytes in the blood. The symptoms of respiratory embarrassment are often extreme, the mortality rate is high, and death occurs early. After a number of animal passages the picture becomes quite different. The respiratory embarrassment is less violent, the reduction in leukocytes less marked, and the exudation of leukocytes in the lung is the domi-



nant picture (figs. 6 a and 7 a) as hemorrhage and edema become less prominent. The lungs are not so voluminous (figs. 2 and 3). Degeneration and desquamation of the epithelial cells and necrosis of the capillaries are slight (fig. 8 b) and the bacteria are diffusely distributed throughout the exudate instead of along the alveolar lining (figs. 6 b and 7 b). The difference in amount of leukocytic infiltration depends not on the duration of the experiment, but varies with the number of animal passages. The diminution of virulency of these strains from

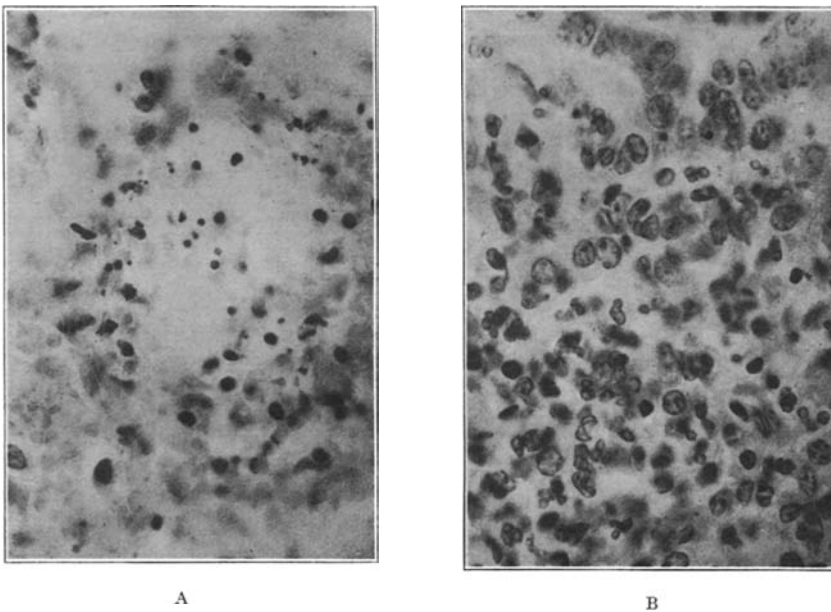


Fig. 8.—High-power magnification of sections of lungs shown in figures 4 and 7, illustrating type of lesions obtained during the first few and higher numbers of animal passages. a. Note the marked fragmentation of nuclei of alveolar epithelium and capillaries, the marked hemorrhage and edema, and the almost complete absence of leukocytic infiltration. b. Note the marked leukocytic infiltration and the relatively slight necrosis of cells of alveolar walls. Hematoxylin and eosin,  $\times 500$ .

cases of influenza as a result of successive intratracheal injections is contrary to the result following successive intraperitoneal injection of strains of the pneumostreptococcus group. The latter method was tested to determine whether the green-producing streptococcus from influenza is peculiar in this respect.

In chart 2 is given a summary of the results of successive intraperitoneal and intratracheal injections of a series of strains of green-

producing streptococci from influenza. In the forced experiment on intraperitoneal injection the virulence of the green-producing streptococci increases as that of streptococci and pneumococci from other sources. But when the former micro-organisms are applied successively to the normal mucous membrane of the lower respiratory tract their invasive power increases only during one or two animal passages; it then becomes progressively less during three or four subsequent passages. Most of the strains that were passed through animals were cultivated on artificial mediums for one generation and in one strain

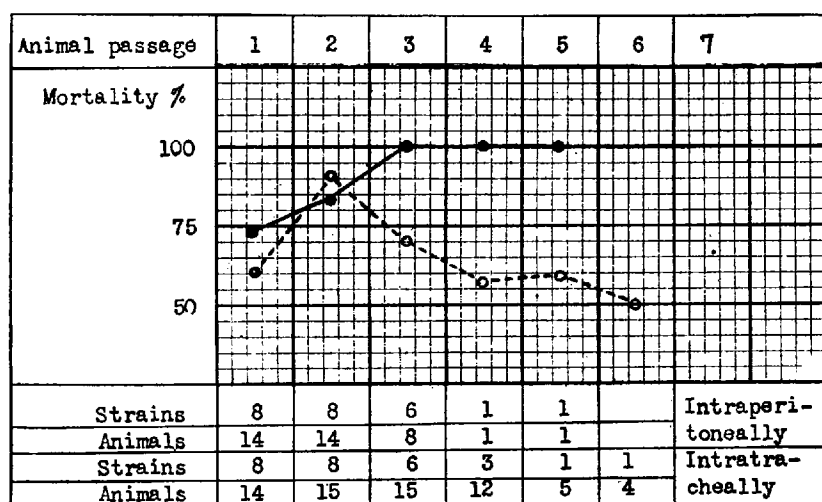


Chart 2.—Mortality in guinea-pigs following successive intraperitoneal and intratracheal injections of streptococci from influenza. The straight line denotes intraperitoneal injection; the broken line denotes intratracheal injection.

(case 2800), for two generations between each passage. In order to make sure that the diminution in invasive power on successive intratracheal application was due to effects exerted on the micro-organism by the body cells or fluids and not due to the culture mediums, control experiments were carried out; emulsions of the consolidated lung were injected directly from animal to animal. The results were similar. The diminution in infecting power was even more rapid than when intervening cultures were injected. This result, contrary to what might be expected, considering what usually happens after direct successive intraperitoneal injections, is in keeping with what has been observed repeatedly when emulsions of lung and pleural fluid from man and from animals are injected directly into the trachea and compared with

the results following the injection of the corresponding cultures. The severity of reaction and the mortality following injections of the cultures were greater even when the number of viable bacteria was no larger than that in the exudate. The cells or fluids in the exudate seemed to rob the bacteria of their bite, as it were, a property which they often regained during the growth in suitable artificial mediums. Moreover, in harmony with this idea is the fact that the mortality was higher following the injection of sputum during life than following the injection of the bloody lung exudate after death. During the course of the experiments on intratracheal injection it was also noted that cultures of the green-producing streptococci from the blood and pleural exudate after death in cases of long standing were not so virulent as those from the sputum or throat. Thus in 22 guinea-pigs injected intratracheally with the strains cultured from the sputum of one patient the mortality was 64 per cent., whereas in a series injected with the strains cultured from the blood the mortality was 33 per cent.

Hirsch and McKinney found that the pneumococci at the height of the epidemic at Camp Grant were far more virulent when injected intraperitoneally or intravenously in animals than those isolated as the epidemic was subsiding, and, moreover, the strains isolated from the blood at the height of the epidemic were less virulent than those from the sputum.

#### EXPERIMENTS SUGGESTING THE OCCURRENCE OF MUTATION IN VIVO

It has been shown elsewhere that marked changes occurred in the bacterial flora in the later stages of influenzal infection, that similar changes often occurred in vitro, and that the new strains or variants were not only virulent on injection in animals, but also tended to produce leukopenia. Following intratracheal injection the changes in the lung closely simulated those of influenzal infection.

Owing to the instability of the streptococci from influenza noted at the onset of this work, we have taken particular pains to observe whether the changes noted in patients and in vitro might occur in the body of the experimental animal. A striking example of the mutation of a hemolytic streptococcus into a green-producing streptococcus simultaneously in a series of guinea-pigs has been detailed in the experiments recorded in case 2800. The lesions produced by the intratracheal injection method resembled closely those observed following injection of the green-producing streptococcus from influenza.

On the other hand, the green-producing streptococcus often appeared to become a hemolytic streptococcus. Thus in case 2749 one of four guinea-pigs injected intravenously with a pure culture of green-producing streptococcus from the blood of a guinea-pig injected subcutaneously with the sputum, showed a moderate number of hemolytic streptococci and *Staphylococcus aureus* in the pleural fluid, hemorrhagic areas in the lung, hemorrhagic mucus in the uterine horns, and in the pancreas. The pancreas showed a moderate number of green-producing streptococci. The green-producing streptococcus from 2 animals in the second-passage series were injected in various ways into 7 guinea-pigs, 2 rats, 2 dogs, and a mouse. The organism injected was isolated in pure form from all the guinea-pigs, one rat, and one dog. One rat injected subcutaneously yielded a large number of the green-producing streptococci from the blood and subcutaneous tissues, and from the latter a moderate number of *Staphylococcus aureus* also. The mouse injected intraperitoneally died in forty-eight hours from hemorrhagic peritonitis and pleuritis. The blood and pleural fluid yielded a moderate number of green-producing streptococci, slightly hemolytic streptococci, and *Staphylococcus aureus*. The one dog which aborted following intravenous injection had a large number of moist spreading hemolytic streptococci, and a moderate number of staphylococci in the bloody mucus of both uterine horns, and the blood yielded a few colonies of green-producing streptococci. The culture in dextrose-brain broth of the hemolytic streptococcus from the uterus, and the green-producing streptococcus from the blood were injected intratracheally into one guinea-pig each. The first animal showed leukopenia for forty-eight hours, then leukocytosis, and died on the third day with distended lungs (17 c c) showing marked exudative pneumonia of pseudolobar type (14 gm.). The lung and pleura yielded green-producing streptococci. The second animal showed a progressive and marked leukopenia and died with similar lung findings the day of injection. Cultures from the blood and kidney showed a few colonies of green-producing streptococci in pure form, while the pneumonic lung and mucus in the right horn of the uterus showed the green-producing streptococci and *Staphylococcus aureus*.

The results in case 2851 are similar to those observed in others. The patient developed an attack of influenza of ordinary severity, but on the sixth day became suddenly worse and died on the eighth day from acute hemorrhagic edema and bronchopneumonia. The sputum on

the second day was mucoid and contained a large number of the green-producing streptococci, a few slightly hemolyzing streptococci, and a few staphylococci. On the seventh day the sputum was blood-tinged but purulent, and showed *Staphylococcus aureus* in pure form in large number. Permission for necropsy could not be obtained, but a syringe-full of bloody fluid was withdrawn from the left pleural cavity. The blood-agar plate inoculated with this fluid contained a large number of *Staphylococcus aureus* and a few moist slightly hemolyzing colonies of streptococci. The culture in dextrose-brain broth from a single well isolated colony of the slightly hemolytic streptococcus from the pleural fluid was injected into the tracheas of 3 guinea-pigs. Pure growth of slightly hemolyzing colonies of streptococci was obtained from the blood-agar plate of the culture injected. In all 3 guinea-pigs there was a reduction in leukocytes in from twenty-four to forty-eight hours after injection; the average count before injection was 12,900, twenty-four hours after injection 7,600, and forty-eight hours after injection 8,260. The animals seemed quite well three days after injection, when they were chloroformed. All had bronchopneumonia, and the one female had endometritis with hemorrhagic mucus in both uterine horns. None had pleuritis. In 2 the cultures from the blood were sterile; cultures from the blood of the female contained a few colonies of streptococci. Cultures from the pneumonic lung of all yielded a pure culture of *Staphylococcus aureus*. The hemorrhagic mucus in the uterine horns yielded *Staphylococcus aureus* and a few colonies of green-producing streptococci. Cultures from the adrenal, kidney, spleen and liver were sterile.

The culture in dextrose-brain broth from a single well isolated colony of *Staphylococcus aureus* which showed no streptococci in smears and only staphylococcus colonies on plating was injected intratracheally into a guinea-pig; it died in twenty-four hours from hemorrhagic edema of the lung. The pleural cavity was filled with hemorrhagic fluid, and the visceral pleura was covered with a thin fibrinous film. The leukocyte count of 12,400 before injection dropped to 3,400. Blood-agar-plate cultures from the blood, pleural fluid, lung, kidney and liver showed *Staphylococcus aureus* in large numbers, those from the adrenal and spleen a few. A few colonies of moist spreading green-producing streptococci in addition to *Staphylococcus aureus* were isolated from the pleural fluid and lung. A culture in a tall tube of dextrose broth from a single well isolated green-producing colony of

the streptococcus from the pleural fluid was made and injected intratracheally into 2 guinea-pigs and intraperitoneally into 1. The latter showed a drop in leukocytes of from 19,000 to 7,600 twenty-four hours after injection, and then recovered. One of the former, a female, died in two days from hemorrhagic edema of the lung, bronchopneumonia, a large amount of hemorrhagic fluid in the pleural cavity, and hemorrhagic endometritis. Cultures from the blood, uterus, lung and pleural fluid revealed a large number of moist spreading green-producing streptococci, and a few *Staphylococcus aureus*; those from the adrenal, spleen, liver, and brain contained a smaller number of both. The leukocyte count dropped from 6,600 before injection to 2,000 twenty-four hours after injection. No count was made after death. The other pig injected intratracheally had a drop of from 14,200 to 10,000 leukocytes, and increased respiration for a few days; it then seemingly recovered, but died seventeen days after the injection. Serosifibrinous pleuritis and peritonitis were found. There were no lesions of the lung. Cultures from the blood remained sterile, while those from the peritoneal exudate contained many colonies of *Staphylococcus aureus* in pure form. The blood-agar plate made at the time the culture was injected into these animals showed a pure growth of a green-producing streptococcus, and after the same tube was incubated for fifteen days a blood-agar plate showed a moderate number of rather dry green-producing and slightly hemolyzing colonies of streptococci and a large number of colonies of staphylococci.

A dextrose-brain broth culture from a single green-producing colony from the blood of the female guinea-pig that died from hemorrhagic edema and pleuritis was injected into the trachea of one guinea-pig. The leukocyte count dropped from 15,800 to 4,000 in twenty-four hours, respiration was increased moderately for a few days, and the animal then recovered. The strain was lost.

This case is an example of a predominant green-producing streptococcal flora noted early in influenza being replaced by *Staphylococcus aureus*. Hemorrhagic pleural fluid after death showed a preponderance of the *Staphylococcus aureus* and a few slightly hemolytic streptococci. A subculture from a single colony of the latter proved only moderately virulent, and in all the guinea-pigs staphylococci only were isolated from the lesions in the lung as recovery seemed assured. The staphylococcus culture from a single colony was extremely virulent. The hemorrhagic lung and pleural fluid yielded, in addition to the

staphylococcus, a few colonies of green-producing streptococci. A culture from a single colony of the latter, which showed no staphylococci when injected, yielded staphylococci in both of 2 guinea-pigs as well as in the culture tube after prolonged cultivation. The green-producing strain in the next animal passage produced marked leukopenia, increased respiration for a time, and then was lost as recovery ensued.

In case 2608 the sputum was injected directly intraperitoneally into a guinea-pig. It died from peritonitis. The blood showed the green-producing streptococcus in pure culture; the peritoneal fluid showed this organism and *Staphylococcus aureus* in moderate number. A well isolated single colony of the former was inoculated into glucose-brain broth. The twenty-four-hour culture was injected intraperitoneally into a guinea-pig. The blood-agar plate of this culture yielded only green-producing streptococci. The guinea-pig died in three days of hemorrhagic fibrinous peritonitis, pericarditis and pancreatitis. The leukocyte count dropped from 6,000 before injection to 4,480 in twenty-four hours, and to 3,600 in forty-eight hours. The cultures from the blood showed a pure growth of green-producing streptococci, whereas the pericardial and peritoneal fluid showed these together with a moderate number of the *Staphylococcus aureus*. In order to test whether or not the staphylococci found in the pericardial exudate possessed virulence, a culture in dextrose-brain broth from a single colony was injected intraperitoneally into a guinea-pig in the usual dosage (0.5 c.c. per 100 gm. weight). It died in three days from hemorrhagic peritonitis, with localized areas of hemorrhage and edema in the lungs. The leukocyte counts were 9,200 before injection, 3,200, 6,000, and 3,240, respectively, twenty-four and forty-eight hours later, and after death. Cultures from the peritoneal fluid and blood yielded a pure growth of *Staphylococcus aureus*.

A summary of a large number of experiments in animals injected with cultures proved to be pure by plate cultures, reveals that apparent mutations occurred in 11 of 75 injected intraperitoneally and intravenously, and in 14 of 73 injected intratracheally.

It is realized that the finding in these animals of bacteria that were not introduced might be interpreted as secondary invasion, if it were not for the fact that these mutation forms develop in the test tube under controlled conditions. Indeed until the pure line requirement and the remote possibility of contamination from the air in the test-tube



experiments are met, conclusions with regard to the mutation of streptococci into staphylococci and mutation of influenza bacilli cannot be drawn. However, from a consideration of the precautions which have been taken to exclude accidental contamination, the regularity of its occurrence under certain conditions, and the high virulency of the staphylococci which at times displace the streptococcal flora in fulminating cases of influenzal pneumonia and the high and peculiar virulency of the mutants, the observations are believed worthy of record.

#### MORTALITY FROM INFLUENZAL INFECTION IN RELATION TO THE RISE AND FALL OF EPIDEMIC WAVES

It has been noted by physicians who have seen many cases of influenza that the attacks were more severe during the height of the epidemic and milder as the epidemic subsided. The mortality statistics of infectious diseases now available are based almost wholly on the number of patients who develop the disease and the number of deaths within a certain number of days, weeks or months. No records of epidemic diseases have come to my notice in which the mortality rate is studied strictly in relation to the time in the epidemic at which the disease was contracted. Owing to the changes noted on successive intratracheal application in the invasive power of streptococci from influenza it was thought worth while to determine the mortality in the patients with influenza admitted to the hospitals according to the period in the epidemic the disease was contracted. In chart 3 each black column represents the number of patients who developed influenza on that day. These columns show that there were four distinct waves and two lesser recrudescences between September, 1918, and April, 1919, and that each wave spent its force in about six weeks. Each wave was divided into three two-week periods, namely, two weeks before and including the day of the crest of the wave, the first two weeks following the crest, and the second two weeks following the crest of the wave. The first row of figures at the bottom of the chart indicates the number of persons with influenza admitted to the hospital in each of these periods. The second row indicates the percentage of deaths from influenzal infection, not during the two weeks, respectively, but during that time or later. In other words, 16 per cent. of the 43 persons contracting influenza during the first two weeks of the first wave ultimately died, 20 per cent. of the 112 persons contracting influenza during the second two weeks died, and 13 per cent. of the 54 persons contracting influenza during the third two weeks died, and so on. By a study of the mortality



according to the time the disease was contracted, it was discovered that the highest mortality rate occurred in each of the three main waves in the second two weeks, the time when the largest number of cases developed. It was lower during the first two weeks as the epidemic was on the increase, and in each instance lowest the third two weeks as the wave subsided. The number of cases during the third wave was small and the mortality low; accordingly, the marked rise and fall in mortality did not occur. The slight recrudescence in November also carried with it a low mortality (15 per cent.). The mortality during the recrudescence in April was highest (26 per cent.) when the number of cases was largest (36), and much lower (12 per cent.) as the epidemic disappeared. The curve to the extreme right in the chart represents the average mortality percentage of the four waves, during the three periods of two weeks each, 14, 21 and 12 per cent., respectively.

Besides the change in mortality rate, there was a noticeable difference in the type of the disease during the early part, or the height of each wave, and that found as the wave subsided. The incidence and degree of exudation into the lung was more marked during the middle part of the epidemic. Thus, during the first two weeks of the first wave 24 per cent. of the patients admitted developed pneumonia, during the second two weeks, 30 per cent., and during the third two weeks, 27 per cent. The average percentage incidence of influenzal pneumonia during the four waves for the three biweekly periods was 30 per cent., 37 per cent., and 41 per cent., respectively. The lesions in the lung found at necropsy in our cases as in those of other observers were distinctly different early and late in the waves.

Voluminous lungs with marked hemorrhagic edema and relatively slight true consolidation were the rule at the height of the waves, while exudative pneumonia of the bronchopneumonic type with relatively slight hemorrhage and edema dominated the picture as the waves subsided.

In a previous paper <sup>4</sup> I have shown that the tendency to a persistence of leukopenia in patients contracting the disease late in epidemic waves is less marked than at the height of the waves. In the light of the animal experiments might not this difference as well as the greater tendency to true consolidation of the lung late in the waves be an expression of a diminished virulence on the part of the infecting micro-organisms?

In another paper <sup>4</sup> I have shown also that as patients recover from influenza and especially influenzal pneumonia the leukocyte count goes up. Exceptionally this is true also in protracted cases in which the patient dies. This is generally considered to be due to secondary invasion or to a winning fight by the defensive mechanism of the host. The possibility that this is due to changes in the parasite must, in the light of the experiments on successive intratracheal injection, be taken into consideration. Leukocytosis following an initial leukopenia was noted commonly in guinea-pigs injected with sublethal doses of the green-producing streptococcus, and prolonged contact with the body fluids and cells was found to rob these strains of the power to produce leukopenia. The mortality curves in the epidemics studied represent in a general way those noted by others and indicate a rise and a fall of virulency of the infecting micro-organism. The severity of influenza as it passes through smaller groups, such as large families, often shows the same rise and fall.

The difficulties, however, in studying the severity of influenza in sequence in individual families in which quarantine is not strictly observed are obvious. Authentic information regarding the severity of attacks has been obtained, however, in the case of a number of families living in the country. The findings in a family of eleven living in isolation 15 miles from a railroad station are especially instructive in this connection. The date of onset, date of death in the fatal cases, the age of the patients, and the attacks according to severity are arranged chronologically in Chart 4. It will be noted that fifty-three days elapsed from the time the first became ill (September 27) until the last one contracted the disease (November 19). The interval between the groups of cases was about four, twelve, ten, twelve and fifteen days, respectively. The epidemic spent its force in the surrounding community during the same time. The first person to contract the disease had a mild attack, but because he persisted in working, he developed severe symptoms, was in bed with fever for six days, and then recovered. The 3 persons who came down last had mild attacks, and all recovered without developing pneumonia or other complications. The 7 who contracted the disease during the interval between the first and the last cases all had severe attacks; 3 died from influenzal pneumonia; 2 of those who recovered developed pneumonia, and 1 phlebitis of the leg; the fourth had a severe attack, but did not develop outspoken signs of pneumonia. The source of the infection was not known. From the dates of onset of symptoms in these cases, it seems that the

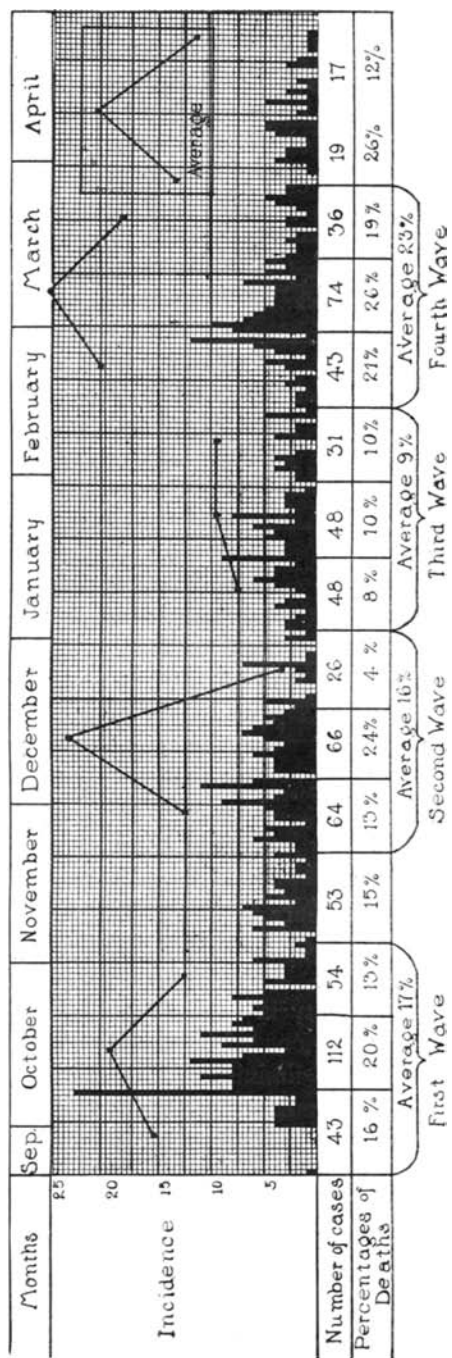
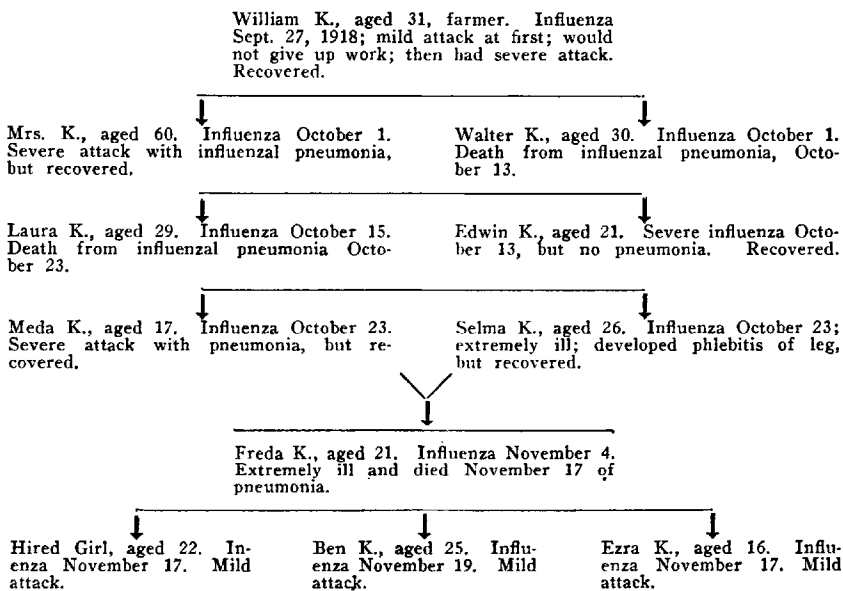


Chart 3.—Mortality from influenza in relation to the rise and fall of epidemic waves of the disease. The black columns show the number of cases of influenza. The curves show the percentage of deaths in persons who developed influenza during two weeks prior to the crest, first two weeks following the crest and second two weeks following the crest of the waves.

organism passed through five persons, and as this occurred the virulency, just as on successive animal passage on tracheal application increased during the earlier passages and then decreased, and was lost in the sixth passage.

#### CHART 4

THE RISE AND FALL IN VIRULENCY OF INFLUENZAL INFECTION AS IT PASSED THROUGH A LARGE FAMILY



#### SUMMARY

The results reported elsewhere following the intratracheal injection of the green-producing streptococcus from influenza have been verified and extended in this study.

The virulency and mortality in animals increased for one or two successive intratracheal injections of this organism, and on further animal passage progressively diminished. At the peak of virulency the symptoms of respiratory embarrassment are frequently violent and often resemble those of anaphylaxis. Cyanosis and leukopenia are marked, and death from hugely dilated lungs filled with hemorrhagic edema fluid with relatively slight exudative pneumonia frequently occurs. Microscopically, necrosis of alveolar epithelium and endothelium of the capillaries of all grades with marked hemorrhagic edema and little leukocytic infiltration are the chief findings. In subsequent

intratracheal injection respiratory embarrassment becomes less marked, reduction in leukocytes less pronounced or wholly absent, and as this occurs the dilatation of the lung becomes less, exudation of leukocytes in the lung more pronounced, and extensive pneumonia with little hemorrhage and edema is the dominant picture.

TABLE 1  
EFFECT OF SUCCESSIVE INTRATRACHEAL INJECTION OF STREPTOCOCCI FROM INFLUENZA,  
THE LEUKOCYTE COUNT AND MORTALITY

Animal Pas- sage	Strains	Ani- mals In- jected	Average			Percentage Showing			Mor- tality per Cent.
			Before Injec- tion	After Injection (24 hours)	Reduc- tion, %	Leuko- penia	Leuko- cytosis	No Change	
First.....	5	14	14,800	7,200	51	92	4	4	57
Second.....	5	8	12,300	4,200	66	88	0	12	100
Third.....	3	7	17,100	8,500	50	70	15	15	57
Fourth.....	3	13	14,700	13,300	9.5	47	25	25	38

From a study of the four epidemic waves as they occurred in Rochester, it has been found that a similar rise and fall in severity of symptoms, mortality and character of lung lesions occurred as the epidemic waves appeared and disappeared. The symptoms, cyanosis and leukopenia were most pronounced, and the mortality was the highest at the peak of the waves when the lungs were of huge size, and at necropsy hemorrhagic edema with relatively slight exudative pneumonia was the striking picture. Later as the symptoms became milder, leukopenia less persistent, and the mortality rate lower, exudative pneumonia became more common. Experimental evidence has thus been obtained to show that (1) the change in the type of the disease early and late in epidemics, (2) the rise and fall in mortality rate in the same epidemic and the virulency of different epidemics, and (3) the lesser tendency to leukopenia late in epidemic waves may be due, in the main, to changes in virulency and other properties of the green-producing streptococci isolated so constantly in influenzal infection.

These facts do not exclude the possibility that the influenza bacillus may play a rôle in the production of symptoms and lesions in influenza. In some cases they rather suggest the possibility that this organism may undergo similar changes, and that it may acquire peculiar and high infecting powers. Indeed the recent work of Blake and Cecil, in which symptoms and lesions simulating influenza have been produced experimentally in the monkey with the influenza bacillus made highly virulent by repeated monkey passages, supports this view.

Throughout the work the well marked examples in which green-producing streptococci suddenly acquired hemolytic power and hemolytic streptococci suddenly became green-producing streptococci, both in vitro and in vivo, suggest strongly that the complete or partial displacement of one type of streptococcal flora by another throughout, especially late in the epidemic waves, may be due to the development of mutation forms rather than the result of superimposed infection from the upper respiratory tract.

Since the mutants have been found to possess the power of producing the characteristic lesions in the lung and a sharp leukopenia on intratracheal application, might not the green-producing streptococcus isolated so constantly early in influenza and influenzal pneumonia, since it has high and peculiar invasive and other properties, be a mutation form of the pneumococcus-streptococcus group which humans normally harbor? Moreover, might not the sudden appearance and rapid "spread" of influenza among isolated groups and often almost simultaneously over wide areas be in part due to this cause?

1. Blake, F. G., and Cecil, R. L.: The Production of an Acute Respiratory Disease in Monkeys by Inoculation with *Bacillus Influenzae*. A Preliminary Report, Jour. Am. Med. Assn., 1920, lxxiv, 170-172.

2. Hirsch, E. F., and McKinney, M.: An Epidemic of *Pneumococcus Bronchopneumonia*, Jour. Infect. Dis., 1919, xxiv, 594-617.

3. Rosenow, E. C.: Studies in Influenza and Pneumonia. II. The Experimental Production of Symptoms and Lesions Simulating Those of Influenza with Streptococci Isolated During the Present Pandemic, Jour. Am. Med. Assn., 1919, lxxii, 1604-1609.

4. Rosenow, E. C.: Studies in Influenza and Pneumonia. VI. The Leukocytic Reaction in Influenza and Influenzal Pneumonia. (In press.)