THE INFLUENCE OF RESISTANCE ON THE CHARACTER OF DISEASE IN EXPERIMENTAL RESPIRATORY INFECTION.

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It has become well recognized that organisms of considerable virulence may be introduced into the uninjured respiratory tract without producing recognizable disease. Arloing, Cornevin, and Thomas[1] called attention to the fact that injection of fluids containing the bacilli of symptomatic anthrax into the trachea of calves resulted in immunity but failed to produce disease, provided there was no leakage of material into the peritracheal tissues. Snel's[2] experiments with the anthrax bacillus gave similar results. He injected into the trachea of guinea pigs the vegetative and spore forms of the anthrax bacillus. When the organisms were introduced by means of a catheter passed through the larynx the animals remained well. Guinea pigs injected by means of a hypodermic needle thrust between the rings of the trachea died of septicemia. Snel pointed out that a locus occurred about the site of injection, from which the organisms entered the circulation.

A number of workers have succeeded in producing pneumonia in animals by intratracheal injection of various organisms. In most instances it has been necessary to force considerable amounts of culture well down into the trachea before positive results have been obtained. Wadsworth[3] was able under certain conditions to produce pneumonia in rabbits by the injection of 1 cc. of pneumococci between

[1] Arloing, Cornevin, and Thomas, cited by Kitt, T., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1912, iv, 819.

[2] Snel, J. J., *Z. Hyg. u. Infectionskrankh.*, 1902, xl, 103.

[3] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851.

the tracheal rings. It is of interest that in his first experiments the rabbits either developed septicemia without lung involvement or remained well. Kline and Winternitz[4] produced pneumonia in rabbits with the pneumococcus by intratracheal injection of large amounts of culture. They pointed out that the production of pneumonia depended on the introduction of the organisms into the alveoli. In order to accomplish this, it was necessary to insert a catheter as deeply into the bronchus as possible. The liquid culture was injected with considerable force.

During the past year the writer[5] has developed a simple and rapid method for introducing fluids into the trachea. A properly bent metal tube with a rounded end may be inserted by way of the mouth through the larynx. By attaching a syringe varying amounts of fluid may be injected. Aside from the extreme simplicity of the procedure, no apparent injury is produced. In addition, the rabbit is placed in the natural position so that it is necessary to exert only a slight pressure on the piston of the syringe to insure that the material reaches the lungs.

The pathogenicity of a number of organisms introduced in this manner has been tested. Members of the Pasteurella group associated with pneumonias in animals, such as the swine-plague bacillus, certain of the hemorrhagic septicemia organisms of bovine origin, and *Bacillus lepisepticus*, possess considerable pathogenicity when introduced beneath the skin or into the circulation. They rarely produce disease when introduced into the uninjured trachea. The animals usually develop a temperature reaction for a few hours, but fail to manifest further symptoms. The recent experiments of Stillman[6] are of considerable interest in this connection. He exposed mice to an atmosphere containing cultures of various organisms in the form of a fine mist. He was able to show that the organisms reached the lower respiratory tract. The pneumococcus usually disappeared from the lungs within a few hours. Hemolytic streptococci persisted for longer periods and gave rise to septicemia in many instances.

[4] Kline, B. S., and Winternitz, M. C., *J. Exp. Med.*, 1915, xxi, 304.
[5] Jones, F. S., *J. Exp. Med.*, 1923, xxxvii,789.
[6] Stillman, E. G., *J. Exp. Med.*, 1923, xxxviii, 117.

Organisms of the chicken-cholera group are usually extremely virulent for rabbits. The writer is in possession of a culture of *Bacillus avisepticus* which when introduced into the trachea in small quantities gives rise to a fatal septicemia. 1/100 cc. of a 24 hour bouillon culture suspended in 1 cc. of sterile bouillon injected into the trachea always gives rise to septicemia which terminates fatally in a little over 24 hours. With this amount of culture the average length of time between inoculation and death has been 28 hours, the minimum 17 hours and the maximum 37 hours.

It seemed possible that under certain conditions such an organism, which is capable of passing through the walls of the lower respiratory tract, might be made to localize within the lung. Ample evidence exists that other members of the hemorrhagic septicemia groups, *Bacillus lepisepticus*, *Bacillus bovisepticus*, and others, are associated with pneumonia in their respective hosts. In addition, it might be possible by experimentation to throw some light on the general problem of pneumonia. With these points in view a number of experiments were undertaken.

EXPERIMENTAL.

Rabbits from a stock which is relatively free of spontaneous respiratory disease were used throughout the experiments. Those of approximately the same age, breed, and weight (2,000 gm.) were employed. Intratracheal injections were made with the curved metal tube. Sufficient ether was given to insure complete relaxation. 1/100 cc. of a 24 hour bouillon culture suspended in 1 cc. of sterile bouillon was always used as the infecting dose. It has been stated that this dose always proved fatal; the initial experiments dealing with this point will be omitted, since protocols given later include animals which received only the infecting dose.

Smith and Moore[7] were able to vary by vaccination the type of disease produced by injection of highly virulent cultures of the swine-plague bacillus. Unvaccinated control rabbits rapidly succumbed to septicemia after subcutaneous inoculation with living cultures. Rabbits that had received four injections of killed culture were subsequently inoculated with live culture. They developed large

[7] Smith, T., and Moore, V. A., *U. S. Dept. Agric., Bureau of Animal Industry, 8th and 9th Ann. Rep.*, 1891–92, 45.

TABLE I.

The Protocols of Rabbits Immunized with Killed Culture by Various Routes and Subsequently Inoculated with Living Culture.

Rabbit 1. Vaccinated intraperitoneally.	Rabbit 2. Vaccinated intratracheally.
1922	*1922*
Dec. 28. 1 cc. of vaccine.	Dec. 27. 1 cc. of vaccine.
1923	*1923*
Jan. 2. 1 " " "	Jan. 2. 1 " " "
" 6. 1.5 " " "	" 6. 1.5 " " "
" 15. Agglutinins +++ 1:200.	" 15. Agglutinins + 1:100.
" 16. 1/100 cc. of living culture intratracheally.	" 16. 1/100 cc. of living culture intratracheally.
" 17. Blood culture negative. Animal normal.	" 17. Blood culture negative. Animal normal.
" 23. Agglutinins ++ 1:500.	" 23. Agglutinins + 1:500.
1/10 cc. of living culture intratracheally.	1/10 cc. of living culture intratracheally.
" 31. 1 " " " " "	" 31. 1/2 " " " " "
Feb. 7. 1/100 " " " " subcutaneously.	Agglutinins + 1:500.
" 9. Abscess.	Feb. 7. 1/100 cc. of living culture subcutaneously.
Animal recovered.	" 9. Abscess.
	Animal recovered.

Rabbit 3. Vaccinated subcutaneously.	Rabbit 4. Vaccinated intraperitoneally.
1923	*1923*
Jan. 29. 1 cc. of vaccine.	Jan. 29. 1 cc. of vaccine.
Feb. 3. 1 " " "	Feb. 3. 1.5 " " "
" 12. Agglutinins complete 1:50.	" 12. Agglutinins ++++ 1:100.
1/100 cc. of living culture intratracheally.	1/100 cc. of living culture subcutaneously.
Remained well.	" 13. Animal very sick.
	" 14. Large abscess. Animal very sick. Later the abscess ulcerated. Animal finally recovered.

Rabbit 5.
Unvaccinated control.*

1923	
Jan. 16.	1/100 cc. of living culture intratracheally.
" 17.	Blood culture positive.
	Died of septicemia 29 hrs. after inoculation.

* Normal rabbits injected subcutaneously with 1/100 cc. of bouillon culture succumbed within 48 hours.

abscesses about the site of inoculation and survived much longer than the controls.

The first phase of the problem consisted in establishing whether rabbits could be protected against the experimental respiratory infection with this strain of *Bacillus avisepticus*. In addition, it was essential to show whether the general infection could be limited to a local process about the site of inoculation. With these points in view two lots of rabbits were tested after various treatments. Inasmuch as the series are small and the results similar, a brief record of all rabbits is given in Table I.

The vaccines used throughout the experiments were prepared by suspending the surface growth from 24 hour agar slant cultures in small quantities of salt solution to which 0.5 per cent phenol had been added. The heavy suspensions were heated for 1 hour at 58–60°C. Before use sufficient sterile salt solution was added to reduce the turbidity to 2.5 with the Gates apparatus.

It is evident from the data given in Table I that a more or less complete immunity to a subsequent intratracheal infection may result from intraperitoneal, intratracheal, or subcutaneous vaccination with killed cultures. Although it is not known how little vaccine is required to produce complete resistance, in the case of Rabbit 3, 2 cc. of vaccine administered beneath the skin completely protected. Rabbits 1 and 2 were able to tolerate many times the lethal dose, provided however the living culture was introduced into the respiratory tract. Such was not the case when the organisms were injected beneath the skin, since the bacteria multiplied in the subcutaneous tissue and produced an abscess. It is evident that immunity to respiratory infection may be readily induced provided the integument is not seriously injured. Here the natural mechanism is not interfered with and the added resistance stimulated by the vaccine soon enables the individual to overcome the infection.

The experiment also parallels Smith and Moore's findings with the swine-plague bacillus that the type of disease could be changed from a rapidly fatal septicemia to a process local in character which spread by continuity.

As a basis for further experiments three points had been established: (1) 1/100 cc. of a 24 hour bouillon culture when introduced into the trachea of normal rabbits will always produce a rapidly fatal septi-

cemia. (2) Infection through the respiratory tract may be completely prevented by previously administering small doses of vaccine subcutaneously, intraperitoneally, or intratracheally. (3) By stimulating resistance the process may be changed from a septicemia to a local process beneath the skin.

On the basis of these facts, it was postulated that a partial protection or resistance somewhat greater than that possessed by the normal individual, but considerably less than that of complete protection, would, on intratracheal injection, tend to produce a localization within the lung. The later experiments resolved themselves into attempts, by raising the resistance slightly above the normal by means of vaccination with killed cultures, to change the disease from an acutely fatal septicemia to a less acute pneumonia.

Cecil and Steffen[8] were able to show that intratracheal injections of killed cultures rendered monkeys insusceptible to later intratracheal injections of living pneumococci. Among other experiments they attempted to produce immunity by spraying the vaccine into the pharynx. They were unable to establish immunity by this method. Such a method seemed desirable for experimenal purposes. Attempts were made to increase the resistance of rabbits to subsequent intratracheal injection by spraying killed cultures into the nostrils and throat.

Experiment 2.—As in the preceding experiments, rabbits averaging 2,000 gm. in weight were employed. Vaccine prepared in the usual manner but of greater turbidity (1.5) was sprayed into the nostrils of No. 6 and into the pharynx of No. 7. No. 8 served as a control. The results are given in Table II.

The experiment was repeated with the same results. It was not possible by means of sprays of vaccine to increase the resistance appreciably. The blood failed to show agglutinins throughout the experiment. Temperature reactions or leucocytosis did not occur following the administration of vaccine. Apparently insufficient antigen is absorbed to afford measurable protection against the subsequent intratracheal infection.

In the third series of experiments it was decided to inject subcutaneously a number of rabbits with 1 cc. of vaccine (turbidity 2.5)

[8] Cecil, R. L., and Steffen, G. I., *Pub. Health Rep., U. S. P. H.*, 1922, xxxvii, 2735.

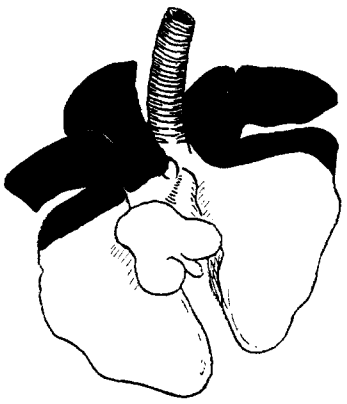
TABLE II.

Attempts to Increase the Resistance of Rabbits against Intratracheal Injection by Spraying Killed Cultures on the Mucous Membrane of the Nostrils and Pharynx.

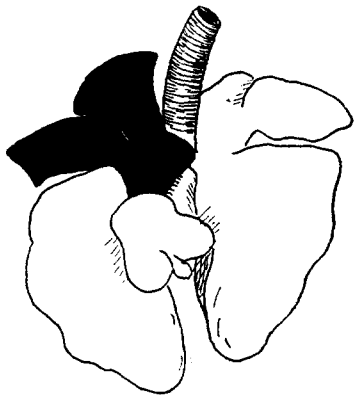
Rabbit 6. Vaccine sprayed into the nostrils.	Rabbit 7. Vaccine sprayed into the pharynx.	Rabbit 8 Control.
1923	*1923*	
Mar. 26. Nasal mucosa sprayed with vaccine.	Mar. 26. Throat sprayed with vaccine.	
" 27. " " " " "	" 27. " " " "	
" 28. " " " " "	" 28. " " " "	
Apr. 2. " " " " "	Apr. 2. " " " "	
" 3. " " " " "	" 3. " " " "	
" 4. " " " " "	" 4. " " " "	
" 11. 1/100 cc. of living culture intratracheally.	" 11. 1/100 cc. of living culture intratracheally.	Apr. 11. 1/100 cc. of living culture intratracheally.
" 12. Died (36 hrs. after injection). Septicemia.	" 12. Died (22 hrs. after injection). Septicemia. Fibrinous pericarditis. Pleuritis with effusion.	" 12. Died (23 hrs. after injection). Septicemia.

and after definite intervals to inject into the trachea 1/100 cc. of living culture.

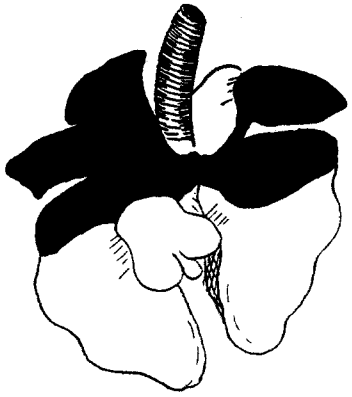
In one experiment three rabbits were vaccinated and 2, 4, and 7 days later injected intratracheally with 1/100 cc. of living culture. An unvaccinated rabbit



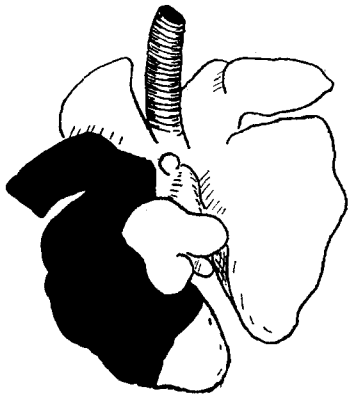
TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIG. 3.



TEXT-FIG. 4.

Consolidation is shown in black.

TEXT-FIG. 1. The ventral aspect of the lungs of Rabbit 9.
TEXT-FIG. 2. The ventral aspect of the lungs of Rabbit 10.
TEXT-FIG. 3. The ventral aspect of the lungs of Rabbit 12.
TEXT-FIG. 4. The ventral aspect of the lungs of Rabbit 16.

served as control. In another experiment three rabbits were vaccinated and infected 3, 5, and 6 days later. A fourth rabbit served as a control. Inasmuch

as some of the clinical evidence is of considerable interest an outline of temperature reactions, the results of blood cultures, and in certain instances the findings after pleural puncture are appended.

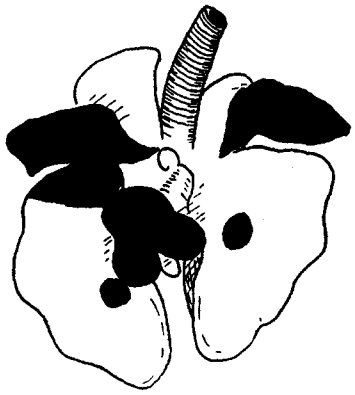
Blood cultures were always made from the peripheral circulation. 1 cc. of blood may be readily drawn from the ear artery or marginal vein with a 2 cc. Luer syringe, provided the ear is warmed to increase the peripheral circulation. The median artery is better for the purpose, since the pressure may be sufficient to fill the syringe. The advantage of the use of the vessels of the peripheral circulation over the cardiac method for blood culture in diseases involving the thoracic organs is obvious, especially when the organisms are numerous in the exudates covering the pericardium and pleura.

Rabbit 9.—(Text-figs. 1 and 7, *a*.) Vaccinated 2 days before infection. Septicemia developed during the first 24 hours. The rabbit died 56 hours after infection. Autopsy revealed severe pleuritis and pericarditis. The pleural cavity contained 5 cc. of serous fluid. The ventral and cephalic lobes and adjacent portions of the caudal lobes of both lungs were completely consolidated. The solid portions were deep red in color. The air-containing portions of the lung were congested and edematous. Microscopically the air cells of the involved lobe were filled with red cells, red cells and fibrin, and a few round cells. The vascular system was engorged. The epithelium of the air tubes was degenerated and often the more superficial layers had exfoliated.

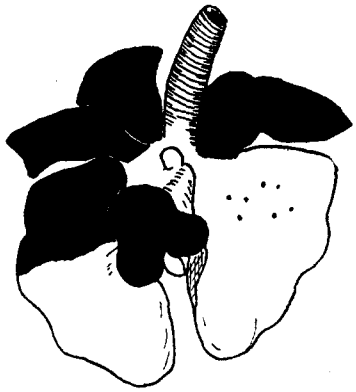
Rabbit 10.—(Text-figs. 2 and 7, *b*.) Vaccinated 4 days before infection. Septicemia did not develop during the first 24 hours. The animal reacted severely and died 69 hours after the intratracheal injection. On autopsy 4 cc. of turbid exudate were found in the pleural cavity. There was a marked fibrinous pericarditis and pleuritis. The ventral and cephalic lobes of the right lung were much enlarged. On section they were firm and varied from reddish gray to red in color. The cephalic lobe of the left lung was shrunken and deep red. The other lobes were congested and edematous. Microscopically the process in the cephalic lobe was that of intense pleuritis. Leucocytes had invaded the pleura. Hemorrhages had occurred in portions of the sections beneath the pleura. In the center of the lobe many of the alveoli were filled with red cells. The vascular engorgement was intense. The ventral lobe manifested two well defined types of pneumonia rather sharply differentiated. In one portion the exudate which filled the alveoli was largely fibrinous; in the remainder of the lobe the air spaces were packed with leucocytes. Severe degeneration of the epithelium of the air tubes was constant in both sections.

Rabbit 11.—(Text-fig. 7, *c*.) Vaccinated 7 days before infection. The animal showed no particular reaction to the usual intratracheal dose of culture. 2 days after the first infection the rabbit was again injected with a larger amount of culture. A characteristic temperature reaction developed for a few hours after the second injection. It was of short duration. 8 days after the last injection the rabbit was killed. Autopsy failed to reveal abnormalities.

Rabbit 12.—(Text-figs. 3 and 8.) Vaccinated 3 days before infection. Septicemia was not manifested during the first 24 hours. The organisms were present in the blood after this time. The rabbit died 88 hours after infection. Postmortem examination revealed 6 cc. of turbid, serous exudate in the pleural cavity. The usual fibrinous pericarditis and pleuritis were encountered. Extensive

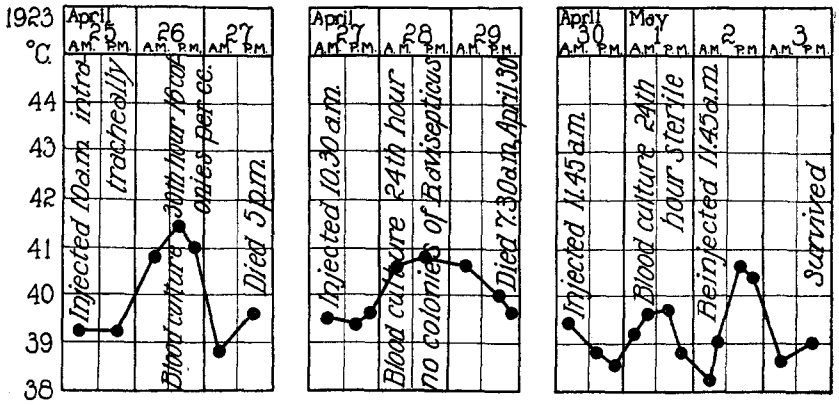


TEXT-FIG. 5.



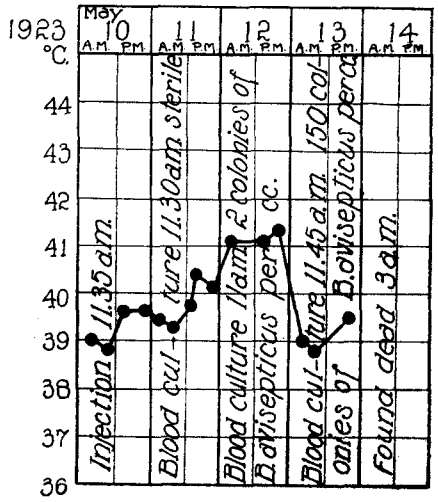
TEXT-FIG. 6.

TEXT-FIG. 5. The ventral aspect of the lungs of Rabbit 20.
TEXT-FIG. 6. The ventral aspect of the lungs of Rabbit 21.

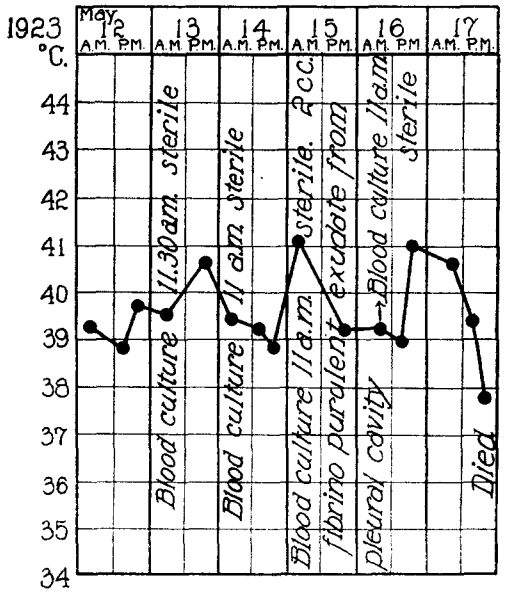


a *b* *c*

TEXT-FIG. 7, *a* to *c*. Temperature curves. (*a*) Rabbit 9, vaccinated 2 days before intratracheal injection. (*b*) Rabbit 10, vaccinated 4 days before intratracheal injection. (*c*) Rabbit 11, vaccinated 7 days before intratracheal injection.

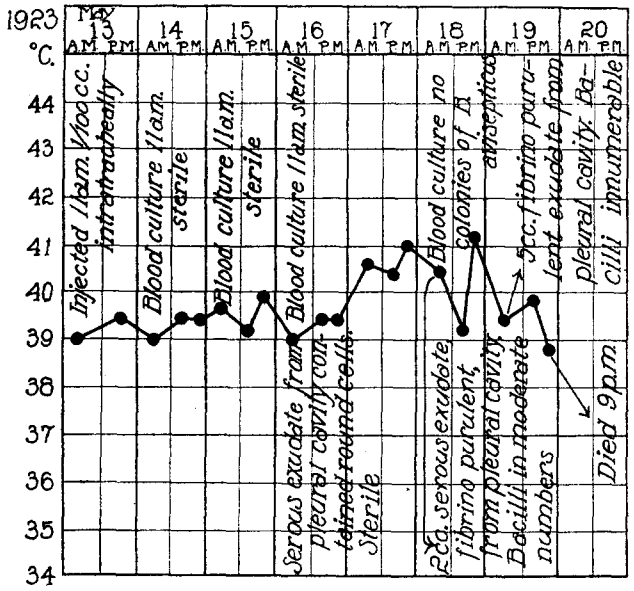


TEXT-FIG. 8. Temperature curve of Rabbit 12, vaccinated 3 days before intratracheal injection.



TEXT-FIG. 9. Temperature curve of Rabbit 13, vaccinated 5 days before intratracheal injection.

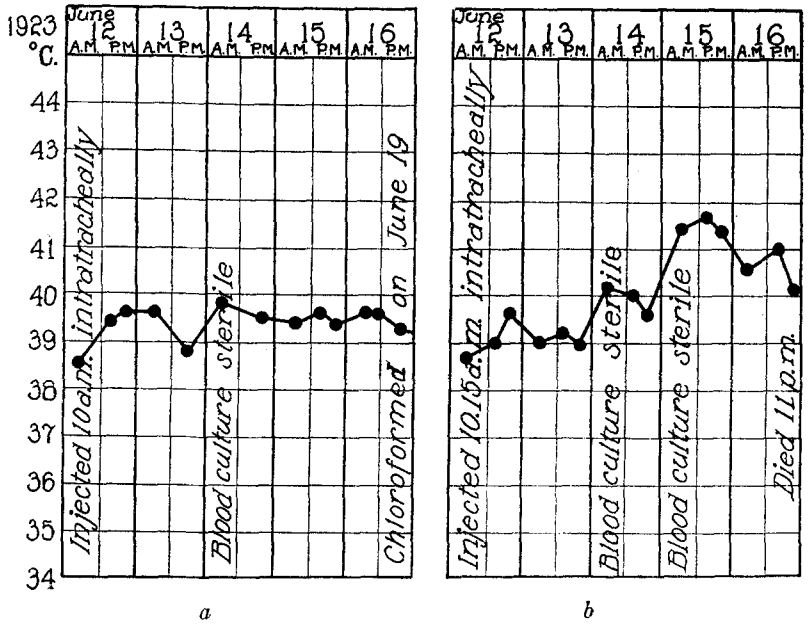
consolidations, varying in color from bright red to gray, of the more dependent portions of both lungs were observed. On the whole the gray type of consolidation was more pronounced in the left lung. Microscopically the character of the pneumonia varied in different lobes and within sections of the same lobes. The more dependent portions of the left ventral lobe revealed very little of the normal lung structure. The whole consisted of a mass of necrotic leucocytes, evidently the remains of the alveolar exudate. In other portions of the lobe the alveoli were filled with fibrin. Here the vascular engorgement was intense. In other lobes the air spaces were packed with red cells. All sections showed severe degeneration or necrosis of the epithelium of the air tubes. Often fibrinous exudate filled the lumen of the air tubes. In many instances the peribronchial blood vessels were engorged and the peribronchial lymph channels were occluded with fibrin.



TEXT-FIG. 10. Temperature curve of Rabbit 14, vaccinated 6 days before intratracheal injection.

Rabbit 13.—(Text-fig. 9.) Vaccinated 5 days before infection. The temperature reaction was irregular. The blood was sterile during the first 4 days of illness. Evidences of empyema were detected on the 3rd day when a serous exudate containing polymorphs and the bacilli was aspirated from the pleural cavity. The animal died 5½ days after inoculation. At autopsy, 5.5 cc. of purulent exudate were obtained from the chest cavity. A heavy, yellowish white, fibrinopurulent exudate was found over the pericardium, the entire right lung, and portions of the left lung. The exudate peeled off with gentle traction, leaving

a roughened surface. The cephalic lobe of the right lung was collapsed. The other lobes were congested and edematous. Microscopically the lesions were essentially those of purulent pleuritis. The endothelium was overlaid with leucocytes. Leucocytes had invaded the subpleural tissues. The alveoli immediately adjacent to the pleura were overrun with leucocytes. Elsewhere the air spaces contained only a little finely granular material or a few round cells. The mucosa of the bronchi and bronchioles revealed only slight degenerative changes. A moderate engorgement of the interalveolar capillaries was also observed.



a *b*

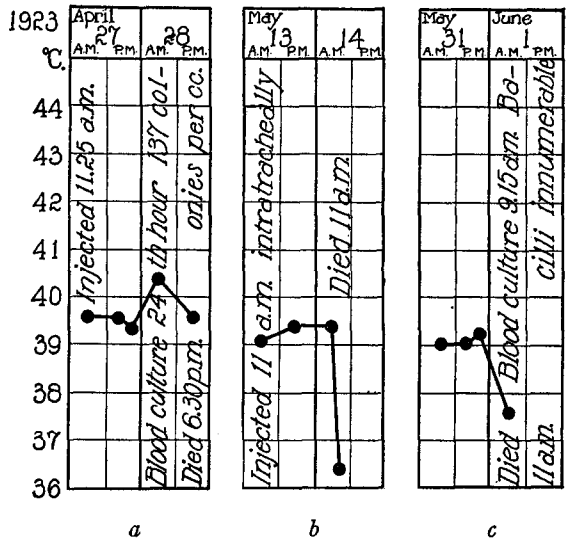
TEXT-FIG. 11, *a* and *b*. Temperature curves. (*a*) Rabbit 15. (*b*) Rabbit 16. Each received two injections of vaccine at 3 day intervals. Intratracheal injection 3 days after second vaccination.

Rabbit 14.—(Text-fig. 10.) Vaccinated 6 days before infection. The onset of symptoms was delayed. The first rise in temperature was noted on the 4th day. The blood remained free from *B. avisepticus* for 5 days. On the 5th and 6th days considerable amounts of purulent exudate were obtained by pleural puncture. The animal succumbed 6½ days after intratracheal injection. The autopsy findings were similar to those encountered in Rabbit 13. In addition there was a recent, deep red consolidation of the ventral lobe of the right lung. Microscopic examination showed a hemorrhage of considerable proportion in this lobe. Pleuritis was marked in all sections. Leucocytes had infiltrated the

pleura and adjoining portions of the lung. The organisms appeared in cloud-like masses in the pleura and subpleural structures. The alveoli and air tubes were not particularly involved.

Rabbit 15.—(Text-fig. 11, *a*.) Vaccinated twice at 3 day intervals. Injected intratracheally 3 days after second vaccination. The animal remained well. On three occasions the pleural cavity was tapped and a little clear, straw-colored fluid obtained. Inoculations of media from this liquid remained sterile. The animal was chloroformed 7 days after intratracheal injection. The lungs and chest cavity were normal.

Rabbit 16.—(Text-figs. 4 and 11, *b*.) Treated the same as Rabbit 15. Reacted on the 2nd day after infection. The blood remained sterile during the first 4 days.



a *b* *c*

TEXT-FIG. 12, *a* to *c*. Temperature curves of unvaccinated controls. (*a*) Rabbit 17. (*b*) Rabbit 18. (*c*) Rabbit 19.

The pleural fluid contained a few leucocytes on the 2nd day, but was clear on the 4th day. Death intervened 4½ days after intratracheal injection. Only a few drops of fluid were present in the pleural cavity on autopsy. The pericardium was free from exudate. The pleura overlying the right lung contained a few patches of grayish white exudate. The consolidation was confined to the ventral lobe and the cephalic four-fifths of the caudal lobe. On section the involved portions varied from reddish gray to gray in color. Sections examined microscopically revealed the oldest lesions in the center of the ventral lobe. Here the consolidation was essentially gray.

Rabbits 17, 18, and 19.—(Text-fig. 12, *a*, *b*, and *c*.) All served as controls. Each received an intratracheal injection of 1/100 cc. of a 24 hour bouillon culture. All developed septicemia and died without particular lung involvements.

It was hoped by further experimentation along similar lines that the experimental disease could be still further varied by increasing the amount of vaccine or the time interval between vaccination and intratracheal infection. On the whole the results obtained were similar to those recorded. In a number of instances more diffuse pneumonias were produced, but even slight departures from the procedures previously described may so enhance the resistance that the animal fails to become infected. As additional evidence a brief record of another experiment is given, which illustrates about the maximum lung involvement obtained in any of the animals.

Rabbits 20 and 21.—(Text-figs. 5 and 6.) Each received a subcutaneous injection of vaccine. Rabbit 20 was injected intratracheally with living culture 7 days later. Rabbit 21 received the intratracheal injection 10 days later. Both reacted severely and succumbed 73 and 74 hours after infection. Necropsy of Rabbit 20 showed consolidation of the ventral and azygos lobes and the cephalic one-fourth of the caudal lobe of the right lung. In addition two round, grayish white consolidated areas were observed in the center of the caudal lobes. The consolidation was more pronounced in the case of Rabbit 21. The cephalic and ventral lobes of both lungs and the azygos were completely hepatized. The cephalic half of the right caudal lobe was also consolidated.

All rabbits were bled before vaccination and just before intratracheal injection. The results of the agglutination tests were not definite in serum dilutions as low as 1:1. In certain instances the serum was tested for opsonin. The serum of rabbits vaccinated 2, 3, or 4 days before infection failed to show any appreciable increase in opsonin. Tests of the serum from rabbits vaccinated 7 and 10 days prior to bleeding gave more definite results. In these observations about 60 per cent of the leucocytes contained the organisms. The tests were made by adding equal parts of a moderately heavy salt solution suspension of the organism to an equal part of the rabbit serum. After 2 hours' incubation a suspension of leucocytes obtained from a guinea pig was added. In addition a little fresh guinea pig serum was employed as complement. The whole was mixed and incubated for 2 hours. Films were made from the sediment.

DISCUSSION.

The results obtained by Wadsworth for the pneumococcus agree with those obtained in the experiments cited. Wadsworth injected rabbits with the pneumococcus by means of a needle introduced through the tracheal wall. He states that the inoculations led to variable results, either the animals were unaffected or they developed a rapidly fatal septicemia. In later experiments he first immunized rabbits with various products of the pneumococcus and in certain instances employed living cultures. After definite periods the animals were injected through the walls of the trachea with a highly virulent culture. Only a few manifested acute symptoms, but when killed 3 and 4 days after the infection pneumonia was found on autopsy.

In the writer's series of observations the lesions have been more pronounced. The rabbit apparently possesses little natural resistance for certain of the Pasteurella, so that at will one may so stimulate the resistance artificially that a variety of processes may be encountered. The normal rabbit reacts characteristically. The organism is extremely invasive and makes its way into the circulation from the lungs. The process is radically changed by the acceleration of the animal's resistance. The invasiveness of the organism is checked for a time and the process remains local. The principal lesion is that of fibrinous pneumonia with severe involvement of the bronchial epithelium. Rabbits whose resistance has been increased to a greater degree live longer. In these cases the alveolar exudate is fibrinopurulent or purulent. The epithelium of the air passages is severely involved. Perhaps during localization the protective substances are consumed and the organisms gain access to the circulation. From the results obtained one is inclined to believe that in animals possessing only a slight resistance the localizations occur close to the point of entry and are manifested as consolidations of the more dependent lobes. In animals of greater resistance the organisms evidently do not cause the pronounced reaction at the point of entry. The bacilli, however, to judge by the microscopic processes, invade the lymph channels and make their way to the pleura, but even in such conditions the process is essentially local. The blood remains sterile although the organisms exist in the pleural exudate in large numbers. Attempts to produce a long standing empyema or a more acute

pneumonia with resolution have failed. The relatively great invasiveness of the organism, its power to produce large necrotic foci, and the rather poor general resistance of the rabbit may offer a partial explanation.

To what degree resistance under natural conditions has so modified the type of disease resulting from respiratory infection can only be surmised. The experimental evidence is suggestive that organisms capable of producing a rapidly fatal septicemia may be made to localize within the chest cavity for definite periods and leads one to believe that organisms now associated with pneumonia are encountering a more resistant host which localizes their greatest activities at or near the site of infection. Certain of Blake and Cecil's[9] findings in their study of experimental pneumonia in monkeys suggest that even in such a highly susceptible species as the monkey considerable variations in natural resistance occur. Among a larger series of monkeys which were inoculated through the walls of the trachea with a Type I pneumococcus, two died of septicemia without particular lung involvement. A few others were entirely resistant, but a great proportion developed pneumonia. It is well recognized that certain of the Pasteurella do under natural conditions give rise to septicemia and are frequently associated with pneumonia. In the instance of bubonic plague, two well recognized types of disease exist, the septicemic form in which the organisms are introduced beneath the skin and the pneumonic type. It is probable that in the latter type the respiratory tract is the portal of entry and the organisms are localized for a time within the lung.

The distribution of the experimental pneumonia is similar to that encountered in natural infections in animals with organisms of this group. The infection apparently involves first the more dependent portions of the lungs, such as the ventral and cephalic lobes, and spreads from them. The pathology is essentially the same in both spontaneous and experimental disease. The consolidations vary from red to gray. Pleuritis and edema are also common to both.

One or two other points should be emphasized. The protocols in the first experiment indicate that the animals by vaccination with killed cultures may be made completely resistant to a subsequent inoculation into the trachea. It is to be inferred that the infecting

[9] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

doses in the experiments contain many more organisms than are introduced into the lower respiratory tract under natural conditions. That this immunity does not necessarily imply complete resistance when the organisms are artificially introduced beneath the integument, as in the case of subcutaneous injections, is also recorded in the protocols. It seems reasonable to presume that under natural conditions animals may be made greatly resistant to natural respiratory infections by vaccination. Protective substances are evidently formed with considerable rapidity, since the rabbit vaccinated only 2 days before infection proved more resistant than the unvaccinated control. The extreme delicacy of the balance between infection and resistance in experimental respiratory disease is illustrated in the instance of Rabbit 11 vaccinated 7 days before intratracheal injection. That the animal possessed sufficient resistance to prevent active infection is shown by subsequent injection of a larger dose of culture.

SUMMARY.

An organism of the Pasteurella group (*Pasteurella avicida*) has been shown to possess considerable pathogenicity for rabbits when injected into the trachea. 1 cc. of bouillon containing 1/100 cc. of a 24 hour bouillon culture introduced by means of a curved metal tube passed through the larynx gives rise to a rapidly fatal septicemia in which the lungs are not particularly involved. Rabbits may be made completely resistant to this intratracheal infection by means of intratracheal, intraperitoneal, or subcutaneous injections of killed cultures. Vaccination and subsequent injection of living culture beneath the skin resulted in extensive abscess formation about the site of inoculation. Vaccines sprayed on the mucous membranes of the nose and throat failed to raise appreciably the resistance against subsequent intratracheal injection.

Rabbits whose resistance has been stimulated within narrow limits by a small dose of vaccine beneath the skin become more resistant to the experimental respiratory infection. The character of the disease is changed from a rapidly fatal septicemia without lung involvement to a more prolonged pneumonia. The blood remains sterile for varying periods. Consolidations of the more dependent lobes occur with considerable regularity. In two instances a characteristic empyema developed in the course of the experiments.