# A STUDY OF THE EFFECT OF SENSITIZATION ON THE DEVELOPMENT OF THE LESIONS OF EXPERIMENTAL PNEUMONIA IN THE RABBIT.*

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The analogies between the phenomenon of anaphylaxis in the laboratory experiment and the development and course of spontaneous pneumonia in man are, perhaps, more striking than those shown by any other infection. In pneumonia, moreover, conditions of hypersusceptibility might conceivably be one of the factors or the dominant factor, not only in the systemic febrile reaction but also in the localized and unique lung reaction such as is found only in man. The following study records a series of experiments in which an attempt was made to ascertain whether any definite and constant relationship between conditions of hypersusceptibility and the development of pneumococcus lesions of the lobar type in animals could be established.

Wadsworth,[1] in 1904,—before the phenomenon of anaphylaxis had been associated with infections,—stated that typical lobar lesions developed in a considerable number of partially immunized rabbits after tracheal injection of small quantities of pneumococcus culture. These lesions, absent when normal rabbits had been injected, were also much less extensive or lacking when highly immunized animals were used. Viewed from our present knowledge of anaphylaxis the acute lung reaction might possibly have been due to a state of hypersusceptibility unintentionally caused while attempting to induce a slight immunity as a predisposing condition to the development of the lobar type of lesion.

Typical lobar pneumonia has been successfully incited in the dog by Lamar and Meltzer[2] and others by intrabronchial insufflation of large quantities of pneumococcus culture. In rabbits the reaction has been induced experimentally with difficulty, as, owing to their extreme susceptibility to pneumococcus infection, a general bacteremia without characteristic lung involvement is usual.

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[1] Wadsworth, A., *Am. Jour. Med. Sc.*, 1904, cxxvii, 851.

[2] Lamar, R. V., and Meltzer, S. J., *Proc. Soc. Exper. Biol. and Med.*, 1909–10, vii, 102; *Jour. Exper. Med.*, 1912, xv, 133.

Rasquin[3] stated that tracheal injection of pneumococci gave typical pneumonias in only 7 to 8 per cent. of his rabbits, but when serum from a dog immunized to rabbit serum was added to the culture, 96 per cent. developed typical catarrhal lesions. Winternitz and Hirschfelder,[4] however, by tracheal insufflation of large amounts—4 to 5 c.c.—of concentrated culture were able to obtain extensive fibrinous involvement in a large proportion of fairly small rabbits.

Friedberger[5] and later Schlecht and Schwenker[6] reported a cellular bronchopneumonia produced by spraying horse serum into the tracheas of sensitized guinea pigs. Anaphylactic shock and pneumonic changes in the lungs of sensitized guinea pigs following intratracheal injection of small amounts of horse serum also have been reported by Ishioka.[7]

While the rabbit has proved more resistant to sensitization than the guinea pig, and the results generally less constant and less marked, definite anaphylactic reactions with bacterial proteins have been obtained. Neufeld and Dold[8] and Rosenow[9] incited acute toxic symptoms in the rabbit similar to those of anaphylaxis by using a pneumococcus culture and normal or immune serum-complement mixture, such as Friedberger had obtained with other bacteria. The toxic split products of pneumococci obtained by Vaughan, the autolysates of Rosenow, and the lytic bile extracts of Cole have all caused acute toxic reactions in the rabbit. Apparently in none of the experiments with bacteria has the toxic dose been given tracheally, nor has the local reaction called forth by it been studied.

In the present study two hypotheses have been kept in mind: one which would be strengthened by negative results, that in lobar pneumonia, notwithstanding its extraordinarily rapid and exceptional course, the progress is still a definitely progressive one and not dependent on conditions such as give rise to the sudden and eruptive phenomena of anaphylaxis; the second, suggested by the abrupt onset and sudden termination of the disease, by certain local reactions, and by the not infrequent clinical history of previous infection suggestive of sensitization, that its unique and striking features are of an anaphylactic nature. In the latter case pneumonia may be considered either as mainly a bacteremic or toxemic condition, the lung lesions being a local and comparatively unimportant feature of the disease as a whole, and the anaphylactic reaction a general and systemic phenomenon. Or, the lungs with their system of tubes

[3] Rasquin, E., *Arch. de méd. expér. et d'anat. path.*, 1910, xxii, 804.
[4] Winternitz, M. C., and Hirschfelder, A. D., *Jour. Exper. Med.*, 1913, xvii, 657.
[5] Friedberger, E., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 481.
[6] Schlecht, H., and Schwenker, G., *Deutsch. Arch. f. klin. Med.*, 1912, cviii, 405.
[7] Ishioka, S., *Deutsch. Arch. f. klin. Med.*, 1912, cvii, 500.
[8] Neufeld, F., and Dold, H., *Berl. klin. Wchnschr.*, 1911, xlviii, 55.
[9] Rosenow, E. C., *Jour. Infect. Dis.*, 1911, ix, 190.

and air spaces may provide a particularly favorable environment and may act as a coöperating test-tube in which the cell complex furnishes the antibody, and the invading pneumococci the toxic agent. The resulting toxic substances, acting locally, might then incite exudative reactions in the lung, and on being absorbed give rise to the characteristic febrile reaction, the bacteremia being a secondary and transitory phenomenon.

*Methods.*—The pneumococcus organisms used were A, a virulent strain (the Neufeld culture), obtained through the kindness of Dr. Cole of the Hospital of The Rockefeller Institute; B, one of moderate virulence, but which had given evidence of unusual toxicity; and AA, an avirulent culture of A, attenuated by long continued growth on agar. Derivatives of eighteen to twenty-four hour meat infusion broth cultures were used for sensitization. The culture was centrifugalized and the supernatant fluid passed through a Berkefeld and then a Pasteur filter. The sediment was washed twice, suspended in salt solution equal to one-half or one-quarter of the original culture, and heated for thirty minutes at 52° to 55° C. Usually 0.1, 0.5, 7.5, and 15 cubic centimeters of filtrate or dead cell suspension were given intravenously to groups of rabbits. Two weeks later one cubic centimeter of eighteen to twenty-four hour live broth cultures was given tracheally to the rabbits. The same or a larger volume of concentrated suspension of living pneumococci cultivated aerobically in broth, or anaerobically under oil, or in large Petri dishes, was later used in a second series of rabbits. The effect of repeated sensitizing or toxic doses was also tested.

In studying the reactions incited by tracheal injection of serum and culture, mixtures were made of one cubic centimeter of live broth culture, and 0.1 or 0.5 of a cubic centimeter of sera from normal rabbits, or from animals immunized with pneumococcus filtrates, or with dead cells. These mixtures were usually injected immediately, and after incubation at 37° C. for periods of one to four hours.

The rabbits were etherized lightly and at the time of the tracheal injection tilted so that the natural course of the fluid would be into the left lung. Temperatures were taken before tracheal injection and once or twice a day afterward. If not already dead, the ani-

mals, with few exceptions, were killed at the end of forty-eight hours. At autopsy smears were made from the heart's blood and cultures from the pleural cavity, heart, liver, spleen, and trachea. Paraffin sections were made of all lungs, which had been previously distended and preserved in 95 per cent. alcohol or 10 per cent. formalin.

In the first series of experiments preliminary intravenous injection of culture filtrates or dead cells for purposes of sensitization was followed after an interval of two weeks by tracheal injection of living organisms.

### SENSITIZATION WITH CULTURE FILTRATES.

*Experiment 1.*—A series of 20 rabbits received intravenously 0.1, 0.5, 7.5, or 15 c.c. of culture filtrate of the attenuated culture AA. Two weeks later 1 c.c. of a living broth culture of the same avirulent organisms was injected tracheally. None developed symptoms of anaphylaxis and all were killed after 48 hours.

While there was considerable variation in the lung reaction, in no instance was the lobar type of lesion approached, nor was there any apparent connection between the amount of the preliminary inoculation and the extent and nature of the reaction.

*Experiment 2.*—Similar preliminary treatment of 8 rabbits with filtrates of the same avirulent organisms, but with the virulent culture substituted in the tracheal injection, resulted in even less lung involvement.

*Experiment 3.*—In a third series 6 rabbits received both sensitizing and tracheal injections of the moderately virulent, though highly toxic strain B. The febrile reaction was acute. Only one rabbit died within 48 hours, although the lung involvement was much more diffuse and extensive than in the other series. The reaction, however, was apparently independent of the amount of filtrate previously received, and the unsensitized controls developed similar though, on the whole, less marked reactions.

*Experiment 4.*—In striking contrast was the almost complete absence of diffuse exudative lesions in the series of 16 rabbits receiving filtrates of the virulent strain A followed by tracheal injection of live organisms of the same virulent strain. The animals given the larger sensitizing doses had acquired considerable immunity, as they were still alive after 48 hours, whereas those sensitized with similar doses of the avirulent strain in experiment 2 died.

### SENSITIZATION WITH DEAD PNEUMOCOCCUS CELLS.

*Experiment 5.*—19 rabbits which had received 0.1, 0.5, 7.5, and 15 c.c. of a killed suspension of the avirulent organisms AA were injected tracheally with 1 c.c. of a live broth culture of the same avirulent culture.

While there was more congestion and somewhat more lung involvement than in the corresponding filtrate series, the reactions did not differ materially in the different groups.

*Experiment 6.*—Eight additional rabbits which had been treated with the same dead cell suspension of the avirulent culture, but injected tracheally with the virulent organisms of the same strain, failed to develop marked exudative lesions. Treatment with large doses of dead cells had apparently a stronger protective action than similar treatment with filtrates of the avirulent culture, as two rabbits in this series were alive after forty-eight hours.

*Experiment 7.*—The reaction incited by the moderately virulent strain B, when given in both sensitizing and tracheal injections, was studied in 12 rabbits. The majority developed extensive exudative lesions in which areas of hemorrhage and necrosis were quite numerous. In four unsensitized controls, similar, but on the whole less extensive, areas of consolidation were found. The lesions, while more marked, closely resembled those in the corresponding filtrate series.

*Experiment 8.*—In this experiment in which sensitization with dead cells of the virulent culture was followed by tracheal injection of the same strain in the virulent state, 4 out of 14 rabbits developed extensive exudative lesions, in which fibrin and polymorphonuclear leucocytes were abundant. These lesions, however, were not confined to rabbits which had received the same sensitizing doses. While the two untreated controls died in 24 hours without marked lesions, the protective action of the sensitizing injections was shown by the survival for at least 48 hours of a number of the treated rabbits which had received the larger doses.[10]

In the following series concentrated cultures and culture material were used under similar conditions of experiment.

*Experiment 9.*—Of 16 rabbits treated with the usual concentration of dead cells, 4 received intravenous, and 12 tracheal injections of a concentrated suspension of living virulent organisms grown on agar. None showed anaphylactic symptoms. The lung reaction when present resembled that usually associated with foreign body pneumonias.

*Experiment 10.*—17 rabbits which had already received sensitizing and toxic injections were reinjected tracheally with a concentrated suspension of pneumococci grown in broth anaerobically under oil. No extensive lesions developed.

*Experiment 11.*—8 rabbits received single or repeated sensitizing injections of dead cells followed by intravenous injections of a concentrated suspension grown on agar. In one rabbit which had received a single sensitizing injection of 5 c.c., the toxic dose of 2 c.c. was immediately followed by symptoms resembling marked anaphylactic shock followed by complete recovery in about 30 minutes. The lungs at autopsy, 48 hours later, showed practically no involvement. In another series of 7 animals, one rabbit sensitized with 5 c.c. of concentrated dead cells and reinjected with 3 c.c. of a concentrated suspension of live organisms grown on agar died in less than 5 minutes, with symptoms resem-

[10] Additional sensitized rabbits in this or other series died in the latter part of the interval before tracheal injection; a number died of an infection present at the time, but less fatal to normal rabbits, suggesting that the animals, while they had acquired more tolerance to the pneumococcus, were so injured that they had become more susceptible to other infections.

bling anaphylaxis. No unusual reaction was found in the lungs on microscopical examination.

*Experiment 12.*—For the purpose of comparison 12 rabbits were sensitized with repeated injections of horse serum and reinjected at intervals intravenously or tracheally with the same serum. Anaphylactic shock developed in most of the animals receiving the toxic injection intravenously. Of those injected tracheally one was prostrated immediately after the injection, but recovered in half an hour. Killed 48 hours after the last tracheal injection. The sensitized rabbits' lungs showed possibly more peribronchial reaction than the unsensitized controls which had received tracheal injection, but the difference was not striking and no diffuse lesions of the lobar type were found.

In the preceding experiments active sensitization with filtrates or dead cells of strain A in its highly virulent or avirulent state followed by tracheal injection of live virulent or attenuated cultures of the same race did not result in a definitely increased lung involvement. When a second strain B, of much less virulence, was used, marked exudative lesions, varying to some extent, developed in the rabbits previously sensitized with filtrates or dead cells of the same strain, but the untreated controls also developed similar though possibly less extensive reactions. Since these experiments with active sensitization failed to bring out any very definite relationship between sensitization and the character of the lung lesions, the effect of passive sensitization by means of tracheal injection of different sera and culture mixtures was next studied.

### TRACHEAL INJECTION OF MIXTURES OF SERUM AND CULTURE.

*Experiment 13.*—Mixtures of 0.5 c.c. of fresh pooled normal rabbit sera and 1 c.c. of living attenuated culture, freshly mixed or incubated, were given tracheally to 8 rabbits. The animals almost at once developed marked signs of discomfort, such as restlessness, dyspnea, and prostration, their temperatures falling at least 3.2° F. The control rabbit, however, receiving 1 c.c. of culture diluted with 0.5 c.c. of sterile broth developed similar, though less marked, symptoms. All recovered in half an hour. The control and the rabbit receiving the mixture incubated for over 3 hours died in about 48 hours. The seven others died within 4 hours of each other in sudden and violent paroxysms, about 24 hours after the tracheal injection. Pneumococci were found in all cultures. A small rabbit injected intravenously with 1 c.c. of a transfer from the culture used in this experiment was unaffected.

The control's lungs showed some peribronchial infiltration. Two rabbits which had received serum mixtures developed extensive, two less marked diffuse fibrinous lesions. Repetition of the experiment failed to incite unusual symptoms, though in the lung of several rabbits small lesions of a fibrinous character were found.

*Experiment 14.*—Similarly mixtures of normal rabbit sera and virulent living culture were given tracheally to 21 rabbits. No immediate symptoms developed, but a number died in acute paroxysms 24 hours after tracheal injection. In the first series of these rabbits receiving a mixture containing 0.5 serum, which had stood at room temperature for about 2½ hours, one rabbit developed a typical fibrinous lobar pneumonia, the other smaller diffuse lesions. In later series the proportion of rabbits developing diffuse and fairly extensive lung involvement was, however, much smaller. Incubation of the mixtures for 2 to 3 hours appeared to favor a slightly increased lung reaction.

*Experiment 15.*—Of 12 rabbits receiving mixtures of avirulent living culture and sera from rabbits immunized with culture filtrates of the virulent strain, none showed immediate symptoms, but 7 died within 24 hours of injection—the majority in violent convulsions. The control which had received culture without serum died while not under observation in less than 48 hours. While four serum mixture rabbits developed considerable diffuse fibrinous involvement, the remainder, including the control, showed little or no exudative reaction.

*Experiment 16.*—The effect of virulent cultures and sera from rabbits immunized with filtrates of the same virulent strain A was tested on 26 rabbits. Of four receiving mixtures which had stood at room temperature 2 to 3 hours and in which the sera was three weeks old, two developed typical fibrinous lobar involvement. In the other series one rabbit showed extensive, and quite a number smaller diffuse fibrinous lesions. While the animals receiving the larger amount of sera possibly showed more lung reaction, the period of incubation apparently exerted little or no effect in this experiment.

*Experiment 17.*—The experiment was repeated, 11 rabbits receiving attenuated cultures and sera from animals immunized to dead pneumococcus cells. As before, the mixtures were incubated for varying periods. All the rabbits were still alive at the end of 48 hours. The lung reaction was distinctly less than in the corresponding normal or filtrate sera and avirulent culture experiments.

*Experiment 18.*—A series of 22 rabbits was given similar mixtures of virulent culture and sera from rabbits immunized to dead cells. The sera would seem to have exerted a certain amount of protective action, as over half of the rabbits were alive at the end of 48 hours. None developed extensive lesions.

The effect of intravenous inoculation of normal and immune sera immediately before tracheal injection of live culture was tested in the following experiment.

*Experiment 19.*—11 rabbits were inoculated intravenously with 0.1 or 0.5 c.c. of normal rabbit or horse sera, or with sera from rabbits immunized with filtrates or with dead cells. This was immediately followed by tracheal injection of living virulent organisms. Of the three rabbits, which had received injections of sera from rabbits immunized with filtrates, all died practically 20 hours after injection in acute paroxysms. One of three rabbits, receiving sera from rabbits immunized with dead cells, was alive after 48 hours; the others died while not under observation. None developed diffuse lesions.

The distribution in the lungs of material injected through the trachea, and the resulting injury, must necessarily vary considerably. While these mechanical factors were undoubtedly responsible for certain differences in the bronchopneumonic reaction, they did not obscure the results. Tracheal injections of only 1 or 1.5 cubic centimeters had been given to avoid reactions caused by excessive quantities of concentrated culture such as Winternitz and Hirschfelder had obtained in normal rabbits, and also because it seemed desirable to approach more closely conditions of spontaneous pneumonia in man. Similarly, small sensitizing and non-concentrated toxic doses had been carefully tested, as it was thought a hypersensitive condition favorable to the development of a definite cellular reaction in the lung might be present, although acute or even mild anaphylactic symptoms were entirely lacking.

Active sensitization by previous intravenous injection of pneumococcus filtrates or dead cells failed either to hasten death or to increase markedly the lung reaction, although beginning with as small sensitizing doses as 0.1 of a cubic centimeter the amount injected had been increased until there had been produced an immunity sufficient to protect. In fact, the controls which had not received preliminary treatment were usually the first or among the first to die, indicating that the chief effect of the preliminary treatment was to increase materially the resistance of the animals, especially when large doses were used. While filtrates of the non-virulent culture AA, although originally derived from the extremely virulent strain A, failed, even in large doses, to protect from tracheal injection of virulent organisms of the same strain, and while the protective action of the moderately virulent strain B could not be gauged, as both controls and treated rabbits, with one or two exceptions, live for forty-eight hours, in no instance was death hastened as a result of sensitization.

Comparison of the histological changes in the lungs of treated rabbits and untreated controls also failed to show striking or constant differences, though it appeared that preliminary treatment, especially with dead cells, possibly favored a somewhat more diffuse reaction. In the two experiments in which extensive lesions were found, not only in the treated animals but also to a lesser extent

in the untreated controls, the strain used, though of moderate virulence, was considered especially toxic as compared with the more virulent strains studied. Extremely virulent organisms, on the other hand, apparently encountered little resistance from the lung tissues, but passed immediately into the circulation without inciting exudative reactions. In certain instances where large preliminary doses had been given, the lung involvement was also proportionately greater.

These results would seem to bear out in some measure the view that in pneumonia the lung reaction is partly dependent on a favorable equilibrium obtaining between the natural or acquired resistance of the host and the degree of virulence and toxicity of the organisms rather than on an anaphylactic state.

The reactions, both systemic and local, incited by tracheal injection of serum culture mixtures were somewhat more marked. A supposedly attenuated culture to which normal sera had been added caused immediate but transient symptoms suggesting mild anaphylactic shock. Seven of the eight rabbits, however, died in violent convulsions twenty-four hours later. These acute paroxysms, which closely resembled those of fatal anaphylaxis, also occurred in other series about twenty to twenty-four hours after serum mixtures had been injected.[11] The animals suddenly showed extreme restlessness, threw themselves about their cages, making violent running or jumping motions and often crying out. Death occurred within a few minutes of the onset of the symptoms, the heart continuing to beat for some time after respiration had ceased. Examination of the lungs failed, however, to show any definite connection between these paroxysms and the development of exudative reactions.

It is difficult to account for the sudden death of such a large number of the animals at practically the same time, although the interval after inoculation varied in the different experiments, except as the result of some phase of delayed anaphylactic shock. This delayed reaction might be due to several factors, but not to the tracheal method of injection alone, because similar reactions also

[11] In other experiments similar results were noted as early as 10 to 12 and as late as 32 to 34 hours after inoculation.

occurred after intravenous inoculation. The capsule of the pneumococcus cell which is protective according to Welch, and the interval required for the production of sufficient toxin, whether from the body fluids or from the pneumococcus cells through growth, might be important factors.

The local tissue reactions incited by the serum culture mixtures, particularly those containing normal serum or serum from rabbits immunized to filtrates, were also somewhat more marked and developed in a larger proportion of animals than in the experiments with active sensitization. Several rabbits developed typical fibrinous lobar pneumonia, and a number of others less extensive but diffuse exudative lesions. These were partly offset, however, by the partial or almost complete absence of exudative reactions in the other rabbits of the experiments, which had received similar treatment.[12]

Throughout the experiments the absence of uniformity in the lung reaction was especially striking. Controls in a few instances developed more involvement than certain of the treated rabbits of the same experiment, while in several series the test animals ran the whole gamut of reaction without apparent relation to the special character of preliminary treatment or tracheal injection.

The extreme sensitiveness of the pneumococcus to any alteration in its environment was repeatedly noticed. While the causes of fluctuation in virulence and toxicity are obscure, they not infrequently seemed closely associated with growth activity. That extremely subtle reactions occur within the body seems probable, and until the laboratory methods more nearly duplicate conditions present in the body, where the cellular elements and body fluids undoubtedly play an important part, similar and constant results can hardly be expected. In the present experiments when body conditions were more nearly approached the lung reaction was generally increased. It is of interest that in Wadsworth's experiment with partial immunization, the pneumococci used in the tracheal injection were grown in equal parts of normal rabbit serum and broth, while Winternitz and Hirschfelder used a 5 per cent. serum medium.

[12] For purposes of comparison tests with heterogeneous sera were made. Three rabbits received tracheally mixtures of culture and sera from a horse immunized to live cultures. Two developed diffuse and fairly extensive exudative lesions, one a very slight reaction.

Although an anaphylactic or hypersensitive condition in the host may possibly enable the invading pneumococcus to gain its first foothold, similar conditions might equally well underlie the development of a streptococcus infection, but would not necessarily determine in either case the type of lesion that later develops. In this study the essentially progressive character of the lung lesion was frequently brought out, all degrees of exudative reaction being found from small peribronchial foci merging into patchy or confluent lesions to the typically lobar involvement.

## SUMMARY.

While preliminary treatment with culture filtrates or dead cells of the extremely virulent strain A gave rise to varying degrees of immunity, the exudative lung lesions developing after tracheal injection with live organisms of the same virulent strain were not strikingly increased in any group or series of these rabbits. Despite carefully graduated dosage in the preliminary treatment, none of the animals developed symptoms of a definitely anaphylactic nature.

In similar experiments following sensitization with the attenuated avirulent culture AA, tracheal injection of virulent or avirulent organisms of the same strain failed to incite any definite increase in the exudative lung reaction. In none of the rabbits were symptoms resembling anaphylaxis noted. The immunity which was induced by the larger sensitizing doses of culture filtrates of the strain in the virulent state was lacking when similar doses of the culture filtrates of organisms in the non-virulent state were used.

Extensive lesions developed in both sensitized and unsensitized rabbits when the strain used in the tracheal injection was one apparently combining moderate virulence with exceptional toxicity, indicating that the exudative lung reaction was one of adjustment rather than of acquired hypersusceptibility.

When, in the experiments, small amounts of sera from normal rabbits, or from animals immunized to culture filtrates, were added to the culture before tracheal injection, an increased fibrinous lung reaction was frequently found.

The present study would seem to give some ground for the view that while in pneumonia a hypersensitive condition probably takes

some part in the inception of the infection, the subsequent development of the diffuse exudative reaction in the lung is not directly due to an acquired hypersusceptibility, but to intrinsic qualities possessed by the pneumococcus itself.

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