A Search for Pneumococcidal Agent During Crisis in Pneumonia.

(Thesis presented in partial fulfilment of the requirements for the Ph.D. degree.)

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Protective substances in the serum. That the serum of convalescent lobar pneumonia patients would protect animals against otherwise fatal doses of pneumococci was first demonstrated by ~~Gand~~ G. and H. Klemperer (Berlin klin. Wchnschr. 1891. XXVIII 833-835), later by Romer (Arch. f. Opthal. 1902 LIV. 99-200), Dochez (J. Exper. Med. Lanchester Pa. 1912 XVI, 665-679) and Clough (Johns Hopkins Hospital Bulletin 1913 XXIV 295-306)

Boettscher and Einleitung (Deutsches Arch. f. klin. Med. Leipiz, 1910. XCVIII 93-121) Seligmann and Klopstock (quoted by Clough, Johns Hopkins Hospital Bulletin 1913, XXIV 296-306) and Strouse (J. Exper. Med. Lanchester Pa. and N. Y. 1911 XIV 109-115) were unable to demonstrate any protective substances in the serum of pneumonia patients after crisis.

Clough (Johns Hopkins Hospital Bulletin, 1913, XXIV 295-305) found that serum during the acute stage of the disease or serum from fatal cases showed no protective power.

Phagocytosis. Issaeff (Ann. d l'Inst. Pasteur, Paris 1893. VII. 260-285) was the first to discover the part played by leucocytosis in pneumococci infection. Mennes (Ztschr f. Hyg. n. Infektionskiankh. Leipiz 1897., XXV 413-437) first studied the phagocytosis of pneumococci in immune serum in vitro. His work was repeated by Huber (Berl. klin. Wchnschr. 1903 XL 359-360). Wright and Douglas (quoted by Jordan. 7th edition p. 167, 1903-4) discovered the opsonins and independently Neufeld and Rimpeau (Deutsch Med. Wchnschr. 1904. XXV 1458-1460) discovered bacteriotropins in streptococci and pneumococci immune serum. Foa (quoted by Clough, Johns Hopkins Hospital Bulletin 1913 XXIV. 295-306) is the only investigator who has observed in the serum of pneumonia

patients after crisis the phagocytosis of virulent pneumococci notphagocytable in normal serum.

Boettscher (Deutsches Arch. f. klin. Med. Leipiz XCVIII 193-121) and Boni (quoted by Clough, Johns Hopkins Hospital Bulletin 1913, XXIV 295-306) reported finding increased phagocytic activity with the serum of pneumonia patients after crisis

Strouse, however, (Jour. Exper. Med. Lanchester Pa and N. Y. 1911. XIV 109-115) obtained negative results with the serum of pneumonia patients after crisis with the homologous and stock strain of virulent non phagocytable pneumococci.

Wolf (quoted by Hektoen, Jour. Am. Med. Assn. 1914 LXII 294-257), Rosenow, (Jour. Inf. Dis. 1906 III 683-700) Potter and Krumweide, (Jour. A.M. Assn. 1907 LXIX 1815-1824) and Tunnicliff (Jour. Inf. Dis. 1911 XIII 302-315) found that as a rule there is a low opsonic index during the height of the pneumococcus infection, a rise above normal at the time of crisis, and a return to normal during convalescence. In fatal cases the opsonic index may fall much below normal.

Rosenow (Jour. Inf. Dis. 1911. VIII 500-503) examined lung exudate obtained by lung puncture and found that the amount of phagocytosis is never very great. Before crisis there is no phagocytosis; after crisis there is a moderate amount of phagocytosis However, phagocytosis occurs only after the pneumococci have undergone degenerative changes to such an extent that they no longer take basic dyes. He also found that the number of colonies of pneumococci obtained from culturing the exudate on blood agar decreased gradually at the time of crisis in cases that recovered and increased in fatal cases.

Agglutinins. Bensaçon and Griffon (Ann. d'l'Inst. Pasteur. Par. 1900. XIV 449-463) demonstrated agglutinins in the blood of pneumonia patients in 1900.

Chickering (J. Exper. Med. 1914 XX. 599-606) found agglutinins in the blood of pneumonia patients after crisis in 73.8 per cent of the cases due to pneumococci types I, II and IV. Clough (Johns Hopkins Hospital Bulletin 1919 XXX. 167-176) found

agglutinins in 79 per cent of the pneumonia patients examined after crisis He found no agglutinins in the serum of pneumonia patients before crisis

Bactericidal action. Much and Dold (quoted by Clough, Johns Hopkins Hospital Bulletin. 1913. XXIV. 295-306) found that normal human serum had a slight bactericidal action on virulent pneumococci. They did not find this activity present in susceptible animals like the mouse, rabbit and the horse.

Clough repeated Dold's experiments with several specimens of rabbit serum and plasma and with the serum and plasma of four pneumonia patients after crisis. His results confirmed Dolds. The reduction of the number of colonies of pneumococci was not greater in the pneumonic serum than in normal serum and was not greater for the homologous strain than for a heterologous strain.

Virulence. That there is no decrease in the virulence of pneumococci during the course of pneumonia has been demonstrated by Rosenow,(Jour. Inf. Dis. 1904. I 280-312) and by Graham ,(Jour. Inf. Dis. 1908. V. 273-278) and by Leutscher (Jour. Inf. Dis. 1911. IX. 287-323)

Sensitizing substances. Weil and Torrey(Exper. Med. 1916. XXIII. 1-10) found that the serum from pneumonia patients before crisis contains substances which sensitize guinea pigs passively. Patients serum after crisis failed to give this reaction, as also normal individuals.

Weiss and Kolmer (Proc. Path. Soc. Phila. m.s. 1918. XXI. 10-11) using pneumotoxin washed pneumococci dissolved in sodium choleate, as an antigen, did skin tests on pneumonia patients and got positive results as early as the fifth and as late as five days after crisis. Negative results were obtained on children immediately or a day or two after crisis. Patients with broncho pneumonia or with acute or chronic infections not of pneumonic origin as well as healthy adults and children got no reaction.

Ferment changes in serum. Other changes in the serum at the time of crisis are ferment changes. Aseoli (Berl. klin. Wchnschr. 1903. XL. 392) investigated antiferment changes during crisis and noted an early rise in antiferment titer with a decline after crisis.

Dick (Jour. Inf. Dis. 1912. X 383-387) in 1912, found that proteolytic ferments develop in the blood during pneumonia about the time of crisis. These ferments split pneumococcus proteins and the digestive product could be detected by means of the polariscope.

Rosenow (Jour. Inf. Dis. 1912. XI. 94-108) found that the rotary activity with polarized light of a disintegrated salt suspension of pneumococci, and the amine acid, determined by formol titration, increased as toxicity appeared and then disappeared. In an immunized guinea pig, he found the undigested proteins to be very rapidly split into non toxic material, so that the pig showed few or no toxic symptoms. In a normal guinea pig the digestion went on at a much slower rate so that the toxic symptoms lasted longer. After digestion of the pneumococcic proteins proceded far enough the toxicity disappeared for both normal and immune guinea pigs.

Vaughn (Boston Med. and Surg. Jour. 1906. CLV. 243-247) found that protein digestion products may cause many of the general symptoms present in pneumonia. The crisis, he believes, may be due to the large quantity of toxic proteins set free at this time and rapidly broken up into non toxic products.

Jobling, Peterson and Eggstein (J. Exper. Med. 1915. XXII. 568-589, 590-595, 597-602, 603-611) did their work on ferments in the serum in 1915. They found that the crisis in pneumonia was usually accompanied by a decrease in the serum antiferment, the appearance of non specific proteoses in the serum, an increase in the serum lipose, and a decrease of non coagulable nitrogen and of proteoses in the serum.

They came to the conclusion that the beginning of active autolysis associated with crisis depends on the altered antiferment-ferment balance. The fibrin and leucocytic debris was considered as one of the potential sources of toxic substances;with rapid autolysis proceding only non toxic materials are absorbed.

Kline and Winternitz (J. Exper. Med. 1915. XXI. 311-319, 320-329) believe that the crisis in pneumonia is largely local. They found that there was a marked impairment of the circulation in the lung in pneumonia due to relative uniform capillary thrombi. This impairment of circulation is of importance in bringing about resolution. Only enough blood is allowed to seep through the vessels to nourish the alveolar walls. Consequently very little serum escapes into the alveoli, and the autolysis of the exudate is unhindered by the leucocytes.

Ornstein and Braunstein, (New York M.J. 1923, CXVII. 466-468) also believe that crisis is brought about by the formation of fibrinous plugs and thrombi in the blood vessels of the involved area. With the cessation of circulation, absorption of toxic substances from the involved pneumonic lung ceases, and the crisis occurs. Lysis is a more gradual interference with the circulation in the pneumonic area. Toxemia is probably caused by a soluble substance absorbed into the circulation. The symptoms are shown from the inception of the disease until the pneumonic area becomes avascular.

Tongs (Jour. Inf. Dis. 1922. XXX. 323-332) working with type I pneumococcus injected into rabbits, found that the leucocytic reaction is somewhat dependent on the virulence of the organism. A pneumococcus with low virulence producing leucocytosis and a pneumococcus of high virulence produces leucopenia. After the intraperitoneal inoculation of guinea pigs with virulent and non virulent pneumococci the leucocytes as a rule show phagocytosis. In certain instances the failure of leucocytes to take up very virulent pneumococci seemed to be due to toxic changes in the leucocytes as evidenced by the degenerative changes. The virulent pneumococci seemed to produce achemotactic substance, for although the leucocytes failed to ingest virulent pneumococc they were usually surrounded by them.

Hiss believed that the leucocytes contained a substance which was responsible for immunity reactions and used aqueous extracts of leucocytes, obtained by injecting aleronot into the peritoneal cavity of animals, against a number of infections. The results seemed quite promising but further investigation proved that their use could not be justified.

Williams and Youland (Jour. Med. Res. 1914-1915 XXXI. 391-407) repeated Hiss' work on the treatment of lobar pneumonia and likewise got negative therapeutic results

Opié, (Amer. Med. 1905. IX. 1028-1029) in 1905 obtained exudates by injecting suspensions of aleronot into the pleural cavities of dogs and rabbits and allowed them to autolyse. The Keldohl method was used to determine the nitrogen of coagulable protein converted by digestion into soluble form.

He found that the inflammatory exudates removed one or two days after injection of the irritant underwent very little change, while those removed three or four days after the onset of inflammation exhibit appreciable though slight autolysis. There was no relation found between the amount of digestion and the number of cells which were present. Jobling, Peterson and Eggstein also found that the leucocytic curve has no relation to the serum ferment changes. If the cells were separated by centrifugalization from the serum and suspended in normal salt solution well marked autolysis was demonstrable. By recombining cells and serum it could be shown that the serum inhibits autolysis. When this inhibitory action is prevented by heating serum to 100 degrees C. leucocytes acting upon the coagulated serum may cause very active digestion. The anti-enzymatic action of the serum is unaffected by a temperature of 65 degrees C, but is destroyed at 75 degrees C. The proteolytic ferments of the leucocytes act in weakly and alkaline medium but are most efficient in the latter. The antienzymatic action of the serum is favored by an alkaline reaction but is completely lost in acid medium.

The serum of the exudate contains a proteolytic ferment which is active only in an acid medium.

The antienzymatic power exhibited by the serum of the inflammatory exudate is possessed by the serum of the blood from which it doubtless passes into the exudate. In the later stages of inflammation produced by aleronot and in exudates caused by bacteria there is some diminution of the antienzymatic action.

This is essentially what Lord, in 1919,(J. Exper. Med. 1919. XXX. 379-388; 1921 XXXIV 199-200, 201-205) found in pneumonia exudates. Lord obtained from the lung, in pneumonia,a proteolytic enzyme digesting coagulated blood serum at hydrogen ion concentration of Ph 7.3 to 6.7 and in active at higher concentrations. A proteolytic enzyme splitting peptone to amino acids at hydrogen ion concentration Ph 8.0 to 4.8 but most active at Ph. 6.3 to 5.2 were ,also, found to be present.

This proteolytic enzyme was active after eighteen months preservation and was active at incubator temperature before and after heating to 65 degrees C. for one hour. It was slightly active at room temperature and inactive after heating to 75 degrees C. for one hour. Dialysis of the enzyme was not demonstrable. This activity persisted when the enzyme was mixed with concentration of sodium chloride varying from normal to thirty two times normal.

Cellular material from the pneumonic lung in an early stage of lobar pneumonia failed to erode the surface of Loeffler's blood serum until washed with normal saline solution. Mixtures of washed cellular material and normal human serum failed to erode Loeffler's blood serum when the amount of cellular material was less than one part of cells to approximately three parts of serum. Erosion occurred when the cellular material exceeded this amount in the ration. Opie, it will be recalled, likewise found that the serum prevented enzyme action.

Almagia (Centralb.Biochem. n. Biophys. 1913-1914. XVI. 283-) found that the growth of pneumococci were completely inhibited by autolysing fibrin.

Lord and Nye, (J. Exper. Med. 1922. XXXV. 699-701) in 1922,found that pneumococci

types I, II and III undergo dissolution when mixed with cellular material from the pneumonic lung at Ph 6.9 and 5 5, but not at Ph. 4.5.

That enzymes are liberated from the pneumococci on disintegration has been demonstrated by Avery and Glenn (J. Exper. Med. 1921. XXXIII. 547-569). With acetone extraction they obtained an enzyme which would hydrolize saccrose, starch, and inulin at Ph. 5.0 to 8.0 but best at Ph. 7.0, and in a sodium choleate solution of pneumococci, they got a protease and a peptonase in Ph. 7.0 to 7.8. At Ph 5.0 the acid death point of the pneumococcus, the action of the enzyme was suspended, but was restored by adjusting to optimum Ph. value. Attenuation of virulence had no measurable quantitive effect on the enzyme acticity.

This bile solution of pneumococci, also, showed marked lipolytic activity as shown by its action on tributyrin at the optimum hydrogen ion concentration for growth of pneumococci.

Lord (J. Exper. Med. 1922. XXXV 689-701) studied the dissolution of pneumococci at varying hydrogen ion concentrations. Suspensions of living pneumococci in approximately isotonic bouillon with a Ph. varying from about 4.0 to 8.0 after incubation show dissolution of organisms in these solutions having a Ph. 5.0 to 7.0. Some dissolution took place towards the alkaline end of the scale. The addition of fresh human serum to the suspensions of pneumococci at varying Ph. prevented dissolution. Lord ascribed this dissolution to enzymes derived from the bacteria themselves.

Lord (J. Exper. Med. 1921 XXXIV. 207-209) obtained a specific precipitin test when the pneumonic exudate was mixed with the homologous antipneumococcus serum.

Specific agglutinins, according to Lord, (J. Exper. Med. 1921. XXXIII. 211-216) were lacking, or present only in small amounts, in the pneumonic exudates due to the fixed types of pneumococci. Suspensions of fixed types of pneumococci in the supernatent fluid obtained after centrifuging the mash of the pneumonic lung gave positive results not higher

than equal parts of suspensions and supernatent fluid. The pneumonic lung contained a soluble substance which inhibited agglutination of the fixed types by the homologous anti-pneumococcus serum.

While the enzymatic activity, agglutinins and precipitins of the exudate have been studied I have found no literature on the presence or absence of a bacteriophage for such exudates or the presence or absence of a protective value of such exudates for animals

Experiments on the Presence or Absence of a Bacteriophage in Pneumonic Sputum.

Experimental.

Judging from the course of cases of dysentery, barbone etc. as discussed by d'Heulle (The Bacteriophage -Williams Wilkins Co. 1922) it seemed possible that the crisis in pneumonia might be due to the sudden development of a highly virulent bacteriophage for the pneumococcus.

A case of lobar pneumonia was selected and the first specimen of sputum was obtained January 26, 1925. This was cultured in meat infusion broth for 6 hours and the bacteria isolated on blood agar. Thereafter, specimens of sputum were obtained almost every day, ground in a mortar, mixed with about four to five parts of meat infusion broth Ph 8.0, incubated over night at 37.5 degrees C. and filtered through a sterile Berkefeld filter. The sterile filtrates were then stored in an ice box until ready for use.

The filtrates for each day were tried out on blood agar slants with the homologous pneumococcus (type IV) The results were difficult to read. If a bacteriophage was present it did not seem to be very active.

January 30, 1925 the first test was carried out in vivo. The pneumococci were grown on blood agar slants and suspended in 2 cc. of meat infusion broth Ph.8.0 just before injection. Dilutions were then made in broth so that the dose of pneumococci was contained in 0.2 cc. This suspension of pneumococci with 0.5 cc. of broth Ph. 8.0 for the control series and 0.5 cc. of January 22nd. filtrate (the first filtrate after crisis) for the test series was injected intraperitoneally into white mice. The results are given in Table I.

Table I.

No.	Dose of pneumococci.	Survival in hours of broth control mice.	Survival in hours of Jan. 22 filtrate mice.
1	0.1 cc	20	20
2	0.01 cc	20	25
3	0.001 cc	20	40

Table I (continued)

No.	Dose of pneumococci.	Survival in hours of broth control mice.	Survival in hours of Jan. 22 filtrate mice.
4	0.0001 cc.	20	20
5.	0.00001 cc	30	40
6	0.000001 cc	36	Saved.

(Note: In this and following tables the figures are the time of survival in hours.)

January 31, 1925 this experiment was repeated using a broth control series January 16 filtrate (a filtrate before crisis) and January 22 filtrate (a filtrate after crisis) The results are given in Table II.

Table II.

No.	Pneumococci dose	Broth control mice	Jan. 17 filtrate mice	Jan. 22 filtrate
1	0.1 cc	20	20	30
2	0.01 cc.	20	30	40
3	0.001 cc	20	30	Saved.
4	0.0001 cc	20	31	"
5	0.00001 cc	31	40	"

In this test the Jan 22 filtrate, the filtrate after crisis, seemed to exert more protective power than the filtrate before crisis.

February 2 this experiment was repeated using Jan. 23 filtrate, another filtrate after crisis. The results are given in Table III.

Table III.

No.	Pneumococci dose	Broth control.	Jan. 23 filtrate
1	0.1 cc	20	20
2	0.01 cc	20	32
3	0.001 cc	20	96
4	0.0001 cc	30	Saved.
5	0.00001 cc	36	96

Jan 23 filtrate also exerted some protective power.

January 5, 1925 the experiment was repeated using broth control series, a broth culture of the homologous pneumococci filtered, Jan. 19 filtrate, Jan. 22 filtrate boiled and not boiled, Jan. 23 filtrate and Jan. 28 filtrate. The results are given in Table IV.

Table IV.

No	Pneumococci dose	Broth control	Broth culture filtrate	Jan 19 filtrate	Jan 22 filtrate boiled	Jan 22 filtrate	Jan 23 filtrate	Jan 28 filtrate
1	0.1 cc	20	20	20	20	20	20	20
2	0.01 cc	20	26	22	20	20	43	42
3	0.001 cc	22	20	42	20	76	Saved	42
4	0.0001 cc	26	42	26	42	50	"	22
5	0.00001 cc	42	42	42	42	Saved	"	Saved
6	0.000001 cc	42						

In this experiment the filtrate from the Jan 23 specimen showed the most protective action The boiled filtrate of Jan 22 showed less protective power than the filtrate which was not boiled.

Case 2.

A culture of pneumococci was obtained from the hearts blood of a mouse injected with sputum January 31, 1925.

The crisis occurred and a second specimen of sputum was obtained. A filtrate was prepared from this specimen as in the previous case -

The culture from this case was lost and protection experiments were carried out in mice with this filtrate and the pneumococci type IV from case 1. The results are given in Table V.

Table V.

No.	Pneumococci dose	Broth culture.	Feb.2 filtrate.
1	0.1 cc	20	20
2	0.01 cc.	20	20
3	0.001 cc	22	42
4	0.0001 cc.	26	42
5	0.00001 cc.	42	42
6	0.000001 cc.	42	

There was almost no protective power exhibited by this one filtrate after crisis for the pneumococcus from Case 1.

Case 3, was due to a type I pneumococcus infection. The pneumococci were isolated from the hearts blood of a mouse injected with Feb. 10, 1925 sputum. The filtrates were prepared as usual from sputum specimen obtained Feb. 12, 13, 14, 15, 16, and 17. The temperature in this case fell by lysis. It began falling Feb. 13 and was normal Feb. 15. March 4, 1925 mice were injected with the filtrates and the homologous pneumococcus. The results are given in Table VI.

Table VI.

No.	Pneumococci dose	Broth control	Feb.11 filtrate	Feb.12 Fil.	Feb.13. Fil.	Feb.14 Fil.	Feb.15 Fil.	Feb.15 Salt Fil.	Feb.16 Fil.	Feb.17 Fil.
1	0.1 cc	6	20	20	20	20	20	20	20	20
2	0.01 cc	20	20	20	20	20	20	20	20	20
3	0.001 cc	20	20	20	20	20	20	44	20	30
4	0.0001 cc	20	20	20	30	22	20	20	24	64
5	0.00001 cc	20	44	30	96	Saved	Saved	20	Saved	Saved
6	0.000001 cc	26								

A feeble protective power is shown by the filtrates obtained from the sputums after the temperature had gone down.

Cases 4 and 5. Type I.

The sputum specimens of Case 4 before crisis, April 15, 16, and 17, 1925 were combined with one sputum specimen of Case 5, also before crisis. The sputum was ground in a mortar and the following portions were added to broth incubated and filtered - pure sputum (no broth), 75% sputum in broth, 50% sputum in broth, 25% sputum in broth and 12% sputum in broth.

The sputum specimens from Case 4 after crisis, April 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30 were also combined and filtrates were obtained after incubating- 100% sputum, 50% sputum in broth, 25% sputum in broth and 10% sputum in broth.

Also to 82 cc. of the combined late sputum 164 cc. of ether was added, mixed, and placed in the icebox for ten days. The ether was then removed and placed in a test tube with a cotton stopper. By May 16 the ether had evaporated. May 8, 1925 mice were injected with the late filtrates and a heterologous type I pneumococcus. The results are given in Table VIII.

Table VIII.

No	Pneumococci dose	Broth control	10% sputum filtrates	50% sputum filtrate	100% sputum filtrate.
1	0.1 cc.	20	-	-	-
2	0.01 cc	24	20	20	24
3	0.001 cc	24	20	44	20
4	0.0001 cc	44	44	44	44
5	0.00001 cc	44	-	-	-
6	0.000001 cc	50	-	-	-

There was no protective power exhibited by the late filtrate of this case for another type I pneumococcus.

May 11, 1925. The early filtrates from the two cases were injected into mice with the type I pneumococcus used above. The results are given in Table IX.

Table IX.

No.	Pneumococci dose	Broth control	100% sputum filtrate.	50 and 75% sputum filtrate combined.
1	0.01 cc.	44	20	44.
2	0.001 cc.	44	44	44.
3	0.0001 cc	44	44	44
4	0.00001 cc	44	-	-

There was no protection shown by the early filtrates from this case. The residue of the ether extract was mixed with 5 cc. of broth and 0.5 cc. of this was injected into mice with culture of pneumococci diluted to 0.2 as usual. A type I pneumococcus was used but it was a heterologous strain. The results are given in Table X.

Table X.

No.	Pneumococci dose.	Broth control.	Ether extract mixture.
1	0.1 cc.	20	20
2	0.01 cc	20	20
3.	0.001 cc	44	46
4	0.0001 cc	46	46
5	0.00001 cc	46	-

This extract was very concentrated. Therefore the protective substances in the sputum of lobar pneumonia for mice not a lipin extractable by ether or else there were no protective substances in the sputum of this particular case for the pneumococcus used.

Cases 6 and 7. Types III and II.

From Case 6 two specimens of sputum were obtained the first and second day after crisis. Filtrates were prepared from these specimens in the usual way and combined.

Case 7 was a fatal case of lobar pneumonia due to a type II pneumococcus. One sputum specimen was obtained the day before death, April 16, 1925. A filtrate was prepared in the usual way.

April 21, 1925 the filtrates from the two cases were injected along with a type II pneumococcus into mice The results are given in Table XI.

Table XI

No.	Pneumococci dose.	Broth control.	Filtrate from case 7.	Filtrate from case 8.
1	0.1 cc	17	17	17
2	0.01 cc	23	17	17
3	0.001 cc	34	17	34
4	0.0001 cc	34	23	47
5	0.00001 cc	34	44	47

There was no protection by the filtrate from a fatal case of similar type of pneumococcus. Neither was there any protection from the sputum after crisis for the pneumococcus of another type.

Case 8. Type II.

Pneumococci were isolated from the hearts blood of a mouse injected with sputum April 18, 1926. Filtrates were prepared in the usual way from sputum specimens obtained on April 20, 21, 22, 23, and 24.

April 26, 1926 These filtrates were injected with the homologous pneumococci into white mice. The results are given in Table XII.

Table XII.

No.	Pneumococci dose	Broth control.	April 20 & 21 filtrates	April 23 filtrate	April 24 filtrate boiled.	April 24 filtrate.
1	0.1 cc	15	18	18	15	37
2	0.01 cc	23	37	37	20	37
3	0.001 cc	37	-	62	37	41
4	0.0001 cc	37	37	62	37	44
5	0.00001 cc	41	44	62	37	45

The crisis in this case occurred April 20 but there was a slight rise in temperature

April 21. There was a protection, if it could be called that, of a few hours from the filtrates made the next two days. There was not this latency in the death of the mice injected with the boiled filtrate.

Case 9.. Type I.

Pneumococci were isolated from the hearts blood of a mouse injected with sputum October 16, 1926. Filtrates were prepared from sputum specimens obtained Oct. 16, 17, 18, 19, 20, 21, and 22.

October 26, 1926 mice were injected with the filtrates and homologous pneumococci. The results are given in Table XIII.

Table XIII.

No.	Pneumococci dose	Broth control	Oct.16 filtrate	Oct.17 fil.	Oct.18 fil.	Oct.20 fil.	Oct.21 fil.	Oct.22 fil.
1	0.1 cc	12	14	12	12	17	12	15
2	0.01 cc	12	16	13	11	13	16.5	16
3	0.001 cc.	17.5	17	16	18.5	14.5	14.5	36
4	0.0001 cc	14	19	17	18.5	17	36	36
5	0.00001 cc	16.5	36	36	36	19	21.5	36
6	0.000001 cc	20						

The crisis in this case occurred October 20. There is almost no evidence of protection exhibited by the filtrates. Every mouse was autopsied soon after death and smears of the hearts blood and peritoneal fluid were stained with Grams and Hiss capsule stain. There were no striking differences noted between the control mice and those which received filtrates.

Case 10. Type IV.

Pneumococci were isolated from the hearts blood of a mouse injected with sputum October 27, 1926. Filtrates were prepared from sputum specimens October 27, 28, 29, and 30.

November 4, 1926 the filtrates and homologous pneumococci were injected into mice. The results are given in Table XIV.

Table XIV.

No.	Pneumococci dose	Broth control	Oct. 27 filtrate	Oct.28 fil.	Oct.29 fil.	Oct.30 fil.
1.	0.1 cc	11.5	14.5	16.5	11	36
2.	0.01 cc	36	36	11	36	19
3.	0.001 cc	36	36	11	36	36
4.	0.0001 cc	36	36	36	36	36
5.	0.00001 cc	36	36	39	96	Saved

The crisis in this case occurred October 27 and 28. There was very little protective action exhibited by the later filtrates.

November 8, 1926. The broth for controls and the filtrates were mixed with the graduated doses of the homologous pneumococci one hour before injecting into mice. The results are given in Table XV.

Table XV.

No.	Pneumococci dose	Broth control	Oct.27 filtrate	Oct.28 filtrate	Oct. 29 filtrate.	Oct. 30 filtrate.
1	0.1 cc	17	15	12	17	12.5
2	0.01 cc	25	15	18	20	12.5
3	0.001 cc	19	19	25	24.5	24.5
4	0.0001 cc	20.5	25	25	24.5	35
5	0.00001 cc	25	12	35.5	35	35
6	0.000001 cc	25				

Mixing the filtrates and pneumococci an hour before injecting did not cause the protective action of the filtrates to be exhibited in a more striking way.

Case 11. Type III.

Pneumococci were isolated from the hearts blood of a mouse injected with sputum November 20, 1926. Filtrates were prepared from sputum specimens November 21, 22, 24, 26 and 27.

December 5, 1926 The filtrates and homologous pneumococci were injected into white mice. The results were given in Table 16.

Table XVI.

No.	Pneumococci dose	Broth control	Nov.21 filtrate	Nov.24 filtrate	Nov.26 filtrate	Nov.27 filtrate.
1	0.1 cc	17	24	18	17	
2	0.01 cc	17	24	36	36	
3	0.001 cc	23.5	36	36	36	
4	0.0001 cc	36	36	36	36	
5	0.00001 cc	36	36	36	36	Saved.
6	0.000001 cc	36				

The crisis was November 20. With the exception of the one mouse there was almost no protection shown.

Case 12. Type IV.

Pneumococci were isolated from the hearts blood of a mouse injected November 20, 1926. Filtrates were prepared from sputum specimens April 20, 21, 22, 23, 24, and 27.

At the beginning this case seemed to be a typical lobar pneumonia due to pneumococci. Later (Nov. 24) the character of the sputum changed - became instead of tenaceous and rusty sputum, very thin and white. On direct smear many small Gram negative bacilli were present. November 24 a mouse was injected with 0.5 cc. of the sputum and Gram negative bacilli as well as pneumococci were cultured from the hearts blood. The patient's temperature went up and down. He signed himself out of the hospital November 28.

November 30. The filtrates and homologous pneumococci were injected into mice. The results are given in Table XV.

Table XVII

No.	Pneumococci dose	Broth control	Nov.20 fil.	Nov.21 fil.	Nov.22 fil.	Nov.23 fil.	Nov.24 fil.	Nov.26 fil.	Nov.27 filtrate.
1	0.1 cc	14.5	21	14	18	17.5	14.5	17	17
2	0.01 cc	36	36	36	36	36	24	36	20
3	0.001 cc	36	36	36	36	36	36	20	23.5
4	0.0001 cc	36	36	36	36	36	36	36	36
5	0.00001 cc	24	36	36	36	36	36	36	36
6	0.000001 cc	36							

There was no protective substances in the sputum filtrates from this case.

There is in the majority of cases a constant latent period in the time of death of the mice receiving the sputum filtrate after crisis as compared to the time of death of the control mice One would expect some individual variation in the resistance of the mice to pneumococci but such differences would appear in the control mice and in the mice receiving filtrates made bdfore crisis as well as in the mice receiving the filtrates after crisis. This latent period in the time of death of mice does not appear for mice injected with sputum filtrates made before crisis or for mice injected with filtrates made after crisis and a pneumococcus of another type. It does not appear when the filtrates of sputum after crisis is boiled or when the filtrates are made from the sputum of fatal cases.

In this feeble protective power due to a weakly virulent bacteriophage, is it due to some substance liberated upon the disintegration of the polymorphomuclear leucocyte, or the pneumococci, or is it due to some other substance in the exudate. The method used to demonstrate this protective power was a purely arbitrary one. Could some other method be used to demonstrate a greater protective power.

The bacteriophage. 1 cc. of October 30 filtrate from Case 10 which had shown some protection for mice against the homologous pneumococcus was added to 9 cc. of the same pneumococci suspended in broth, incubated 20 hours and filtered through a sterile Berkfeld filter. 1cc of this filtrate was added to another 9 cc. of a fresh suspension of the homologous pneumococci, incubated and filtered as before. From December 9 to December 22, 1926 fifteen filtrations were made with incubation periods of 8 to 20 hours. After that number of filtrations if a bacteriophage were present its virulence should be enhanced. The final filtrate was then injected into mice with the pneumococci isolated from the case from which the first filtrate was made
The results are given in Table 18.

Table XVIII.

No.	Culture	Broth control	Last filtrate.
1	0.1 cc	21	20
2	0.01 cc	28	30
3	0.001 cc	72	72
4	0.0001 cc	-	72
5	0.00001 cc	96	96

There was no protection shown by the last filtrate.

Lord found that proteolytic enzymes are in the pneumonic exudate and that the enzymes will erode the surface of a Loeffler blood serum slant. Could these enzymes have anything to do with the protection of mice against the pneumococcus?
April 27, 1927 1 cc. of each of the filtrates from the following cases were placed on Loefflers blood serum slants and incubated.

Case 9. Filtrates Oct. 16, 17, 18, 20, 21.

Case 10. Filtrates Oct. 27, 28.

Case 11. Filtrates Nov. 21, 24, 26.

Case 12. Filtrates Nov. 20, 21, 22, 23, 24, 25, 26, 27.

Case 13. (No protection experiments carried out) Filtrates Nov. 2,3,4,5.

Case 14. " " " " " Filtrates Nov. 9,10

Case 15. (No protection experiments carried out) Filtrates Nov. 20, 21, 22, 24, 26, 27.

Case 16. (No protection experiments carried out) Filtrates Nov. 23, 24. This was a case which terminated fatally.

Case 17. (No protection experiments carried out) Filtrates Nov. 22, 24, 26, 27.

There was no erosion of Loefflers blood by the filtrates either before or after crisis. As will be noted there was quite a period of time between the preparation of these filtrates and the test for proteolytic enzymes. However, Lord found that the proteolytic enzymes were still active after eighteen months preservation. It is evident that the manner in which these filtrates were prepared is not suitable for demonstrating the presence of proteolytic enzymes.

Enzymes. That such enzymes as Lord described are present was demonstrated in two cases:

Cast 18. The method was as follows. Sputum specimens before crisis were obtained, ground in a mortar and divided into equal quantities. One volume was mixed with four volumes of physiological salt solution and centrifuged. The suspended salt solution was removed and one volume of fresh salt solution and a few drops of chloroform was added. A few drops of chloroform was also added to the supernatent fluid. The other portion was ground with an equal volume of physiological salt solution and a few drops of chloroform were added. The entire sputum specimens after crisis were ground and added to an equal volume of physiological salt solution in the same way. If any of the salt sputum mixtures did not turn brom - thymol-blue a green color 5% sodium bicarbonate solution was added until it did. A loop of each mixture was then placed on Loefflers blood serum slants from which the water of condensation

had been removed. The results are given in Table XX.

Table XX.

March 27.	Sputum washed	Digestion.
" "	" not washed	No digestion.
" "	Supernatent liquid from washed sputum	No digestion.
" "	" " and washed sputum	" "
March 28.	Sputum washed	Digestion.
" "	" not washed	No digestion.
	Supernatent liquid and washed sputum .	No digestion.
March 30.	Sputum not washed.	Digestion.
" 31	" " "	"
April 2.	Sputum not washed.	"

The crisis occurred March 28. There was no proteolysis of Loefflers blood serum before crisis except when the sputum was washed. After crisis there was proteolysis without washing the sputum.

Case 20 was a fatal case. Washed and unwashed sputum salt mixtures on the two days preceding death were prepared as in Case 19. The washed sputum mixture caused the erosion of a Loefflers blood serum slant while the unwashed did not.

Discussion: While protective substances, agglutinins, opsonins etc. have been shown to be in the serum of pneumonia patients after crisis, and to some extent in the lung exudate after crisis, it is well known that similar antibodies occur in the convalescent serum of patients suffering from other infections, during the course of which no crisis occurs. The cause of crisis cannot, therefore, be due to the formation of such antibodies. Rather such antibodies must make their rather sudden appearance because of some other factor which must be present. About the only difference between lobar pneumonia and other diseases, in which no crisis occurs, is that the important

foci of infection, the lungs, become avascular, due to the formation of uniform capillary thrombi (Kline and Winternitz - and Ornstein and Braunstern) at the event of which the phenomonen known as crisis occurs. When toxic symptoms are manifest in the body the serum exhibits its inhibition effect on agglutinins and enzymes (Lord). It is at this time that the lung is vascular. After the lung becomes avascular so many toxic substances cannot be absorbed the serum cannot exhibit its inhibitory action, and dissolution of the exudate by enzymes present are free to take place as are also other antibody reactions.

Conclusions.

1. In the majority of cases of lobar pneumonia there is a latent period in the time of death or protection of mice injected with sputum filtrate after crisis with a fatal dose of pneumonocci as compared to control mice.

2. Protection or a latent period in the time of death of mice does not occur with sputum filtrates before crisis, with sputum filtrates from fatal cases, with sputum filtrates after crisis injected with a pneumococcus of different type from that infecting the patient, or with the sputum filtrate boiled and injected with the homologous pneumococci.

3.A bacteriophage which might account for crisis was not recovered from the sputum.

4. Proteolytic enzymes which would erode Loefflers blood serum as Lord described were demonstrated in the sputum of two pneumonia patients.