

## STUDIES ON THE PNEUMONIC EXUDATE.

### IV. THE PRESENCE IN THE PNEUMONIC LUNG OF A SOLUBLE SUBSTANCE INHIBITING AGGLUTINATION BY THE HOMOLOGOUS SERUM.

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(Received for publication, March 28, 1921.)

Besançon and Griffon,<sup>1</sup> Chickering,<sup>2</sup> Blake,<sup>3</sup> and others have shown that agglutinins appear in the blood of lobar pneumonia patients during the course of the disease or during convalescence. In Blake's series, agglutinins for the homologous pneumococcus appeared in the blood in all patients who recovered, just before, during, or after crisis or lysis. Their concentration increased rapidly at the time of recovery and remained elevated during convalescence in the milder cases and showed a tendency to fall off in the more severe cases. Reference to his charts shows that in one case the amount of agglutinin, as measured by the highest dilution in which a positive test occurred, reached such a concentration that the homologous organism was agglutinated by a dilution of 1:32 of the patient's serum and that in two other cases it reached 1:16. Fatal cases failed to develop demonstrable agglutinins in the blood.

Cole<sup>4</sup> has noted that empyema fluids due to pneumococcus infection contain large amounts of soluble substances which have the property of neutralizing pneumococcus antibodies. For the purpose of his investigation, as indicated by the protocol of one of his experiments, 0.4 cc. of empyema fluid due to Type I pneumococcus, cleared by centrifuging, and mixed with an equal volume of an increasing dilution of antipneumococcus serum, was tested for its power to cause agglutination of Type I pneumococcus. The admixture of pneumonic

<sup>1</sup> Besançon, F., and Griffon, V., *Compt. rend. Soc. biol.*, 1897, xlix, 551, 579.

<sup>2</sup> Chickering, H. T., *J. Exp. Med.*, 1914, xx, 599.

<sup>3</sup> Blake, F. G., *Arch. Int. Med.*, 1918, xxi, 779.

<sup>4</sup> Cole, R., *J. Exp. Med.*, 1917, xxvi, 453.

fluid with the homologous antipneumococcus serum in our hands always gave rise to a precipitate which we have had to remove by the centrifuge.

*Agglutinins in Pneumonic Lung Exudate.*—In order to determine to what degree specific agglutinins for the homologous organism may be present in the pneumonic lung, the following experiment was performed with the macroscopic method.

The lung mash from a case of Type I pneumonia was centrifuged and increasing dilutions of the supernatant fluid were transferred to small test-tubes, each containing 5 drops of a suspension of Type I pneumococcus, as shown in Table I. After 2 hours incubation agglutination was noted in a dilution of 1:2 of the exudate, questionable agglutination at 1:10, and none at 1:20. A control series con-

TABLE I.  
*Tests for Agglutinins in Type I Pneumococcic Exudate.*

Tube No.	Suspension of Pneumococcus Type I.	Lung exudate.		Final dilution of lung exudate.	Agglutination.*
		Amount.	Dilution.		
	<i>gt.</i>	<i>gt.</i>			
1	5	5	Undiluted.	1:2	+
2	5	5	1:5	1:10	?
3	5	5	1:10	1:20	0

\* Observation after incubation for 2 hours and in ice box over night.

taining the same strain of Type I pneumococcus and homologous antipneumococcus horse serum showed agglutination in a dilution as high as 1:80 of the serum.

The lung mash from a second case of fatal Type I lobar pneumonia was similarly tested. The clear fluid was mixed in increasing dilution with normal saline solution. To each tube were added 2 drops of a suspension of Type I pneumococcus. No agglutination of the organisms was noted at any dilution, the lowest being 1:4 of the lung fluid. Antipneumococcus horse serum agglutinated the same strain in as high a dilution as 1:40 of the serum.

In a case of Type III pneumonia no agglutination was observed at any dilution, the strongest used being 1:2 of the exudate. In tests simultaneously performed Type III antipneumococcus horse

TABLE II.

*Agglutination Titer with Normal Saline Solution.*

Tube No.	Serum dilution.	Agglutination.
1	1:10	+
2	1:20	++
3	1:40	++++
4	1:80	++
5	1:160	+
6	1:320	0

0.5 cc. of increasing dilutions of Type III antipneumococcus serum + 0.5 cc. of normal saline solution + 3 drops of Type III pneumococcus suspension.

TABLE III.

*Agglutination Titer with Normal Saline Solution and Chloroform.*

Tube No.	Serum dilution.	Agglutination.
1	1:10	+
2	1:20	++
3	1:40	+++
4	1:80	++
5	1:160	0
6	1:320	0

0.5 cc. of increasing dilutions of Type III antipneumococcus serum + 0.5 cc. of normal saline solution + 3 drops of chloroform + 3 drops of Type III pneumococcus.

TABLE IV.

*Agglutination Titer with Normal Saline Solution and Toluene.*

Tube No.	Serum dilution.	Agglutination.
1	1:10	+
2	1:20	++
3	1:40	++++
4	1:80	+
5	1:160	0

0.5 cc. of increasing dilutions of Type III antipneumococcus serum + 0.5 cc. of normal saline solution + 0.5 cc. of toluene + 3 drops of Type III pneumococcus.

serum agglutinated the same strain of pneumococcus in a dilution as high as 1:40 of the serum.

To exclude the possibility that the method of preservation with toluene and chloroform might influence the result, the agglutination titer of Type III antipneumococcus serum against the homologous organism was determined and found not to vary more than might be expected from some slight but indeterminable added dilution of the serum when the serum was mixed with (1) normal saline solution (Table II), (2) saline solution and chloroform (Table III), (3) saline solution and toluene (Table IV), and (4) saline solution, chloroform, and toluene (Table V).

TABLE V.  
*Agglutination Titer with Normal Saline Solution, Toluene, and Chloroform.*

Tube No.	Serum dilution.	Agglutination.
1	1:10	++++
2	1:20	++++
3	1:40	++++
4	1:80	0
5	1:160	0

0.5 cc. of increasing dilutions of Type III antipneumococcus serum + 0.5 cc. of normal saline solution + 0.5 cc. of toluene + 3 drops of chloroform + 3 drops of Type III pneumococcus.

Our previous experiments indicate that there are only very small amounts of, or no agglutinins for the homologous pneumococcus in the pneumonic exudate, although it is still possible that agglutinins may be present and undemonstrable in consequence of neutralization by the large amount of dissolved bacterial extract in the exudate. There is, in fact, evidence that when antipneumococcus serum is mixed with the homologous exudate a considerable amount of agglutinin is removed from the serum, as indicated by the experiment illustrated in Table VI.

In a series of tubes numbered A<sub>1</sub>, B<sub>1</sub>, and C<sub>1</sub>, after mixing varying dilutions of homologous antipneumococcus serum and Type I pneumonic exudate diluted with an equal volume of saline solution, the resulting precipitate was removed by means of the centrifuge. 10

drops of the clear supernatant fluid were then removed from each tube to fresh tubes and to each 2 drops of a suspension of Type I pneumococcus were added. As indicated in Table VI agglutination occurred only in Tube A<sub>1</sub> which contained a dilution of 1:2 of serum and 1:4 of pneumonic fluid.

When, however, to a second set of tubes numbered A<sub>2</sub>, B<sub>2</sub>, and C<sub>2</sub>, containing 0.4 cc. of increasing dilutions of the same lot of Type

TABLE VI.

*Removal of Agglutinin from Antipneumococcic Serum by Homologous Pneumococcus Exudate.*

Tube No.	Type I serum.		Normal saline solution.	Pneumonic fluid + equal volume of normal saline solution.		Final dilution.		Suspension of Pneumococci Type I.	* Agglutination.
	Amount.	Dilution.		Amount.	Dilution.	Serum.	Pneumonic fluid.		
	cc.		cc.	cc.				gtt.	
A <sub>1</sub>	0.4	Undiluted.		0.4	Undiluted.†	1:2	1:4	2	+
B <sub>1</sub>	0.4	1:10		0.4	" †	1:20	1:4	2	0
C <sub>1</sub>	0.4	1:20		0.4	" †	1:40	1:4	2	0
A <sub>2</sub>	0.4	1:10	0.4			1:20		2	+
B <sub>2</sub>	0.4	1:20	0.4			1:40		2	+
C <sub>2</sub>	0.4	1:40	0.4			1:80		2	0
A <sub>3</sub>			0.4	0.4	Undiluted.		1:4	2	0
B <sub>3</sub>			0.4	0.4	1:10		1:40	2	0
C <sub>3</sub>			0.4	0.4	1:20		1:80	2	0

\* The plus signs indicate the presence of agglutination, without regard to its degree.

† Centrifuged to remove precipitate.

I antipneumococcus serum and prepared at the same time, 0.4 cc. of normal saline solution were added in place of the pneumonic exudate and 2 drops of the same suspension of Type I pneumococcus, agglutination took place in as high a dilution as 1:20 and 1:40 of the serum. There are, therefore, substances in the exudate which inhibit agglutination or the agglutinins are removed by the precipitation.

As a control, in Tubes A<sub>3</sub>, B<sub>3</sub>, and C<sub>3</sub>, 2 drops of the same suspension of Type I pneumococcus were added to increasing dilutions of the pneumonic fluid plus an equal volume of normal saline solution. No agglutination took place at any dilution, the highest strength of the pneumonic fluid used being 1:4. The agglutination of the organisms in the mixture of antipneumococcus serum and pneumonic exudate (Tube A<sub>1</sub>) is in consequence to be ascribed to the agglutinins in the serum rather than any in the pneumonic exudate.

#### CONCLUSIONS.

Specific agglutinins for the homologous pneumococcus are lacking or present only in small amount in the pneumonic exudates due to the fixed types of pneumococci. Suspensions of fixed types of pneumococci in the supernatant fluid obtained after centrifuging the mash of the pneumonic lung give positive agglutination tests in dilutions not higher than equal parts of suspension and supernatant fluid (1:2). The pneumonic lung contains a soluble substance inhibiting agglutination of the fixed types of pneumococci by the homologous antipneumococcus serum.