STUDIES ON THE PNEUMONIC EXUDATE.

III. The Presence in the Pneumonic Exudate of a Large Amount of Specific Antigen.

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It is well known that the pneumococcus as well as certain other organisms produces in the media in which it grows a substance which may be precipitated by the homologous immune serum. This specific precipitation occurs in the filtrates of bouillon cultures, in salt solution, and bile extracts. Dochez and Avery[1] have demonstrated such a substance in the cell-free fluid of young cultures and in the blood and urine of experimentally infected rabbits and in human beings. Blake and others[2] showed that a specific precipitation is present in the peritoneal exudate of infected animals. These observations and also Krumwiede and Valentine's finding[3] of soluble antigen in the sputum of patients with lobar pneumonia, made it highly probable that antigen would be readily demonstrable in the pneumonic lung. As we have seen no publication bearing on this matter we present the following simple experiments to determine the presence or absence in the pneumonic lung of antigenic substances capable of causing a specific precipitin reaction with antipneumococcus horse serum.

Soluble Antigen in the Pneumonic Exudate. Detection by the Precipitin Method.—Blocks of a pneumonic lung (Type I pneumococcus)

[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, xxvi, 477.

[2] Blake, F. G., *J. Exp. Med.*, 1917, xxvi, 67. Avery, O. T., Chickering, H. T., Cole, R., and Dochez, A. R., Acute lobar pneumonia. Prevention and serum treatment, Monograph of The Rockefeller Institute for Medical Research, No. 7, New York, 1917, 27.

[3] Krumwiede, C., and Valentine, E., *J. Am. Med. Assn.*, 1918, lxx, 513.

were washed in normal saline solution, passed through a meat cutter, and the resulting mash was preserved in a preserve jar with toluene and chloroform. A part of this material was centrifuged and the supernatant fluid diluted with an equal volume of normal saline solution. This fluid when mixed with diagnostic horse serum showed precipitation in the tubes containing Type I antipneumococcus serum. No precipitation was noted in the tubes containing Type II and III sera.

Another portion of the same lung mash was centrifuged and the resulting supernatant fluid was pipetted off and diluted with an equal volume of normal saline solution from which increasin dilutions were further made. Five tubes were then prepared each con-

TABLE I.

Precipitation Test for Specific Antigen with Increasing Dilution of Exudate.

Tube No.	Type I serum.	Pneumonic fluid diluted with equal quantity of saline solution.		Final dilution.		Precipitation.
				Serum.	Pneumonic fluid.	
	cc.		Incubation at 37°C. for 30 min.			
1	0.4	0.4 cc. of dilution 1:10.		1:2	1:40	++++
2	0.4	0.4 " " " 1:20.		1:2	1:80	+++
3	0.4	0.4 " " " 1:40.		1:2	1:160	++
4	0.4	0.4 " " " 1:80.		1:2	1:320	+
5	0.4	0.4 " " " 1:160.		1:2	1:640	0

taining 0.4 cc. of antipneumococcus horse serum and 0.4 cc. of increasing dilutions of the fluid obtained from the pneumonic lung, as shown in Table I. In making the test a constant amount of antiserum (precipitin) was thus mixed with a progressively diminishing amount of antigen (pneumonic fluid) in the same volume. The tubes were incubated at 37°C. for 30 minutes. The precipitin tests were positive in as high a dilution as 1:320 of the pneumonic fluid.

The reaction is doubtless due to the presence in the pneumonic lung of a large amount of Type I pneumococcus extract derived from the dissolution of the organism. Tests similarly performed with other pneumonic exudates show that infection with Type I,

II, or III pneumococcus can be determined by a precipitin reaction when the exudate is mixed with the homologous serum. The reaction appears to be specific. No group reaction has been observed in these tests.

Similar tests were made maintaining the concentration of the pneumonic fluid constant and increasing the dilution of the serum, as shown in Table II. After incubation at 37°C. for 30 minutes precipitin tests were positive in as high a dilution as 1:20 of the serum.

TABLE II.

Precipitation Test for Specific Antigen with Increasing Dilution of Serum.

Tube No.	Type I serum.	Pneumonic fluid diluted with equal quantity of saline solution.		Final dilution.		Precipitation.
				Serum.	Pneumonic fluid.	
		cc.	Incubation			
1	0.4 cc.	0.4	at 37°C.	1:2	1:4	++++++
2	0.4 " of dilution 1:10.	0.4	for 30	1:20	1:4	++
3	0.4 " " " 1:20.	0.4	min.	1:40	1:4	0
4	0.4 " " " 1:40.	0.4		1:80	1:4	0
5	0.4 " " " 1:80.	0.4		1:160	1:4	0
6	0.4 " " " 1:160.	0.4		1:320	1:4	0
7	0.4 " " " 1:320.	0.4		1:640	1:4	0

CONCLUSION.

In lobar pneumonia due to the fixed types of pneumococci a specific precipitin reaction is obtained when the pneumonic exudate is mixed with the homologous antipneumococcic serum.