THE ELABORATION OF SPECIFIC SOLUBLE SUBSTANCE BY PNEUMOCOCCUS DURING GROWTH.

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(Received for publication, June 1, 1917.)

From the study of bacterial infections in man and the lower animals evidence has been accumulated that pathogenic organisms do not produce harmful effects from their mere physical presence. The general reaction or toxemia of infection is differentiated from the local process which in many instances is the only tangible expression of bacterial invasion. Substances of a harmful nature seem to pass out from the bacteria and through the circulating medium of the animal to injure cells and organs at a distance from the site of infection. An explanation of the nature and mode of action of these substances has been one of the great problems in the study of infection. Certain bacteria, such as diphtheria, tetanus, and others, when grown in artificial media form a soluble toxin whose action when injected into animals differs in no way from that manifested when the same bacteria grow in living tissue. The majority of pathogenic bacteria are not known to form these soluble toxins during their life processes. The capacity of the latter to intoxicate has been explained by their setting free upon death a toxin which during life is retained within the cell body. Some investigators hold the view that intoxication by these bacteria arises in the body of the infected animal from the splitting of the bacterial protein into toxic degradation products. These explanations of bacterial intoxication are not so satisfactory nor so well substantiated as are the facts concerning infection with bacteria which produce known soluble toxins.

Pneumococcus is a highly pathogenic microorganism which is not known to secrete a soluble toxin, and whose harmful effects are supposed to be due either to the setting free of intracellular toxins or

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to the formation on disintegration of toxic split products. In the present paper it is shown that this organism during the early stages of its growth forms a readily soluble substance which diffuses into the culture medium *in vitro*, and in human and animal infections is present in the circulating blood, whence it passes through the kidneys into the urine. We have not as yet been able to demonstrate with certainty that this substance is responsible for the intoxication that accompanies lobar pneumonia.

Formation of a Soluble Substance in Culture Media.

In 1897 Kraus[1] demonstrated for the first time the presence of specific precipitable substances in the germ-free culture filtrates of certain bacterial species. This work was subsequently extended by other observers so that it is now known that a large number of bacteria give rise to these substances in the media in which they grow. The precipitin reaction obtained with these substances is strictly specific and occurs only when an homologous immune serum is used. These culture fluids have been studied after the bacteria have grown in them for 24 hours or more and their presence has been supposed to be due to the passing into solution of the bacterial substance upon disintegration of the cells. The same precipitable substances may be demonstrated in the bacteria-free salt solution or distilled water extracts of organisms grown on solid media.

Neufeld[2] has shown that solutions of pneumococcus obtained by the addition of small amounts of bile to bouillon cultures produce a specific precipitate in the presence of immune rabbit serum. Wadsworth[3] has obtained similar results not only with bile solutions but also with filtered salt solution extracts of pneumococcus. Panichi[4] demonstrated the presence of a specific precipitable substance in the filtrate of bouillon cultures of pneumococcus.

The fact which we wish to emphasize in this study is that pneumococcus from the time it starts to grow elaborates in the medium of its environment a specific substance of bacterial origin in considerable amounts and that the early presence of this soluble substance is not attributable to the death and subsequent disintegration of the bacterial cell, but represents the extrusion into the medium of bacterial substance during the life processes of the organism.

[1] Kraus, R., *Wien. klin. Woch.*, 1897, x, 736.
[2] Neufeld, F., *Z. Hyg. u. Infectionskrankh.*, 1902, xl, 54.
[3] Wadsworth, A., *J. Med. Research*, 1903–04, x, 228.
[4] Panichi, L., *Centr. Bakteriol., Ite Abt., Orig.*, 1907, xliii, 188.

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TABLE I.

Rate of Growth of Culture.

No. of hrs.	Colonies per cc.
0	810,000
2	1,170,000
4	26,000,000
6	245,000,000
8	297,000,000
12	382,000,000
24	No growth from 0.0001 cc.
48	" " " 0.1 "

Titration of Precipitin Reaction in Culture Fluid.

Dilution.	After 0 hrs.	After 2 hrs.	After 4 hrs.	After 6 hrs.	After 8 hrs.	After 12 hrs.	After 24 hrs.	After 48 hrs.
1:1	−*	±	+++					
1:5	−	−	++					
1:10	−	−	±	+++	+++	+++	+++	+++
1:15	−	−	±	+++	+++	+++	+++	+++
1:20	−	−	±	+++	+++	+++	+++	+++
1:25	−	−	±	++	++	+++	+++	+++
1:30	−	−	±	+	++	++	++	++
1:40	−	−	−	+	+	++	++	+
1:50	−	−	−	±	+	+	+	+
1:60	−	−	−	±	±	±	±	±
1:80	−	−	−	±	±	±	±	±
1:100	−	−	−	−	−	±	±	±
1:120	−	−	−	−	−	±	±	±
1:160	−	−	−	−	−	−	−	±
1:200	−	−	−	−	−	−	−	±
1:240	−	−	−	−	−	−	−	±
Control.	−	−	−	−	−	−	−	−

* − indicates no reaction; ±, faint trace; +, visible precipitation; ++, flocculation; +++, heavy flocculation.

In the following experiment a flask of bouillon was incubated with a small amount of an early, rapidly growing culture of pneumococcus. A young culture was chosen in order to avoid the occurrence of bacterial lag, during which some cell death occurs. At varying intervals during the growth of the culture, fractions were withdrawn from the flask, freed from bacteria, and the cell-free fluid was tested for the

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presence of precipitable substances. A bacterial count was made of each specimen in order to determine that the culture was growing at a maximum rate and that little or no cell disintegration had occurred at the time when this substance was already present in considerable amounts. The quantity of precipitable substance present in a given specimen was determined by ascertaining the maximum dilution of the cell-free fluid at which precipitation occurred on the addition of homologous antipneumococcus serum.

A protocol in which Type III pneumococcus was studied is given, since this organism forms a large amount of soluble substance, whereas Type II and Type I form lesser amounts in the order named.

The bacterial counts in the experiment given (Table I) show that the cultures grew at a maximum rate for about 12 hours. Chesney[5] has shown in an elaborate study that during this period the bacteria increase in geometric progression and that the curve of generation time may be plotted as an ascending straight line. From this the deduction may be drawn that during the first 12 hours little or no cell death occurs. Examination of the precipitin reaction with the bacteria-free filtrates of specimens removed from the culture during the first 12 hours of growth reveals the fact that the bacterial substance passes into solution in the culture medium in easily demonstrable amounts during this time. This would seem to indicate that this soluble substance is not the result of bacterial disintegration but represents an actual extrusion of the cell substance into the medium during the life processes of the organism.

Presence of Soluble Substance Derived from Pneumococcus in the Blood and Urine of Infected Rabbits.

The demonstration that pneumococcus during its growth in fluid media gives rise to a soluble substance suggested the likelihood that the same substance might be detected in the body fluids of experimentally infected animals. In order to test this assumption, a rabbit was injected intraperitoneally with 1 cc. of the blood of a rabbit infected with pneumococcus. At varying intervals after infection specimens of blood were collected from the heart, and the serum,

[5] Chesney, A. M., *J. Exp. Med.*, 1916, xxiv, 387.

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freed from cells, was passed through a Berkefeld filter in order to remove the organisms that had reached the blood stream. The bacteria-free serum was then tested for the presence of precipitable substances by the addition of homologous immune serum. The urine of these rabbits was also tested to find out whether the soluble bacterial substance passed through the kidneys and could be demonstrated by the precipitin reaction in the animal's urine. In Table II an example is given in which the rabbit had been infected with a Type II pneumococcus.

TABLE II.

Pneumococcus Precipitin Reaction in the Blood Serum of a Rabbit Infected with Pneumococcus Type II.

Time.	Before infection.		2 hrs. after infection.		4 hrs. after infection.		6 hrs. after infection.		8 hrs. after infection.	
Type serum....	I	II	I	II	I	II	I	II	I	II
Result.....	−*	−	−	±	−	+±	−	++	−	++

* − indicates negative; ±, faint trace; ++, marked flocculation.

Tests of rabbit urine cannot be made at regular intervals because of the failure of the animal to void frequently. Specimens at the end of 24 hours, however, showed a marked precipitate when mixed with the serum corresponding in type with the organism with which the animal was infected.

The experiment given in Table II demonstrates the fact that within a short period of time after intraperitoneal injection of a rabbit with pneumococcus there is present in the filtered blood serum a specifically reacting bacterial substance of pneumococcus origin. This substance readily passes from the blood through the kidneys into the urine and can there be demonstrated in considerable concentration.

Presence of Soluble Substance Derived from Pneumococcus in the Blood and Urine of Patients Suffering from Lobar Pneumonia.

The fact that the pneumococcus forms a readily soluble substance during growth in artificial media and in the body fluids of animals

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experimentally infected makes it not unreasonable to assume that the same substance is formed by pneumococcus during the course of natural infection in human beings. In order to find out whether or not this is so, the blood sera and urine of a large number of patients suffering from pneumonia due to pneumococcus of Types I, II, and III were studied for the presence of this soluble material. Specimens of serum were obtained at varying intervals during the disease and the urine was examined frequently throughout the course of the infection and during convalescence. If the precipitin reaction was not positive with the whole urine, a method of concentrating the urine was employed. It has been found that the soluble substance to which the pneumococcus gives rise is precipitated by alcohol and after precipitation is again readily soluble in water. In order to concentrate the precipitable substance in urine to 25 cc. or more of the 24 hour specimen a few drops of acetic acid are added and the urine is then boiled down to a volume of 5 cc., filtered through paper to remove any precipitate of albumin that may occur, and the filtrate added to eight to ten volumes of 95 per cent alcohol. The precipitate which forms is collected by centrifugalization and dried to remove the excess of alcohol and the residue extracted with 2 or 3 cc. of salt solution which redissolves the specific substance. Any insoluble material is removed by centrifuging and the clear salt solution extract used in the precipitin test. Results of this study are presented in Table III.

In Table III are presented the studies on the presence of the specifically precipitable substance in the blood serum during life of a number of patients suffering from lobar pneumonia. Almost all the patients studied showed a strongly positive precipitin reaction in the urine and were chosen for the purpose of finding out whether at a time when the substance was being excreted in largest amounts it could also be demonstrated in the circulating blood. In all, 25 cases were examined, of which 10 were due to infection with Type I pneumococcus, 11 with Type II, and 4 with Type III. Of the Type I infections, none gave a positive precipitin reaction in the serum, although in all but one the urine was positive at the time the tests were made and in three instances the reaction in the urine was heavy, indicating the excretion of the soluble substance in considerable

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TABLE III.

Pneumococcus Precipitin Reaction in the Blood Serum during Lobar Pneumonia.

Case No.	Blood test.		Blood culture.	Urine.	Serum treatment.	Result of disease.	Remarks.
	Day of disease.	Result.					
Pneumococcus Type I infection.							
2,821	6	−	−	++	+	Recovered.	Total, 10 cases; 2 died.
2,816	8, 9, 10, 11, 12	−	+	++	+	Died.	
2,901	5, 7, 9	−	+	++	+	"	
2,968	6, 7	−	+	+	+	Recovered.	
2,936	7, 9	−	−	±	+	"	
2,815	4	−	−	+	+	"	
2,824	3	−	−	±	+	"	
2,883	5, 6, 7	−	−	−	+	"	
2,858	3, 4, 5	−	−	+	+	"	
2,891	3	−	−	+	+	"	
Pneumococcus Type II infection.							
2,885	3, 4, 6	−	−	+	+	Recovered.	
2,845	6	++	+	++	+	Died.	
2,868	4	−	−	−	−	Recovered.	
	7			+			
2,829	1	−	−	−	−	"	
2,879	2	−	+	−	+	"	
	7			+			
2,892	5, 6	+	+	++	−	Died.	
2,834	6, 7, 9, 10, 11	−	+	++	+	"	
2,922	4, 5, 6	−	−3	+	+	Recovered.	
			+7				
3,006	2, 3	−	−	++	−	Died.	Blood culture positive on 6th day.
3,031	3, 5, 7	+	+	++	+	"	
2,869	3, 4, 5	+	±	+	+	"	
Pneumococcus Type III infection.							
2,898	5, 6, 7	++	+	++	−	Died.	Total, 4 cases.
2,947	6, 10, 11	±	−	+	−	Recovered.	
2,797		+	−	+	−	Died.	
2,783	7	+	+	+	−	"	

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quantity. The failure to demonstrate the substance in blood in Type I pneumonias may be partly attributable to the fact that all these cases were treated with Type I antipneumococcus serum, which is known to cause the disappearance of the substance from the urine in many cases during treatment. It has also been shown by *in vitro* experiments that Pneumococcus Type I forms less of the soluble substance than organisms of Types II and III. The sera of 11 cases of Type II pneumonia were studied. The urine reaction was positive in 10 of these, while the precipitin reaction in the blood was positive in 4 instances. Of these 11 cases 7 were treated with Type II serum. Among the 7 serum treated cases, 3 showed a positive precipitin reaction in the blood. Of the 4 cases not treated with serum, only 1 gave a positive blood test. A positive blood culture was obtained in 7 of the 11 cases studied. Of the 4 cases with a positive precipitin test in the serum, all showed a positive blood culture, while of the 7 cases with a negative precipitin test in the serum, 3 had a positive blood culture. All 4 patients showing a positive precipitin reaction in the blood serum died, whereas of the 7 with a negative serum test, 2 died and 5 recovered.

4 cases of pneumonia due to Pneumococcus Type III were studied. All gave a positive precipitin test in the blood serum. Blood cultures were positive in 2 instances and the precipitin reaction in the urine was positive in all. The infection was fatal in 3 of the 4 patients.

In Table IV are presented the results of the examination of the urine for the precipitable pneumococcus substance in 88 cases of pneumonia due to the fixed types of pneumococcus I, II, and III. Of these 88 cases, 35 were Type I, 28 were Type II, 8 were Type II (atypical), and 17 were Type III. Repeated tests of the urine were made during the course of the disease from within 12 hours after onset in one instance to the 58th day in another. Of the 35 cases due to Type I infection, 20 were positive and 15 negative. A positive blood culture occurred in 13 of the 35 cases. Among the 13 cases with positive blood culture, 9 showed a positive urine reaction and 4 gave a negative result. Of the 20 cases with positive urine reaction, 2 died; of the 14 negative cases all recovered. All these instances of Type I infection were serum treated. The administration of serum

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TABLE IV.

Pneumococcus Precipitin Reaction in the Urine during Lobar Pneumonia.

Case No.	Urine reaction.	Day of disease when urine was first tested.	Day of disease when urine was first positive.	Duration of positive reaction.	Blood culture.	Serum treatment.	Result of disease.	Remarks.
Pneumococcus Type I infection.								
				days				
2,816	++	6	6	6	+	+	Died.	
2,936	+	6	6	18	−	+	Recovered.	
2,968	++	5	5	14	+	+	"	
2,858	+	3	4	1	−	+	"	
2,996	+	3	3	15	−	+	"	
2,891	+	3	3	5	−	+	"	
2,965	+	6	6	16	−	−	"	
2,952	+	2	5	3	−	+	"	
2,804	+	6	6	26	−	+	"	
2,955	++	6	6	26	+	+	"	
2,925	+	1	1	1	+	+	"	
2,908	+	5	13	5	−	+	"	
2,924	+	5	5	22	−	+	"	
2,913	+	5	5	1	−	+	"	
2,945	+	5	5	29	+	+	"	
2,906	+	2	2	31	+	+	"	Delayed resolution.
2,852	+	4	4	2	+	+	"	
2,949	+	8	11	24	−	+	"	
2,901	+	5	5	4	+	+	Died.	
2,917	−	6	−	−	−	+	Recovered.	
2,814	−	2	−	−	−	+	"	
2,944	−	5	−	−	−	+	"	
2,874	−	3	−	−	+	+	"	
2,815	−	4	−	−	−	+	"	
2,824	−	3	−	−	−	+	"	
2,821	−	6	−	−	−	+	"	
2,984	−	7	−	−	−	+	"	
3,020	−	3	−	−	+	+	"	
3,043	−	3	−	−	−	+	"	
3,011	−	5	−	−	+	+	"	
2,883	−	4	−	−	−	+	"	
2,954	−	4	−	−	−	+	"	
2,880	−	3	−	−	+	+	"	
3,019	+	4	8	42	+	+	" (empyema).	
3,033	−	2	−	−	−	+	Recovered.	

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TABLE IV—*Continued.*

Case No.	Urine reaction.	Day of disease when urine was first tested.	Day of disease when urine was first positive.	Duration of positive reaction.	Blood culture.	Serum treatment.	Result of disease.	Remarks.
Pneumococcus Type II infection.								
				days				
2,869	++	2	2	2	+	+	Died.	
2,922	+	3	3	40	+	+	Recovered.	
3,006	+	2	2	8	−	−	Died.	
2,885	+	2	2	2	−	+	Recovered.	
2,845	++	5	5	2	+	+	Died.	
2,868	+	4	7	3	−	−	Recovered.	
2,899	−	1	−	−	−	−	"	
2,879	−	2	7	1	+	+	"	
2,892	++	5	5	2	+	−	Died.	
2,834	++	5	5	5	+	+	"	
2,991	+	1	2	58	−	−	Recovered.	
2,854	−	3	5	1	−	−	"	
3,003	+	7	7	13	−	−	"	
2,827	++	3	3	1	+	−	Died.	
2,746	+	3	6	9	+	−	"	
2,881	+	6	6	2	−	−	Recovered.	
2,786	−	6	−	−	−	−	"	
2,896	−	7	−	−	−	−	"	
2,926	−	4	−	−	−	−	"	
2,897	+	3	4	1	−	+	"	
2,886	+	3	3	6	−	−	"	
2,890	+	3	3	4	+	+	Died.	
2,971	+	7	14	10	−	−	Recovered.	
2,825	++	1	1	5	+	−	Died.	
3,031	+	3	3	5	+	+	"	
3,047	+	3	3	?	−	−	Recovered.	
2,937	−	1	−	−	−	−	"	
2,934	−	4	−	−	−	−	"	
Pneumococcus Type II (atypical) infection.								
2,861a	−	3	−	−	+	−	Recovered.	
2,878x	−	4	−	−	−	−	"	
2,822b	−	5	−	−	−	−	"	
2,831x	+	4	4	3	−	+	"	
2,864b	+	8	8	2	+	−	Died.	
2,960x	−	1	−	−	−	−	"	
2,963b	+	4	7	13	−	−	Recovered.	
2,935b	−	1	−	−	−	−	"	

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TABLE IV—*Concluded.*

Case No.	Urine reaction.	Day of disease when urine was first tested.	Day of disease when urine was first positive.	Duration of positive reaction.	Blood culture.	Serum treatment.	Result of disease.	Remarks.
Pneumococcus Type III infection.								
				days				
2,838	−	3	−	−	−	−	Recovered.	
2,889	−	7	−	−	−	−	"	
2,797	++	2	2	5	−	−	Died.	
2,898	++	5	5	3	+	−	"	
2,947	++	5	5	30	−	−	Recovered (empyema).	
2,812	+	2	5	1	+	−	Died.	
2,783	+	7	7	1	+	−	"	
2,919	+	5	6	4	−	−	"	
2,800	+	3	3	3	+	−	"	
2,849	+	3	3	2	+	−	"	
2,927	+	2	6	4	−	−	Recovered.	
2,973	+	3	10	4	−	−	"	
2,911	+	2	2	13	−	−	"	
2,837	−	1	−	−	−	−	"	
2,972	+	1	6	2	−	−	"	
2,485	−	6	−	−	+	−	Died.	
2,918	−	2	−	−	−	−	Recovered.	

Summary of Urine Reaction.

Type.	Total No. of cases.			Per cent positive.	Positive blood culture.	Positive urine reaction.		Negative urine reaction.		Fatal cases showing urine reaction.	
	Examined.	Positive.	Negative.			With blood culture positive.	With blood culture negative.	With blood culture positive.	With blood culture negative.	Positive.	Negative.
				per cent							
I	35	20	15	57.1	13	9	11	4	11	2	0
II	28	20	8	71.4	11	10	10	1	7	10	0
II (atypical)	8	3	5	37.5	2	1	2	1	4	1	1
III	17	12	5	70.5	6	5	7	1	4	7	1
	88	55	33	62.5	32	25	30	7	26	20	2
IV	10	0	10	0	2	0	0	2	8	0	2
Cases of respiratory disease due to organisms not pneumococcus.	14	0	14	0	2	0	0	2	12	0	2

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in Type I pneumonia often results in the temporary disappearance of the substance from the urine. Upon cessation of treatment the soluble substance may reappear in the urine.

Of 28 cases of Type II infection, 20 gave positive precipitin reaction in the urine, and 8 were negative. A positive blood culture occurred in 11 of the 28 cases. Among the 11 cases with positive blood culture, 10 gave a positive urine reaction and 1 a negative result. Of the 20 cases with a positive urine test, 10 died; of 8 negative cases all recovered.

Among 8 cases of infection with atypical Type II pneumococcus, 3 gave a positive precipitin reaction in the urine and 5 a negative reaction. Inasmuch as a normal Antipneumococcus Serum Type II was used in determining the presence of soluble substance in the urine of individuals infected with atypical Type II pneumococcus, a lower percentage of positive urine reactions should be expected in this series, since the precipitin titer of normal Type II serum is low for these atypical organisms.

17 cases of pneumonia due to infection with Pneumococcus Type III were studied. Pneumococcus precipitinogen was demonstrated in the urine of 12 of these instances, and was absent in 5. A positive blood culture was obtained in 6 of the 17 cases. Of the 6 cases having pneumococcus septicemia, 5 showed a positive precipitin reaction in the urine. 7 of the 12 cases giving a positive urine test died, while 4 of the 5 negative cases recovered.

A summary of 88 cases of pneumonia due to the fixed types of pneumococcus shows that the soluble substance of pneumococcus origin was demonstrable in the urine of 55 (62.5 per cent) of these patients at some stage of the disease and in 39 instances was positive on the first examination. Among the 55 cases with a positive precipitin reaction in the urine, 20 had a fatal outcome, giving a mortality of 36.4 per cent, and of the 33 cases with a negative urine test, 2 died; a mortality of 6 per cent. In addition to the 88 individuals suffering from pneumonia due to the fixed Types I, II, and III, 10 cases of Type IV pneumonia and 14 cases of respiratory disease due to other organisms were studied for the presence of a precipitin reaction in the urine. Each urine was tested with standard Antipneumococcus Sera Types I, II, and III. In no instance was a posi-

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tive reaction obtained at any stage of the disease, a fact which establishes beyond doubt the specificity of the reaction.

In addition to the presence of the soluble substance of pneumococcus origin in the blood and urine, it has also been found in other body fluids. In certain cases it can be readily demonstrated in pleural fluids and pericardial exudates and in the spinal fluid of pneumococcus meningitis.

Certain facts have been ascertained concerning the chemical characteristics of this substance. The specific substance is not destroyed by boiling. It is readily soluble in water and is precipitable in acetone, alcohol, and ether, after which it may be easily redissolved in water. It is precipitated by colloidal iron, and does not dialyze through parchment. The immunological reactions of the substance are not affected by proteolytic digestion with trypsin and it is not split by urease. The determination of total nitrogen and nitrogen partition on the active substance obtained by repeated precipitation with acetone and alcohol shows the substance to be of protein nature or to be associated with protein.

One of the chief points of interest in the discovery of the soluble substance of pneumococcus is whether this substance is in any way responsible for the intoxication which attends pneumococcus infection. Studies to ascertain the answer to this question are being actively carried on at the present time but have not as yet progressed to the point at which a definite answer can be given. It may be said, however, that its toxicity is in no way comparable to that of diphtheria toxin. On the other hand, it possesses a degree of toxicity which, exhibited throughout the course of an infection, may account for the signs of intoxication in lobar pneumonia.

DISCUSSION.

The preceding experimental data have shown that a specifically reacting substance of pneumococcus origin occurs in the bacteria-free filtrates of young cultures of pneumococcus and also in the blood serum and urine of patients during lobar pneumonia. The occurrence of specifically precipitable substances in the cell-free filtrate of bacterial cultures has been known ever since the early obser-

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vation of Kraus. It has been abundantly confirmed by other investigators with a variety of bacteria. In general, the presence of this precipitable substance has been demonstrated in culture fluids so old that an opportunity has occurred for cell death and disintegration and consequent solution of bacterial protein. In this paper it is shown that there is present in solution in the culture fluid in which pneumococcus is grown, a soluble substance in considerable amounts at a time when no cell death or disintegration has occurred. Consequently this substance does not represent dead dissolved bacterial protein, but the elaboration and passage into solution of a substance which is the product of the life activity of the cell. In addition to the evidence already cited in support of this fact, it has been demonstrated that the soluble substance is present in culture fluids in considerable concentration at a time when no hemolysin is present. This pneumococcus hemolysin is an intracellular body which does not appear in culture fluids until destruction of the bacterial cell has taken place; hence if the soluble substance described were purely of intracellular origin the curve of its concentration in culture fluids would be coincident with that of the hemolysin. This, however, is not the case, for the curve of hemolysin does not begin to rise until a time when the curve of the soluble substance has almost attained its maximum elevation.

The formation of a soluble substance by the pneumococcus on growth *in vitro* suggested the probability that an analogous substance would be formed on growth of the organism in the animal body and because of the readiness with which the substance passes into solution one would expect no difficulty in demonstrating it in the body fluids of experimentally infected animals. Examination of the blood and urine of rabbits infected with pneumococcus has shown this substance to be present in considerable quantities following intraperitoneal infection. Ascoli and Valenti[6] have demonstrated in the organisms of animals infected with anthrax a substance specifically precipitable with antianthrax serum. Bail[7] has shown the presence of a substance in the exudates of animals infected with anthrax which, when the fluids were freed from bacteria, increased the infectious

[6] Ascoli, A., and Valenti, E., *Centr. Bakteriol., Ite Abt., Ref.*, 1911, xlviii, 243.
[7] Bail, O., *Arch. Hyg.*, 1905, lii, 272; 1905, liii, 302.

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power of anthrax bacilli. This substance he has called aggressin and he considers it to be an excretory product of the anthrax bacillus which favors the invasion of animal tissues by this organism. It is possible that the substances described by Ascoli and Valenti, and Bail are similar in the mechanism of their formation to the soluble substance produced by the pneumococcus. Although our study of other bacteria has been rather limited, it has been demonstrated that certain other species, such as meningococcus, *Bacillus typhosus*, and *Bacillus dysenteriæ*, also give rise to soluble material during their growth in fluid media.

A study of the serum of patients suffering from lobar pneumonia has shown that this soluble specific substance is also present in the circulating blood during the course of the disease in man. It gives a specific precipitin reaction with antipneumococcus serum corresponding in type to the organism with which the individual is infected. This soluble precipitable substance in human serum is less frequently present in demonstrable quantities than in the serum of experimentally infected animals. However, it has been found both when pneumococci are present in the circulating blood and when by blood culture organisms are absent. Complement fixation, as well as the precipitin reaction, may be used for the demonstration of this substance in serum. Although the soluble substance is relatively infrequently present in demonstrable quantities in the circulating blood, it is not unlikely, from the fact that the substance appears in a much larger percentage of cases in the urine, that it is much more commonly present in the blood than observed, but in quantities that are below the threshold of demonstration.

A study of the urine in 112 cases of lobar pneumonia and closely related respiratory diseases has shown that in 62.5 per cent of pneumonia due to Pneumococcus Types I, II, and III, a substance is excreted in the urine which reacts specifically with antipneumococcus serum of the type corresponding to the organism with which the individual is infected. This substance may appear as early as 12 hours after the initial chill, or may appear for the first time at a later stage of the disease, and may continue to be excreted for many days after recovery has occurred. In certain instances in which excretion occurred over a long time, its persistence in the urine could

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be explained by delayed resolution, a condition which represents the passage of the acute pneumococcus infection of the lung into one of a more chronic character. In other instances of continued excretion not explainable on these grounds, the substance must have been stored in the tissues and must have passed into the circulating blood to be excreted by the kidneys without loss of its specific character. It is the rule to find the substance in the urine when pneumococcal septicemia exists. The amount of precipitable substance in the urine seems to be a measure of the severity of the infection. This fact may be dependent upon the quantity of the substance being directly proportional to the actual amount of infection or it may be that the amount of this substance formed bears some relationship to the virulence of the particular strain of pneumococcus responsible for the infection. Most of the instances which fail to show the presence of a precipitable substance in the urine recover, whereas the mortality is high among those in which its presence is demonstrable. If large amounts are excreted the outcome is usually fatal, unless this result is prevented by the administration of antipneumococcus serum. The specific precipitin test in the urine is therefore of considerable prognostic value. It may also be used in making a rapid diagnosis of the type of organism with which an individual is infected and in our experience a positive test in the urine is quite as reliable as the agglutination of the organism isolated from the sputum. The precipitin test in the urine, however, should not supplant the usual diagnostic technique in the determination of the type of pneumococcus.

11 years ago Fornet[8] claimed to have demonstrated in the serum and urine of patients suffering from typhoid fever a substance specifically precipitated by antityphoid serum. From what we now know it would seem likely that his observations were correct despite the fact that subsequent investigators failed to confirm them.

Ascoli[9] has shown that precipitinogen may pass the kidneys and appear in the urine where it exhibits its specific reaction. We have been able to show that if rabbits are inoculated intravenously with

[8] Fornet, O., *Münch. med. Woch.*, 1906, xxxviii, 1862.

[9] Ascoli, M., cited in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, ii, 750.

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soluble pneumococcus material a specific precipitin reaction can be obtained in the urine within 24 hours and that the specific substance continues to be excreted for a number of days. In such an experiment the material injected contained no formed living pneumococci. Pettit[10] has demonstrated that if rats are injected with diphtheria toxin, this substance can be shown to be excreted in the urine in active form following the inoculation.

SUMMARY.

1. A specifically reacting substance of bacterial origin is present in the cell-free fluids of young cultures of pneumococcus. This substance is present when the organisms are growing at their maximum rate and undergoing little or no cell death, and consequently its presence is not dependent upon cell disintegration but represents the extrusion of bacterial substance by the living organism.

2. The blood and urine of rabbits experimentally infected with pneumococcus contain a similar specific soluble substance during the early hours of the infectious process.

3. Human beings suffering from lobar pneumonia have in their blood and more frequently in their urine a specific soluble substance of pneumococcus origin. The amount of this substance present in the urine varies in different individuals and the presence of a large amount is of unfavorable prognostic import. This specific precipitin reaction in the urine is of diagnostic value.

4. Rabbits injected with soluble pneumococcus material continue to excrete this substance for a considerable period of time.

5. The specifically soluble substance obtained from bacterial cultures and from the urine during infection is not destroyed by boiling, by precipitation with alcohol, acetone, or ether, or by trypsin digestion.

6. Studies are in progress at this time on the degree of toxicity and on the antigenic properties of the substance.

[10] Pettit, A., *Ann. Inst. Pasteur*, 1914, xxviii, 663.

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