THE URINARY EXCRETION OF S SUBSTANCE IN LOBAR PNEUMONIA*

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It is well known that the type specific carbohydrate fraction of the pneumococcus, so-called soluble specific substance, or S substance, is excreted in the urine during and following pneumococcus lobar pneumonia. However, the number of careful determinations in which the attempt has been made to relate the presence and amount of urinary S substance to the daily clinical course of the disease is small. Because of the scarcity of such reports, it is the purpose of this study to follow the excretion of S substance in a group of cases of pneumococcus lobar pneumonia due to types I, II, and III, as well as in a group due to the higher types, with special attention to the excretion of S substance in cases of empyema following pneumonia.

Dochez and Avery[1] first called attention to the presence in the urine of a soluble specific substance which they found quite frequently in cases of lobar pneumonia during some stage of the disease. These authors,[2] a little later, reported a series of 112 cases of lobar pneumonia and related respiratory infections, and found that in 62.3 per cent of cases of pneumonia due to types I, II, and III the S substance could be demonstrated in the urine at some time of the disease from 12 hours after the original chill to five weeks after the defervescence. Two cases in this series, one a type I and the other a type III, developed empyema and were found to be excreting S substance as late as the 50th and 33rd days respectively. Long continued excretion in other cases was explained on the basis of non-resolution or a storing of S substance in the tissues. Blake[3] followed 15 cases of lobar pneumonia due to types I and II and one case of pneumonia due to Friedländer's bacillus and reported that 11, or 73.3 per cent, of the types I and II cases excreted the S substance at some time during the early and late stages of the disease, as did also the one case caused by Friedländer's bacillus. One case in Blake's series developed empyema and excreted S substance on the 27th, 28th, and 29th days of the disease.

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On the basis of the frequency with which these authors reported the presence of the S substance in the urine in lobar pneumonia due to the pneumococcus types I, II, and III, many laboratories have attempted to use this knowledge as a means of rapid determination of the type of pneumococcus in cases of lobar pneumonia. It is our belief that this has proved of early diagnostic value in but a small percentage of such cases.

Trask *et al.*,[4] studying pneumonia in children, found that the S substance was not often detected in the urine of cases of lobar pneumonia and was found by them only in cases due to type I pneumococcus. A systematic study was not made in cases due to pneumococci of the other types. Six non-fatal type I cases excreted S substance during the active stage of the disease, as in adults; but in two, the S substance increased coincidentally with recovery, and in the other four it was found in the same amounts throughout the febrile and postcritical periods of the disease.

To our knowledge, there are no reports in the literature which deal with the excretion of S substance in the urine from cases of lobar pneumonia caused by the many types of pneumococci which are recognized as members of the so-called group IV.

Methods

It has been our main object to observe and to quantitate roughly the amount of S substance excreted during early and late stages of lobar pneumonia and to relate its presence or absence to the clinical course of the disease. To do this a practical method for the concentration of S substance in the urine was the first consideration. The method recommended by Dochez and Avery[2] for concentration of the S substance in the urine consisted in evaporating 25 cc. of slightly acidified urine to 5 cc., filtering through paper, and adding the filtrate to from eight to ten volumes of 95 per cent alcohol. The precipitate was collected by centrifugalization, dried, and taken up in 2 to 3 cc. of salt solution. This clear salt solution was then used in precipitin tests. In our present study this method was used only for the purpose of checking the results obtained with other methods and not throughout the study because of the large amount of alcohol necessary to carry out a long series of determinations.

Two other methods were devised. The first was an ultrafiltration method using collodion sacs, Dochez and Avery[2] having shown

that the S substance is not dialyzable. These sacs were prepared from a 7 per cent solution of du Pont's parlodion dissolved in a solution of 75 per cent by volume of ether and 25 per cent by volume of absolute alcohol. The standard adopted as to the suitability of the sacs was that they should be impermeable to hemoglobin. In making a sac the inside of a test-tube was coated with the collodion solution, drained and allowed to dry for 15 to 20 minutes. The tube was filled with 70 per cent alcohol and allowed to stand for one-half hour. Tap water was then substituted for the alcohol for the same length of time. The sacs were then found to have contracted slightly from the tube wall and could be drawn out with relative ease. Each sac was tested for leaks by subjecting it to a negative pressure of 125 mm. of mercury and one in each lot was tested for permeability to hemoglobin. The selected sacs could be kept in water for a period of several days. By ultrafiltration through these sacs, 30 cc. of urine could be reduced in volume to 3 cc. in about 8 to 15 hours, using a negative pressure of about 100 mm. of mercury. This method was used with the idea of eliminating the high concentrations of salts found in boiled or evaporated urine and it was also assumed that it would be less apt to alter the properties of the antigen. The 3 cc. of urine concentrated by ultrafiltration were then filtered through paper and if the filtrate was still cloudy it was shaken with a small amount of kaolin (Merck) and centrifugalized. Precipitin tests were set up with the clear filtrate or supernatant fluid using homologous antipneumococcus serum.* Undiluted typing serum was employed in all tests and for this reason small glass tubes with a lumen diameter of 4 mm. were used. In performing the precipitin tests normal horse serum controls were used with each individual sample of urine to be tested. The precipitin tests were placed in the water-bath at 37° C. for two hours and then left in the ice-box over night to be read the next morning. Each determination represented a test on a single specimen of urine obtained between 5 and 9 A. M. No attempt was made to collect 24-hour specimens.

The second method consisted of evaporating 30 cc. of clear

* The antipneumococcus sera of Types I, II, and III were supplied by the Division of Laboratories, New York State Department of Health; sera used in the Group IV cases were supplied by the Bureau of Laboratories, Department of Health, City of New York.

urine to dryness at a temperature of about 70° C. The residue was taken up in 3 cc. of distilled water, boiled for 2 minutes to remove protein and clear the urine, and passed through filter paper. Precipitin tests were then set up as before using the clear filtrate. This method was found to be useful in some of the group IV cases in which the type of pneumococcus responsible for the disease was not determined until three or four days had elapsed after admission. During this time the evaporated residue could be kept without spoiling.

The efficacy of these two methods was tested by the following experiment. Ten cubic centimeters from an 18-hour broth culture of a virulent type I pneumococcus were boiled for 5 minutes. The solution was then filtered through paper and the filtrate centrifugalized at high speed for 20 minutes; 8 cc. of clear supernatant fluid were then removed and added to 342 cc. of normal urine. This solution gave a positive precipitin reaction when unconcentrated and a suggestively positive reaction in a dilution of 1:2. This urinary

TABLE I

COMPARISON OF THE EFFICIENCY OF TWO METHODS USED IN CONCENTRATING URINARY S SUBSTANCE

	Intensity of precipitin reactions at different dilutions										
	Undiluted	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9	1:10	1:20
I Unconcentrated	+	±	—	—	—	—	—	—	—	—	—
II Concentrated											
a. Ultrafiltration											
5 times ...	+	+	±	—	—	—	—	—	—	—	
10 times ...	++	+	±	±	±	—	—	—	—	—	—
b. Evaporation											
5 times ...	+	+	+	±	—	—	—	—	—	—	
10 times ...	++	+	—	+	+	±	±	±	—	—	—

++ = Strongly positive; + = Positive; ± = Suggestively positive;
— = Negative precipitin reaction.

solution was then concentrated five and ten times by the two methods described above and precipitin tests performed on the concentrated samples as well as on dilutions of the concentrates. The results, as demonstrated in Table I, show that there is a theoretical loss of about 50 per cent of the S substance in the evaporation method and slightly more in the method of ultrafiltration.

Results

Twenty cases of lobar pneumonia were studied and the results appear in Table II. Eleven cases were due to pneumococcus types I, II and III and 9 to pneumococcus group IV. Of the 11 cases 9 were due to type I and 2 of these developed empyema. In but 5 of the cases due to types I, II, and III was S substance demonstrable in an unconcentrated specimen of urine obtained either during or following the febrile stage of the disease. However, it was found in all but one case at some time during the disease in specimens which had been concentrated 5 or 10 times. Of the 9 cases from Group IV, in only one was S substance found in specimens concentrated 10 times. This case was a type V, and S substance was detected in the urine on the 12th to 14th days of the illness. One case due to type VIII died on his second day in the hospital with a heavy blood-stream infection without showing S substance in urine specimens concentrated 10 times. Another case due to type VIII developed empyema also, without excreting detectable S substance either early or late in the disease.

In Table II the presence of S substance has been correlated with the course of the disease. Although the degree of severity of individual cases has not been shown, it is evident here, as in other reports on this subject, that there is little relation, in point of time, to the presence of S substance in the urine except in the two type I cases with empyema. Results obtained in following the two type I cases of empyema seem particularly significant. Both of these cases (D. G. and B. G.) excreted large amounts of S substance late in the disease, one of them until the 41st day and the other until the 27th day. The clinical course of one of these patients, the amounts of S substance detected in the urine, and the agglutinin titer in the patient's blood are shown in Fig. 1. It will be seen that during the four to five days before crisis in which the patient was in the hospital and on which tests were made no urinary S substance

Table II

RELATION OF THE EXCRETION OF S SUBSTANCE IN THE URINE TO THE CLINICAL COURSE OF LOBAR PNEUMONIA

Case	Type	Day of Disease: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21 23 25 27 29 31 33 35 37 39 41
D. G.*	I	L S L L Empyema − − − − − − − 2+ − + + + + + − + − − + +
V. G.	I	C C + + − 2+ − − + − − − − − − − − −
F. V.	I	L L L L L + − + − −
A. M.	I	C + − − −
W. H.	I	L L L − − − − − − − −
B. G.	I	L L L L L L Empyema − − − − − 2+ 2+ 2+ 2+ + + + + − + +
J. G.*	I	4+ 4+ Death
A. N.*	I	S 2+ 2+ − 2+ 3+ Death
J. S.	I	C C + − + + + + − −
W. S.	II	C − + + + +
H. T.	IIa	L L L − − − − − − − − − − − − (Tests made with type II serum)
E. C.	III	L L L + + + +
R. F.	V	L L L L − − − − − − − − − + + + −
F. R.	V	L L L L − − − − − − − − − − −
S. C.	V	C C − − − − − − − −
J. L.*	VIII	− − Death
J. R.	VIII	C C − − −
G. E.	VIII	L L L L Empyema − − − − − − − − − − − − −
F. H.*	XII	L L L − − − − − − − − − − − − −
T. F.	XXVIII	L L L L − −

4+ to + = intensity of positive reaction of urinary precipitin test.
− = negative urinary precipitin test.
All results are given in terms of urine specimens which have been concentrated 10 times.

L = temperature fall by lysis.
C = temperature fall by crisis.
S = Serum.
| = admission to hospital.
* = blood culture positive.

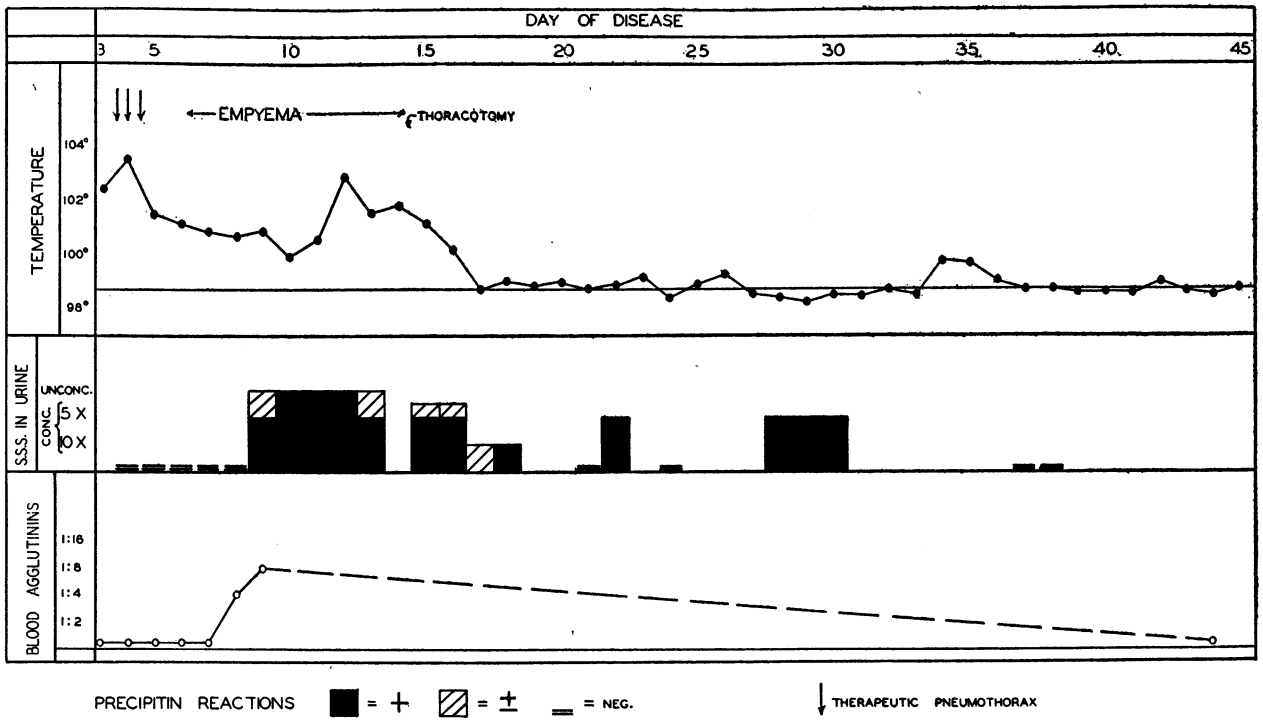
DAY OF DISEASE
3
5
10
15
20
25
30
35
40
45
TEMPERATURE
104°
102°
100°
98°
EMPYEMA
THORACOTOMY
S.S.S. IN URINE
UNCONC.
CONC.
5 X
10 X
BLOOD AGGLUTININS
1:16
1:8
1:4
1:2
PRECIPITIN REACTIONS
= +
= ±
= NEG.
THERAPEUTIC PNEUMOTHORAX

FIG. 1

was detected. At about the time when signs of empyema developed urinary S substance appeared and the amounts detected on subsequent days are shown on the chart. Urinary S substance did not appear until agglutinins for type I pneumococci appeared in the blood.

Discussion

In brief, the results bear out what other observers have found in studying urinary excretion of S substance in cases of lobar pneumonia due to types I, II, and III. In these types there is definitely a relation between the amount of S substance excreted and the severity of the disease, since all cases that died or had a blood-stream infection excreted S substance in such amounts that it was demonstrable in the unconcentrated urine. It is of interest that two type I cases developed empyema and excreted S substance late in the disease. Considering the two cases of empyema in the series of Dochez and Avery and the one case in Blake's series it would appear that late urinary excretion of S substance in empyema due to types I, II, and III pneumococcus is fairly constant.

The study of the higher types did not show the same results. There was only one patient out of the nine in whose urine S substance was found in the concentrated specimens. This is of interest because among the eight cases that did not show S substance, there was one case with a temporary bacteremia, another with a heavy blood-stream infection and early death and a third that developed empyema.

The mechanism whereby S substance is circulated through the body and excreted in the urine is unknown, but the fact that it continues to be excreted in the presence of humoral agglutinins, as shown by Blake, is found also in this series. Unfortunately, although determinations for blood agglutinins were made on most of the patients during the first ten or twelve days in the hospital, no determinations for blood precipitins were carried out.

Summary

Methods for the concentration of S substance in the urine of patients with lobar pneumonia are described and a rough quantitative estimation is given of the amounts of S substance excreted by a

group of patients with lobar pneumonia throughout the course of the disease.

These studies reveal the frequency with which urinary S substance is found in this series of cases during early and late stages of the disease and particularly in cases with empyema, in patients with infection due to types I, II, and III pneumococcus, and the infrequency with which it is found in cases due to the higher types.

References

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