8301 P

Preparative Changes Necessitated by a Quantitative Study of Precipitating Power of Pneumococcus Polysaccharides.*

MICHAEL HEIDELBERGER, FORREST E. KENDALL, AND HENRY W. SCHERP.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

In an extension of the quantitative study of the precipitin reaction between the specific polysaccharide of Pneumococcus III (S III) and homologous antibody[1] it was found that several S III preparations which precipitated the same amount of antibody from horse serum threw down widely varying quantities of antibody from a rabbit serum. The present preliminary report deals with experiments on S II and S III undertaken to find the cause of variations in the precipitating power of these substances toward homologous rabbit antisera.

1. *Specific Polysaccharide of Type III pneumococcus (S III).* A Chamberland filtrate of 24-hour cultures in phosphate meat infusion glucose broth was concentrated about 12-fold *in vacuo* and precipitated several times with an equal volume of alcohol. The caseous S III was redissolved and separated from protein over the barium salt with barium chloride. Barium was removed by repeated reprecipitation of the S III with glacial acetic acid after addition of sodium acetate. The properties of several lots obtained in this way are given in the table. Preparations in which heat and strong acid were avoided showed far greater viscosity than did samples prepared by the original method.[2] Comparison of viscosities was made in 0.9% salt solution.[3] As will be seen from the table a new preparation precipitates approximately the same amount of antibody from serum as does the original culture filtrate. The new preparations precipitate more antibody from Type III antipneumococcus rabbit serum than those prepared by the original method, although all precipitate about the same amount of antibody from homologous horse antiserum. The sera used were first absorbed with "C"-poly-

* The work reported in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital, New York.

1 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **61**, 563.

2 Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 727.

3 Heidelberger, M., and Kendall, F. E., *J. Biol. Chem.*, 1932, **95**, 127.

TABLE I.
Properties of Specific Polysaccharides of Pneumococcus II and III.

Preparation No.	Ash as Na	Nitrogen*	$[\alpha]^*_D$	Neutral equivalent	η rel. 0.10% soln. in 0.9% NaCl	Antibody N pptd. from 1 ml. serum by slight excess S: Horse 607	Rabbit B53	Rabbit 350-1	Rabbit 718	
	%	%	Degrees			mg.	mg.	mg.	mg.	mg.
S III										
A 66[1]	0.2	.06	—37.3	350	1.09	1.34	.62	1.12		
S III Ba[2]	0.7	.10	—38.0	351	1.04	1.34	.33	0.86		
S 102[3]	3.4	.43	—30.7	383†	1.18	1.32	.56	1.10		
S 105 B_1[4]	6.3	.22	—32.5	365†	2.64	1.37	.78	1.25		
					1.34[5]			1.22[6]		
S 107B[4]	6.1	.27	—32.8	379†	2.06	1.37		1.18	.80	
								1.17[7]		
105 cult. filtrate									.73	
									Rabbit Pn II RSI	
						Horse B83A	Rabbit R377		heated *in vacuo*	heated in air
S II										
Cu purified	0.0	.16	+52	1000	1.04		.64			
S 80A[3]	0.1	.20	+61	960	1.04	1.27	.65	0.63		
S 83E[4]	2.2	.46	+53	956†	1.31			0.94	0.94	0.84
Culture filtrate 1						1.27	.87			
" " 4							.93	1.07	1.02	0.86

*Calculated to the ash-free basis.

[1]Prepared as in Reference 2.

[2]Prepared as in Reference 2 with additional purification with $Ba(OH)_2$.

[3]Prepared by concentrating broth on steam bath, isolating S without the use of strong acid and alkali. S III obtained as the acid sodium salt by precipitating with acetic acid.

†Calculated from the ash content.

[4]Prepared by concentrating broth *in vacuo* and isolating S as neutral sodium salt without use of strong acid or alkali.

[5]After heating 6 hours in boiling water bath.

[6]S III treated with 0.5 *N* NaOH at room temp. for 24 hours.

[7]Amount precipitated after heating S III solution 6 hours at 100°.

saccharide and pneumococcus protein so that the remaining antibody would be mainly anti-S.†

Treatment of the best preparations with 0.5 *N* sodium hydroxide solution[4] at room temperature for 24 hours failed to influence the amount of antibody precipitated from rabbit antisera, nor did heating aqueous solutions in a sealed tube at 100° for 6 hours, although the heat treatment reduced the viscosity markedly. Otherwise, preparations which showed the lowest viscosity gave the lowest values for antibody precipitated from rabbit antisera, a finding consistent with the observation[5] that partial degradation products of S III showing low viscosities as compared with S III precipitated Type III antipneumococcus horse serum but not homologous rabbit antiserum. It would seem that the new S III preparations of exceedingly high viscosity have the longest chain-length, and that the older preparations were partially degraded by the acid used in the purification process and by the initial concentration of the autolyzed culture at 100°. The newer preparations possibly represent the unstable intermediate postulated by Ward[6] as occurring between the native S III antigen and the polysaccharide as then purified.

2. *Specific polysaccharide of Type II pneumococcus (S II).* Similar results have been obtained with S II, and are also summarized in the table. No significant differences were found between an old preparation ("Cu purified") and one made from heated broth without the use of strong acid or alkali (S 80A). After concentration *in vacuo* the S II was precipitated from the culture filtrate by 1 volume of alcohol and extracted with neutralized 20% sodium acetate solution. After several repetitions of these steps treatment with copper acetate at pH 5 precipitated the last of the protein and little S II. The bulk of the S II was precipitated by addition of 5 volumes of glacial acetic acid. The product, S83E, precipitated 50% more antibody from rabbit serum than did the earlier preparations and its viscosity was also much higher.

In the case of S II oxidation appears to be involved as well as hydrolysis when culture filtrates are heated. When either broth or S83E was heated at pH 5.8 at 100°C. *in vacuo* there was little or no change in reactivity toward rabbit serum, but in the presence of air a marked decrease was noted.

† A statement to this effect was inadvertently omitted from Reference 1.

[4] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731; Enders, J. F., and Pappenheimer, A. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 37.

[5] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

[6] Ward, H. K., *J. Exp Med.*, 1932, **55**, 519.