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Immunologic Studies on New Preparation of Type-Specific Polysaccharide from Pneumococcus Type I.

BACON F. CHOW. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

The isolation of chemically different polysaccharides by Heidelberger and Avery[1] from various types of pneumococci places the problem of type-specificity in immunologic reactions on a definite chemical basis. Since the polysaccharide from Type I pneumococcus, called the "Soluble Specific Substance," was not antigenic, it can only be considered as a hapten of the pneumococcal antigen. Later Wadsworth,[2] Enders,[3] and others obtained preparations of polysaccharide from pneumococcus Type I, which were antigenic in mice. Therefore they were believed to be different from the soluble specific substance.

Recently Avery and Goebel,[4] by omitting the alkaline treatment, isolated an acetyl polysaccharide from the autolyzed broth-culture of pneumococcus Type I. Like similar preparations from other laboratories, the acetyl polysaccharide was antigenic in mice. However, the antigenicity was destroyed by treatment with alkali with accompanying loss of acetyl groups. Thus it is probable that Enders' and Wadsworth's polysaccharides may be identical with the acetyl-polysaccharide. Enders and his colleagues[5] have already confirmed the observations of Avery and Goebel.

By using a method which minimized hydrolysis by acid or alkali in the preparation of polysaccharide, we have obtained a product even more complete than the acetyl polysaccharide. An autolyzed broth-culture was not autoclaved, as in Avery and Goebel's procedure, but was concentrated at a reduced pressure so that the temperature was never higher than 37°C. After the subsequent purification according to Avery and Goebel's method[4] a white polysaccharide was obtained. This polysaccharide reacted with a homologous immune-rabbit serum, previously absorbed with the acetyl polysaccharide. On the other hand, the acetyl polysaccharide did

[1] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

[2] Wadsworth, A., and Brown, R., *J. Immunol.*, 1933, **24**, 349.

[3] Enders, J. F., *J. Exp. Med.*, 1932, **55**, 191.

[4] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

[5] Enders, J. F., and Wu, C. J., *J. Exp. Med.*, 1934, **60**, 127; Pappenheimer, A. M., Jr., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 37.

TABLE I.

Precipitin-reaction of newly-prepared and acetyl polysaccharides of pneumococcus type I in homologous antiserum before and after absorption with the polysaccharides. The precipitin-reaction was performed by adding 0.5 cc. of polysaccharides of different dilutions to 0.5 cc. of diluted serum containing 2 cc. of original serum and 3 cc. of saline. The mixtures were incubated at 37° C. for 2 hours and chilled in the ice-box over-night.

Antipneumococcus rabbit serum (Type I)	Final dilutions of the newly-prepared polysaccharide									Final dilutions of the acetyl polysaccharide									
	1:1,000	1:10,000	1:40,000	1:100,000	1:200,000	1:400,000	1:800,000	1:2,000,000	1:5,000,000	1:1,000	1:10,000	1:40,000	1:100,000	1:200,000	1:400,000	1:800,000	1:2,000,000	1:5,000,000	1:10,000,000
Unabsorbed	++++*	++++*	++++	+++	++	+±	+	+	±	+++	++++*	++++	+++	++	+	+	±	—	—
Absorbed with acetyl polysaccharide	++++*	++++*	++++	++±	++	+	+	±	—	—	—	—	—	—	—	—	—	—	—
Absorbed with the newly prepared polysaccharide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

*Disc formed.

not react with its homologous immune-rabbit serum absorbed by the new polysaccharide (Table I). A quantitative precipitin-titration showed that the new polysaccharide precipitated almost 3 times more antibody-protein than did the acetyl polysaccharide.

Although an antipneumococcal serum absorbed with our polysaccharide was still agglutinative and specifically protected mice from an otherwise fatal dose of pneumococci (Type I), the titre was greatly reduced.

The new polysaccharide in small doses produced active immunity in mice. Rats given intravenous injections of the polysaccharide-solution produced sera which protected mice from a fatal dose.

When the polysaccharide solution was heated at 100°C. in 0.05 N NaOH or 0.5 N acetic acid, the hydrolytic product still produced active immunity in mice, but failed to precipitate the immune serum absorbed with the acetyl polysaccharide.

It appears, therefore, that our polysaccharide may be the parent substance from which the acetyl polysaccharide could be obtained by appropriate treatment.

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Metabolism of Ethyl Esters of Fatty Acids.

H. J. DEUEL, JR., L. HALLMAN, J. S. BUTTS AND S. MURRAY.

From the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles.

It has been demonstrated[1] that the conversion of caproic, butyric, and beta-hydroxy butyric acids to acetone bodies in fasting rats by beta oxidation is quantitative, whereas greater amounts of ketone bodies originate after sodium caprylate than after isomolecular quantities of sodium acetoacetate are fed. The latter phenomenon suggests that delta oxidation occurs in the latter case. No ketone bodies are formed when the sodium salts of the fatty acids with an odd carbon chain, as propionic, valeric, heptoic or nonylic acids were fed. It was later demonstrated[2] that the conversion of the odd chain fatty acids into glycogen must represent an approximately quantitative transformation by beta oxidation into propionic acid.

1 Butts, J. S., Cutler, C. H., Hallman, L., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1935, **109**, 597.

2 Deuel, H. J., Jr., Butts, J. S., Hallman, L. F., and Cutler, C. H., *J. Biol. Chem.*, 1935, **112**, 15.