Bacterial Polysaccharides

THEIR CHEMICAL AND IMMUNOLOGICAL ASPECTS

By MARTIN BURGER, Formerly Organic Chemist to the Bureau of Laboratories, Department of Health, New York, New York

This is a concise compilation of data on and methods of isolating carbohydrate bacterial substances. It is an attempt to bring this field, which is slowly but surely becoming more important from the viewpoint of public health, to the attention of physicians, chemists, bacteriologists, hematologists, serologists, allergists, enzymologists, veterinarians, dentists, and immunologsits. Most important, data is given on the use of these carbohydrates as diagnostic reagents.

This book covers, in a comprehensive manner, those polysaccharide substances which are more or less directly related to infection and immunity. For that reason much of the material presented deals with carbohydrate substances which reside in the pathogenic bacteria or are produced as metabolic products by the microoganism, and which have been recognized as the factors responsible for certain immune reactions either in the host (in vivo) or in the test tube (in vitro).

Additional material of great value to the laboratory worker is given in the Appendix which includes a variety of methods used in isolating the many substances.

273 pages 48 tables

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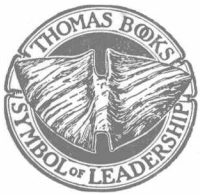

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Chapter One

HISTORICAL AND INTRODUCTION

THE subject of bacterial polysaccharides dates back many years, but it was the famous chemist and father of modern microbiology, Louis Pasteur (1), who applied elementary principles of chemistry and became the first investigator to prove that the slimy "viscous" fermentation of carbohydrate solutions was bacterial in origin. He isolated a gummy substance which, upon chemical analysis, was assigned the empirical formula C_{12} H_{20} O_{10}.

One of the first bacterial polysaccharides that underwent careful investigation was a dextran produced by *Leuconostoc mesenteroides*. The latter in earlier days was generally designated *Streptococcus Leuconostoc mesenteroides*. Scheibler (2), in 1874, isolated the dextran from a gummy mass that had formed during the manufacture of sucrose. He found that the dextran interfered with the crystallization of sucrose and thereby decreased the yield enormously.

By chemical means, Scheibler determined that the gum was an anhydride of glucose, and empirically it resembled the then known cellulose, starch and dextrin. Borscow (3), in 1876, stated that the substance was a pectin. Van Tieghem (4), two years later, described the causal organism quite adequately, and postulated that the substance was not a true cellulose because the gum did not form a soluble cupric ammonium compound.

In 1904 Lippman (5) showed that the dextran was a carbohydrate with an empirical formula of C_6 H_{10} O_5. It was soluble in water, insoluble in cupric ammonia solution and, when hydrolyzed in mineral acids, it was quantitatively split into glucose molecules.

Cellulose; hemicellulose; gums, such as dextrans, levulans, cellulans and galactans; starch; glycogen; chitin; and mucins were among the first polysaccharides isolated from bacteria. The causal organisms

were not restricted to the non-pathogens alone. For example, Tomura (6) revealed that the pathogens *Corynebact. diphtheriae* and *Mycobact. tuberculosis* contained a pentosan which liberated arabinose on acid hydrolysis.

Buchanan and Fulmer's (7) *Physiology and Biochemistry of Bacteria, Volume I,* devotes many pages of worthwhile reading for those who may be interested in the earlier investigations relating to the polysaccharides of bacterial origin.

Although it may be somewhat irrelevant, here, it could certainly be of world wide economic importance to examine the possibilities of utilizing bacterial polysaccharides in the field of agronomy.

For in 1946 Geoghegan and Brian (14) and Haworth, Pinkard and Stacey (15) gathered evidence to show that aggregate formation and moisture conservation of soils were distinctly related to the presence of bacterial mucopolysaccharides of the levan type.

The isolated levans consisted of polyfructose chains linked together by units of an unidentified nitrogenous material.

The purpose at hand, however, is to cover in organized fashion, those polysaccharide substances which are more or less directly related to infection and immunity. For that reason, much of the material to be presented deals with carbohydrate substances which reside in the pathogenic bacteria or are produced as metabolic products by the microorganism, and which have been recognized as the factors responsible for certain immune reactions either in the host (*in vivo*) or in the test tube (*in vitro*).

At the turn of the 19th century, Pick (8) was investigating the chemical composition of typhoid bacilli. In 1902 he demonstrated that when protein material was removed by filtration of trypsin or pepsin digested typhoid cultures, the filtrate contained a polysaccharide. The substance, when mixed with a typhoid antiserum, produced a specific precipitate. This phenomenon was perhaps the first to demonstrate that bacterial polysaccharides as chemical entities, might have some bearing on immune reactions.

Dochez and Avery (9), Toenniessen (10), Zinsser and Parker (11), and Heidelberger and Avery (12) were among the pioneers who devoted their attentions to the possibility that the carbohydrates of pathogenic bacteria, as factors in immunology, were of great importance.

Many investigators now believe that some of the polysaccharides obtainable from bacteria are antigenic in the true sense of the word, that is, they are capable of producing a state of resistance or immunity against those invading organisms which contain the polysaccharides in the intact bacterial cell. There are others who feel that, although the polysaccharides exhibit specific serological reactions, they are not necessarily protective antigens. Obviously, more work is indicated in this direction before the two schools of thought can be reconciled to an agreement which would be favorable to both sides. The book *The Bacterial Cell* by Dubos (13) discusses these questions quite fully.

It is the intention of the writer to present the data in each of the following sections as they were reported in the literature and, whenever possible the compilation shall proceed in chronological order.

The appendix has been included so that some idea of the various methods used in isolating the substances may be available to the laboratory worker.

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Chapter Two

PNEUMOCOCCUS POLYSACCHARIDES

INTRODUCTION

FROM the time that Friedländer (1) in 1883 observed the presence of a mucin-like substance in pneumococcus cells, more experimental work has been reported in the literature on the nature of pneumococcus polysaccharides than on any of the other bacterial carbohydrates of pathogenic origin.

Kraus (2), in 1897, demonstrated for the first time the presence of specifically precipitable substances in cell-free culture filtrates of certain bacterial species. A few years later, Neufeld (3) and Wadsworth (4) showed that bile and saline extracts of pneumococci evoked precipitation in antipneumococcus serum and attributed the reaction to substances native in the pneumococcus cell. Panichi (5) showed that the specific substances could also be present in bouillon cultures of pneumococci. Rosenow (6) and Chickering (7) demonstrated that the substances were responsible for the specific immunological reactions.

Prior to 1917 none of the constituents of pneumococci had been isolated and studied chemically. In that year Dochez and Avery (8) found a soluble substance in cell-free filtrates of broth cultures, in the blood serum and urine of lobar pneumonia patients, and in the blood of experimentally infected animals, which, when mixed with antipneumococcus serum of homologous type, gave a specific precipitate. They characterized the substance as being readily soluble in water, thermostable, impervious to the action of trypsin or urease, non-dialysable through parchment and precipitable by acetone, alcohol and ether.

In 1921 Perlzweig and associates (9) described a substance obtained from pneumococci which was resistant to proteolytic en-

zymes, thermostable in neutral or slightly acid solutions, but readily destroyed in boiling alkali solutions. Their substance precipitated in 70 to 99 per cent alcohol and was insoluble in lipid solvents.

Simultaneously, Zinsser and Parker (10) described substances obtained from the pneumococcus and other bacterial species, which they designated "residue antigens." These residues were protein-free and in the chemically purified state they were non-antigenic *in vivo*. However, in homologous antisera, the "residue antigens" gave specific precipitates.

ISOLATION OF BACTERIAL POLYSACCHARIDES

The "residue antigens" of Zinsser and Parker (10) were obtained by treating ice-cold aqueous extracts of cells with acetic acid. The precipitate, containing phosphoproteins and nucleoproteins, was separated by filtration. The filtrate was then boiled to precipitate other proteins, filtered, and this filtrate when diluted with ten volumes of ethyl alcohol liberated the "residue antigen."

Heidelberger and Avery (11) in 1923, made the first definite chemical investigation on the nature of the pneumococcus carbohydrate isolated from a Type II pneumococcus broth culture filtrate. They revealed that the capsular substance of the pneumococcus was a polysaccharide capable of specific precipitation with antibody of homologous type. Furthermore the polysaccharide (capsule) imparted to the strain of each type its special serological character, that is, cells without capsules lacked virulence and did not determine the exact or specific immunological response elicited by encapsulated cells.

With this groundwork, Heidelberger and associates (12) isolated and studied the polysaccharides which made up the capsular material of Types I, II, III and IV pneumococci. The polysaccharides were designated by Heidelberger as S I, S II, S III, etc. Table I, in summary, shows the analytical data of all the preparations obtained by Heidelberger et al. up to and including the substances isolated by the revised methods reported in the 1936 reference (12). The table also includes the data for the species-specific "C" substance or somatic polysaccharide first isolated by Tillet and Francis (13), and an immunologically inactive polysaccharide.

Noteworthy in Table I are the differences in viscosity for each

TABLE I

Preparation	Nitrogen per cent	$[a]_D$ degrees	Neutral equivalent	Acetyl per cent	Uronic anhydride per cent	Reducing sugars after hydrolysis per cent	n Rel. of 0.1% solution in 0.9% saline	n Rel. of 0.2% solution in water	Amino nitrogen per cent	Phosphorus per cent	Products of hydrolysis
S I old	5.12	+305	417	3.4	65				2.5	0.0	Galacturonic acid, (amino sugar derivative).
S I 91 A	5.22	+294	590	10.0			1.10			0.25	Galacturonic acid, (amino
S I 120	4.62	+278	650	7.1	56	30	1.69	9.0	2.0	0.0	sugar deriv.). acetic acid.
S II old	0.16	+52	1000	1.9			1.04				Glucose.
S II 80 A	0.20	+58	870	3.8			1.04				
S II 83 A	0.46	+53	1020	1.0		100	1.31				
S II 84 A	0.40	+54	980	1.3	19.1	95	1.38				
S II 84 B	0.73	+58	960		17.6	86	1.54				
S II 85 A	0.14	+55	600	0.4	19.8		1.64	10.6		0.0	

S III old	0.10	−38.0	351				1.04				Aldobionic acid, glucose.
S III A 66	0.06	−37.3	350				1.09				
S III 102	0.43	−30.7	351				1.18				
105	0.22	−32.5	340	0.6	48.2	84	2.64	27.6			
107	0.27	−32.8	360	0.9			2.06	24.7			
108	0.08	−36.0	330	0.5	51		3.14	32.9		0.0	
108 acid treated		−36.0	350				2.23	31.6			
S IV	5.5	+30	1550	5.8*		71			0.1	<0.1	(Amino sugar derivative), acetic acid.
"C" substance	6.1	+42	1050	3.7*		36			0.9	4.0	(Amino sugar derivative), acetic acid, phosphoric acid.
Inactive fraction	5.9	+10	4540	5.6*		55			0.0		Glucosamine, (amino sugar derivative), acetic acid.

*Acetyl nitrogen.