Some of the properties of the polysaccharides may be summarized in Table II.

TABLE II.
Chemical and Serological Properties of Polysaccharide Fractions SI, SII and SIII.

Polys. fract.	% nitrogen	Ppt. titre	Complement fixation titre	Molisch test	Immunological properties
SI	1.5	1:4,000,000	1:32,000,000	Positive	Antigenic sensitizing
SII and SIII	1.5	1:4,000,000	1:32,000,000	"	Negative

Demonstration of the antigenic power of polysaccharides in rabbits by means of the complement-fixation reaction has been recorded by Chow[3] for a polysaccharide prepared from Pneumococcus Type I and by Nishimura[4] for inulin, soluble starch and dextrine; the precipitation test was negative. In this paper we have shown that the same may be true of one of the polysaccharide fractions of *B. rhinoscleromatis*. The result of this experiment suggests that the method employed for isolation of the polysaccharides may be of importance in determining its antigenicity.

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Immunological Studies of Polysaccharides of Encapsulated Bacillus. II. Isolation from Rough *B. rhinoscleromatis*.

SAM C. WONG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, China.

It has been claimed by Julianelle[1] that the R-form of Friedlander bacilli does not elaborate any soluble specific polysaccharide and as a result of this fact the organism is only species specific. It appears of interest to us to extend similar investigation to other closely related species of this group with the object of analyzing the antigenic structure of these strains.

Dissociation of the parent strain, *Bacillus rhinoscleromatis*, was carried out according to the method of Julianelle.[1] Rough variants began to appear as small non-mucoid colonies on the eleventh daily

3 Chow, B., *Chinese J. Physiol.*, 1937, **11**, 223.

4 Nishimura, S., *J. Exp. Med.*, 1929, **50**, 419.

1 Julianelle, L. A., *J. Exp. Med.*, 1926, **44**, 683.

transfer on 10% anti-S serum in meat infusion broth. We have found, however, that ageing the culture in 5% immune serum in meat infusion broth, pH 7.6, was equally effective in dissociating our strain, except that dissociants began to appear as early as the seventh day of incubation. Biochemical reactions of the dissociated culture were similar to those of the parent strain except that the production of acid in saccharose and rhamnose was delayed. The culture thus obtained fulfilled all the requirements of a rough strain. Briefly stated, the variant strain was agglutinated by anti-S serum to a titre of 1:512 while the parent encapsulated organism was agglutinated only to a titre of 1:16, it was also agglutinated by the homologous anti-R serum to a titre of 1:1024. By the India ink method, the organisms were found to be without capsules, and their virulence diminished; the parent strain itself was one of low pathogenicity for mice.

The rough culture used for the isolation of the polysaccharides was one which had been transferred daily on meat infusion agar, pH 7.6, for a period of 70 days. The methods employed for preparing the polysaccharides were the same as those reported previously.[2] The following designations will be used for the polysaccharides prepared: (a) Fraction RI, obtained by acid hydrolysis, (b) Fraction RII, obtained by alkaline hydrolysis of the cells previously used for acid hydrolysis, and (c) Fraction RIII, obtained from alkaline hydrolysis of the whole organism.

Immune serum was prepared in the same manner as that previously described.[2]

All the 3 fractions obtained were light colored powders easily soluble in 1% concentration in saline solution. All the usual protein tests were negative; all contained varying amounts of nitrogen, failed to reduce Fehling's solution but did so after hydrolysis with strong mineral acid, and gave a strong Molisch test in a 1:1,000 dilution. Some of the important serological and chemical properties are presented in Table I.

TABLE I.
Properties of Polysaccharides from Rough Strain of *Bacillus rhinoscleromatis*.

Polys. fraction	% yield	% nitrogen	Ppt. titre with anti-R and anti-S sera	Complement fixation titre with anti-R and anti-S sera, diluted 1:10
R I	5.7	2.2	1:2,000,000	1:8,000,000
R II	2.6	3.6	1:10,000	1:100,000
RIII	6.5	2.8	1:500,000	1:4,000,000

[2] Wong, Sam C., in press.

From the table it is apparent that 2 distinct polysaccharides differing in nitrogen content and serological activity are present in the rough organism. Fraction RI prepared by acid hydrolysis, representing the surface polysaccharide, possesses the highest activity, while Fraction RII prepared by alkaline hydrolysis of the remaining intact cells previously treated with acetic acid possesses the lowest activity in spite of the highest nitrogen content. A further differentiation of the 2 polysaccharides was obtained by anaphylactic reactions. Fraction RI elicited symptoms of severe but not fatal shock in guinea pigs sensitized with polysaccharide, Fraction SI, prepared by acid hydrolysis of the smooth organism, when 2 mg. of the substance was administered intravenously, while Fraction RII was inert in similar amount.

It might be stated that a further purification of Fraction RIII did not improve the serological activity of the substance. An explanation of this finding may be obtained from a quantitative determination of the polysaccharide content of the rough organism. The results shown in Column 1, Table I, are the averages of 6 different determinations in 2 different lots based on the dried weight of organisms. In other words, about 70% of the total polysaccharide of the organism is composed of highly active substance which apparently dominates its immunological specificity. It is quite clear that the diminished serological activity of Fraction RIII as compared to Fraction RI prepared by acid hydrolysis may be attributed to a mixture of both active and less active substances.

Serologically, it is difficult to distinguish Fraction RI of the R strain from the polysaccharides prepared from the smooth strain[2] either with anti-R or anti-S serum since all these 3 polysaccharides behave similarly. However, certain differences between Fraction RI and the Fractions SI, SII, and SIII prepared from the smooth organism may be found in immune serum prepared by means of a well washed suspension of the decapsulated organism obtained by acid hydrolysis. Such immune serum agglutinated the R culture to a titer of 1:32 and it gave a very insignificant titre with Fraction RI (see Table II). Against its homologous chemically decapsulated organism an agglutination titre of 1:256 was obtained and against the S culture a titre of 1:16 was found. On the other hand, the chemically decapsulated organism was not agglutinated by anti-R serum. The serological activity of such immune serum with various polysaccharide preparations from the smooth and rough organisms is summarized in Table II.

The above findings seem to indicate that by treatment with acetic

TABLE II.
Serological Activity of Serum Prepared with Decapsulated Organism.

Polys. fraction	Ppt. titre	Complement-fixation titre (serum diluted 1:10)
RI	1:1,000	1:10,000
RII	1:5,000	1:50,000
RIII	1:10,000	1:100,000
SI, SII and SIII	1:2,000,000-1:4,000,000	1:16,000,000

acid the smooth organism was only partially decapsulated. Three facts are difficult to reconcile with this assumption: (a) The chemically decapsulated organism was agglutinated by anti-S serum to a titre of 1:256. (b) Further treatment of the decapsulated organism with acetic acid did not yield any specific substance. (c) Fraction RI prepared by acid hydrolysis of the rough organism reacts poorly with the serum prepared with decapsulated organism in contrast to the high activity with anti-S serum. The exact explanation of these findings is not possible at present. The possibility of Fraction RI being derived from the remains of the capsule can not entirely be ruled out, the available evidence shows, however, that the bacteria from which the substance was prepared possess no demonstrable capsule and, therefore, the substance must have been probably derived from the cell itself.

Summary. Two immunologically distinct polysaccharides have been obtained from a rough strain of *Bacillus rhinoscleromatis,* one serologically active and the other more or less inert. Their chemical and serological properties are described.

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Immunological Studies of Polysaccharides of Encapsulated Bacillus. III. Active Sensitization with Polysaccharide from *B. rhinoscleromatis.*

T. J. KUROTCHKIN AND SAM C. WONG. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, China.

Attempts to produce active sensitization of guinea pigs through the use of killed cultures of different Gram negative microörganisms, have been, so far, uniformly unsuccessful. Since the development of hypersensitivity in infections caused by certain Gram negative bacteria is well established, it appears inconceivable that these bac-