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Polysaccharides of *C. diphtheriæ*.

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Recent serological studies[1, 2, 3] of *C. diphtheriæ* have shown that in addition to the 3 main types, *gravis*, intermediate, and *mitis*, there are many sub-types. Since polysaccharides have been accepted generally to be responsible for the type-specificity of many kinds of bacteria it was considered of interest to determine whether or not polysaccharides determine the antigenic types of *C. diphtheriæ*.

With the exception of one intermediate strain, Park 8, all of the organisms used in this study were isolated locally. All conformed to the cultural characteristics as set forth by Anderson and his associates[4] for *gravis*, intermediate, and *mitis*. One organism from each of the 3 cultural types and one avirulent strain were grown on stomach-digest medium[5] for a week. The methods of preparing the polysaccharides were essentially the same as reported for *B. rhinoscleromatis*[6] except that the extraction-time was one hour instead of half an hour, and 1% potassium hydroxide instead of 0.5% was used in preparing the alkali-fraction. The polysaccharides thus obtained were light brown powders, easily soluble in concentration of 1% and dilute solutions gave a strong Molisch reaction. The total yields were so small that detailed chemical characterization of these polysaccharides can not be made at this time.

Antisera were prepared with heat-killed cultures of 2 strains of each type. Two rabbits for each strain received 3 daily intravenous injections followed by 5 days of rest; this course was repeated twice. Seven days after the last injection serum was obtained and tested with the homologous polysaccharide. Only those sera with a high titer were tested for cross-precipitation. The sera from rabbits immunized with the avirulent organisms did not precipitate with either the homologous or the heterologous types of polysaccharides, and further immunization of these animals did not elicit any response.

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1 Murray, J. F., *J. Path. Bact.*, 1935, **41**, 439.

2 Ewing, J. O., *Ibid.*, 1933, **37**, 345.

3 Keogh, E. V., Simmons, R. T., and Anderson, G., *Ibid.*, 1938, **46**, 565.

4 Anderson, J. S., Happold, F. C., McLeod, J. W., and Thomson, J. G., *Ibid.*, 1931, **34**, 667.

5 Young, C. C., *Chinese Med. J.*, Suppl. No. 1, 1936, 143.

6 Wong, S. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 107.

Most sera reacted with the homologous polysaccharides diluted 1:100,000 excepting one serum which reacted with 1:250,000 (Park 8). Corresponding titers were obtained with heterologous polysaccharides.

For further study of these group reactions, absorption-tests were carried out. Equal volumes of undiluted immune serum and the optimal dilution (1:10,000) of polysaccharide were mixed and incubated at 37°C in the waterbath for 2 hours. Then the treated serum was refrigerated overnight and centrifuged rapidly for half an hour. The supernate was tested with 1:1000 and 1:5000 dilution of the various polysaccharides. Every serum (*mitis, gravis,* or intermediate) was completely absorbed by the homologous and by each heterologous polysaccharide, including the avirulent type.

Conclusion. The polysaccharides of *C. diphtheriæ* appear to be shared by all the types studied.

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Sensitization of Guinea Pigs by a Modified Form of Seibert's Tuberculoprotein Derivative.

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Denaturation of tuberculoprotein is usually accompanied by a decrease in antigenic activity and in special cases, such as in the preparation of OT (old tuberculin) and of PPD (purified protein derivative), antigenicity is usually destroyed.[1] According to Seibert[2] the lack of antigenic properties of PPD is attributed to the use of heat and of trichloracetic acid employed in its preparation. It is here shown that a modified form of Seibert's PPD can sensitize guinea pigs.

Mycobacterium tuberculosis, H37, was grown on Wong's modification of Henley and LeDuc's synthetic medium[3] for 4 weeks. The general procedures of preparing the tuberculoprotein were essentially those used by Seibert, but 3 changes were introduced to shorten the time of preparation: (1) glycerin was not added to the tuberculin during concentration over the waterbath; (2) the concentrated tu-

1 Seibert, F. B., *Am. Rev. Tuberc.,* 1934, **30**, 713.

2 Seibert, F. B., *J. Inf. Dis.,* 1932, **51**, 383; Nat. Tuberc. Assn., 1933, 165.

3 Wong, S. C., *J. Bact.,* 1937, **33**, 451.