bled before each series of injection and the resulting sera were tested with various polysaccharides derived from *C. diphtheriæ*. This might serve to detect any early appearance of type-specific antibodies. It was found, however, that precipitins began to appear after the second week of immunization and that there was no difference in the titers of the sera at any time when they were tested with the polysaccharides prepared from various cultural types. The maximal titer of 1:100,000 for all polysaccharides was reached at the end of the fifth week of immunization.

On the other hand, when rabbits were immunized with living cultures of *C. hofmanni* the sera did not react with heterologous polysaccharides, although a titer of 1:100,000 with the homologous polysaccharides was attained.

The finding of an antigenically distinct polysaccharide in *C. hofmanni* is not out of keeping with the existence of group-specific polysaccharide in *C. diphtheriæ*, especially since the proper classification of *hofmanni* in the genus of *Corynebacterium* is regarded as uncertain by some critical bacteriologists. On the other hand, the presence of a group-specific polysaccharide in *C. xerosis* and other diphtheroid organisms which we have examined during the past year strongly supports the thesis of others that diphtheroids may represent, according to Jungeblut,[2] "degraded" species of true *C. diphtheriæ*.

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Significance of Acetyl Group in Determining Antigenic Activity of Bacterial Polysaccharides.

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The acetyl group[1] has been suspected to be responsible for the antigenicity of the specific polysaccharide derived from Type I pneumococcus. Recent findings by Felton and Prescott,[2] however, seem to indicate that the acetyl group may not be of great importance in determining antigenicity. Because of this discrepancy, investiga-

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

[2] Felton, L. D., and Prescott, B., *Bull. Johns Hopk. Hosp.*, 1936, **59**, 114.

[2] Jungeblut, C. W., *Agents of Disease and Host Resistance*, 1935, p. 949.

tion of other antigenic bacterial polysaccharides appears to be in order.

Two types of polysaccharides, one prepared by acetic-acid hydrolysis and the other by alkali-hydrolysis of the whole organisms, were obtained from *E. coli,* non-capsulated; and *K. rhinoscleromatis,* both the capsulated and the non-capsulated forms. All polysaccharides prepared by acid-hydrolysis possessed immunological properties similar to those of fraction SI[3] of *K. rhinoscleromatis* previously reported, in that they were able to bring about the active sensitization of guinea pigs and the production of the usual antibodies in rabbits. The polysaccharides prepared by alkali-hydrolysis, on the other hand, lacked such properties. Apart from this antigenic difference the acid- and the alkali-prepared polysaccharides of *E. coli* and the capsulated form of *K. rhinoscleromatis* were respectively indistinguishable either by serological or by absorptive tests with homologous immune serum. The polysaccharides prepared from the non-capsulated form of *K. rhinoscleromatis* differed in the precipitin-reaction but not in absorptive tests.[4]

Each fraction was purified by dissolving in distilled water and re-precipitating with alcohol in the presence of a few drops of concentrated hydrochloric acid. The precipitate was washed 5 times with 95% alcohol after which it was dried over a waterbath. One such treatment, as shown by preliminary tests of a few of the polysaccharide preparations, was apparently sufficient to give a substance of constant acetyl value. The serological activity of each polysaccharide when tested against the homologous immune serum was unimpaired by one or several of such treatments. In addition to these polysaccharides, 2 other preparations were included. These were the purified polysaccharides of the capsulated form of *K. rhinoscleromatis* treated in the following manner: The acid-hydrolysis fraction was boiled in 0.5% potassium hydroxide and the alkali-hydrolysis one was boiled in 1% acetic acid, both for 30 minutes, after which each was precipitated in alcohol. Each of them after drying was purified once more by the method outlined above. Both the polysaccharides thus obtained were non-antigenic. The acetyl group was determined by the micro-method of Pregl and Solty[5] and the results are presented in Table I.

It was found that the acetyl group was present in every preparation, the difference between the antigenic and the non-antigenic

[3] Wong, S. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 107, 113.

[4] Wong, S. C., *Ibid.*, 1938, **38**, 111.

[5] Pregl and Solty, *Quantitative Organic Microanalysis,* 2nd edition, 1930.

TABLE I.
Acetyl Content of Bacterial Polysaccharides.

Organism	Acetic-acid hydrolysis method		KOH hydrolysis method	
	Before treatment %	After* treatment %	Before treatment %	After† treatment %
K. rhinoscleromatis (capsulated)	6.45	4.54	1.61	4.95
K. rhinoscleromatis (non-capsulated)	6.18	—	5.75	—
E. coli	10.90	—	3.94	—

— = not done.
*Treatment with alkali.
†Treatment with acetic acid.

polysaccharides being only one of quantity. In the case of *E. coli* the difference was comparatively large (6.96%) while in the non-capsulated form of *K. rhinoscleromatis* the difference was less than 0.5%. In general it may be stated that the antigenic polysaccharides contained more than 6% of the acetyl group while the non-antigenic ones contained less. From these considerations it may seem obvious that a qualitative estimation of the acetyl group can not be employed as an index of antigenicity in the polysaccharides studied.

Whether the acetyl group is responsible for antigenicity in these polysaccharides cannot be determined with certainty. The evidence, however, seems to indicate that the acetyl group may not be of great importance. First, the difference of the acetyl content between the antigenic and the non-antigenic polysaccharides prepared from the non-capsulated form of *K. rhinoscleromatis* is only 0.43%, an amount which in our opinion is too small to explain the marked difference in antigenic activity. Second, when the purified antigenic polysaccharides prepared from capsulated form of *K. rhinoscleromatis* was heated with alkali, there was a loss of only 1.91% of acetyl content, yet its antigenic activity was destroyed. Third, when the purified non-antigenic polysaccharide of the same organism was heated with acetic acid there was an increase of 3.34% in the acetyl content, but the antigenicity of the polysaccharide was not restored.

Conclusion. Evidence is presented which suggests that the acetyl group is probably not responsible for antigenic activities of the polysaccharides prepared by acetic-acid hydrolysis of *E. coli* and the capsulated and the non-capsulated forms of *K. rhinoscleromatis.*

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