

were also done on all sera and it was found that in general, the titer was about 10 to 20 times higher than that obtained by precipitation.

Only sera having the higher agglutinative and precipitative titers were tested for passive protection of white mice. It was found that whereas 0.2 cc of each of the 4 sera prepared with photodynamically inactivated vaccine protected mice against 10,000 to 100,000 MLD, the same amount of 3 sera from each of the other 2 series of animals protected mice against only 1000 MLD.

The superiority of the photodynamically inactivated vaccine over those treated either with formalin or by heat in the immunization of rabbits by the intracutaneous method was further substantiated by the fact that rabbits which had failed to respond to formolized or heat-killed vaccine yielded significantly high agglutinative and precipitative titers when these animals were given another 2 courses of immunization with photodynamically inactivated vaccine by the same route.

The results of these experiments offer further proof that photo-sensitized pneumococcal vaccine is more efficacious than vaccines prepared with formalin or heat.

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Antigenic Activity of Polysaccharides of *B. rhinoscleromatis*.

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Polysaccharide obtained from *B. rhinoscleromatis* through extraction of dried bacterial cells in weak acetic acid solution provokes in immunized rabbits the formation of complement-fixing antibodies and induces in guinea pigs active sensitization. In this experiment an attempt was made to demonstrate that the antigenicity of the polysaccharide of this bacterial species is analogous to that of the whole bacterial cell. It seemed also of interest to compare the immunological properties of this polysaccharide with that prepared by the method in which the treatment of the bacteria by heat was avoided.

A stock culture of *B. rhinoscleromatis* was passed through mice 3 times until its agar culture acquired markedly slimy appearance. The first type of polysaccharide was prepared in accordance with the

method previously reported.¹ The second type of polysaccharide was obtained from the same microorganism by spontaneous autolysis of bacterial suspension in distilled water. Polysaccharides prepared in this manner showed none of the usual protein reaction, the amount of nitrogen in both types of substance being equal to 0.24%. Immunological reactivity of the polysaccharides was determined by testing them against immune rabbit serum prepared by immunization of these animals with formol killed culture of *B. rhinoscleromatis*. It was found that both substances reacted equally well giving positive precipitation and complement-fixation tests in concentration of 1:6,000,000 and 1:64,000,000 respectively. Cross absorption tests showed that the polysaccharide prepared by autolysis of bacterial bodies removed from anti-bacterial serum all precipitin, whereas, the serum absorbed with the polysaccharide prepared by extraction with acetic acid still gave precipitin reaction to the titer of 1:500,000 when tested against the former type of substance. This indicated that the polysaccharide obtained by autolysis is more complex than that obtained by acid extraction.

Eight rabbits were immunized with each type of the substances. Each animal received a total of 9 injections, each of which contained one mg of the substance dissolved in one cc of normal saline. The injections were given on 3 successive days and followed by 4 days of rest. The examination of the sera of all these animals showed that precipitin reaction regularly occurred when 1:1000 concentration of either type of polysaccharides was used. With sera from 2 rabbits a strongly positive test was obtained when 1:10,000 dilutions of the polysaccharides were used. Complement fixation occurred in dilutions from 1:1000 to 1:100,000 of both substances tested against anti-polysaccharide sera in 1:5 dilution. Agglutination reaction performed with a suspension of the rough form of *B. rhinoscleromatis* derived from the smooth culture showed a titer equal to 1:128, whereas the titer of 1:8 was observed when a decapsulated smooth form was used for agglutination. An attempt was made to sensitize guinea pigs with the antipolysaccharide sera giving the best precipitin test. Ten guinea pigs weighing between 150 to 200 g, each received intraperitoneally 3 cc of serum per animal. Twenty-four hours later the animals were tested for hypersensitivity by intravenous injections of 5 mg of either type of the polysaccharides. All but one animal gave negative result. One guinea pig injected with the polysaccharide prepared by acid extraction developed symptoms of typical non-fatal anaphylactic shock.

¹ Wong, S. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 107.

Recent evidence^{2, 3} seems to indicate that antigenic polysaccharides are heat labile. This conclusion is not borne out by our observations. It may seem obvious that the antigenic component of the polysaccharide prepared by acid extraction is heat stable since the use of heat is involved during the process of extraction and final drying of the substance. The heat stability of the antigenic component of the polysaccharide prepared by spontaneous autolysis of bacterial cells is proved by the experiment in which 0.1% solution of the polysaccharide was heated in boiling water either alone or in the presence of 0.5% of potassium hydroxide. Immunization of rabbits with fractions prepared in this manner showed that only the alkali-treated polysaccharide failed to immunize.

The above findings indicate that 2 polysaccharides identical in immunological properties but differing in their absorptive power can be prepared from the cultures of *B. rhinoscleromatis*, and that the rôle played by acetic acid in the process of extraction is merely one of preserving the antigenic property of the substance.

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Isolation of Typhus Fever Virus from House Rats in Peiping.

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In our previous studies¹ on the typhus fever virus isolated from local patients, two distinct types were encountered, one simulating the classical epidemic type and the other, the endemic variety of the New World. At that time it was suggested that these two types of typhus fever might simultaneously exist in Peiping, though the popular impression hitherto was that typhus fever seen here was of the epidemic form. Recently Chung and Chang² studied a strain of virus isolated from a patient and identified it to be *Rickettsia prowazekii*, thus confirming the existence of the classical epidemic type. It occurred to us that if our contention regarding the co-existence of endemic typhus fever should be correct it would be of

² Landsteiner, K., and Chase, M. W., *Ann. Rev. Bioch.*, 1937, **6**, 626.

³ Haworth, W. N., and Hirst, E. L., *Ibid.*, 1937, **6**, 104.

¹ Wu, C. J., and Zia, S. H., *Chin. M. J.*, 1938, Suppl. II, 221.

² Chung, H. L., and Chang, J. H. M., *Chin. M. J.*, 1938, **53**, 513.