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Method for Obtaining Group A Hemolytic Streptococci Sensitive to Reaction of Capsular Swelling.

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The following experiments are an approach to the problem of mouse virulence and type-specificity of Lancefield Group A beta hemolytic streptococci from the standpoint of encapsulation and the reaction of capsular swelling with homologous serum. Many of our stock cultures were avirulent with little or no demonstrable capsules. Attempts to produce capsular swelling with these organisms were unavailing. Further, although capsules were at times noted on our most virulent cultures, all attempts to produce capsular swelling were negative.

It has been shown by Dawson,[1] Seastone,[2] Loewenthal[3] and others that encapsulation of hemolytic streptococci is an attribute always accompanying highly virulent invasive strains. Loewenthal[3] regarding the encapsulated organism as capable of evoking protective antibodies used only this type of organism in producing protective antisera. He succeeded in making antisera from mucoid and non-mucoid strains, but in either case he emphasized the presence of capsules. In an earlier paper[4] he noted the "swelling" of these capsules with the addition of homologous serum to the organisms.*

The logical approach to the problem of increasing virulence is by mouse passage. For our purposes this method offered certain difficulties. Parish and Okell,[5] Pulvertaft,[6] and Hartley[7] have shown that streptococci elaborate toxins which are lethal for mice. Todd[8] further showed a limited protection with an antitoxin (antistreptolysin O) against living cultures of streptococci injected into mice. From these experiments it is clear that toxemia may, in large meas-

1 Dawson, M. H., Hobby, G. L., and Olmstead, M., *J. Inf. Dis.*, 1938, **62**, 138.

2 Seastone, C. V., *J. Exp. Med.*, 1939, **70**, 347.

3 Loewenthal, H., *Brit. J. Exp. Path.*, 1938, **19**, 143.

4 Loewenthal, H., *Brit. J. Exp. Path.*, 1934, **15**, 298.

* It should be noted that these organisms fall serologically into Lancefield's Group B.

5 Parish, H. J., and Okell, C. C., *J. Path. Bact.*, 1927, **30**, 521.

6 Pulvertaft, R. J. V., *J. Path. Bact.*, 1928, **9**, 276.

7 Hartley, P., *Brit. J. Exp. Path.*, 1928, **9**, 259.

8 Todd, E. W., *Brit. J. Exp. Path.*, 1938, **19**, 367.

ure, be responsible for the deaths of mice injected with living streptococci.

Believing that a death for which a septicemia was entirely responsible would yield an organism better suited to our purpose, scarlet fever antitoxin was injected simultaneously with culture into the mouse peritoneum. It was thought that the inhibition of toxin production or the neutralization of toxin as quickly as it was formed would accomplish the desired result.

Procedures. 1. *Mouse Passage.* Thirteen strains originally obtained from Griffith and representing 13 serological types in his classification (types 1, 2, 3, 9, 10, 12, 13, 14, 15, 23, 25, 26, 28) were used. Cultures were taken from vacuum tubes planted in beef-infusion broth with 10% of normal horse serum and incubated at 37° for 18 hours. Five-tenths of a cubic centimeter of the culture, in each case, was inoculated into another tube of serum broth and incubated at 37°C for 4 hours; 0.5 cc of the dilution containing approximately one lethal dose was injected simultaneously with 0.5 cc of unrefined scarlet fever antitoxin† into a mouse. The intraäbdominal route was used.

Immediately upon the death of the mouse (5 to 24 hours) the spleen was excised aseptically and macerated in 1 cc of sterile peptone water. To one-half cc of this suspension, one-half cc of antitoxin was added. This mixture was immediately reinjected into mice.

After excision of the spleen the heart's blood was inoculated into serum broth. When growth was sufficient, a capsular swelling preparation was made and the reaction noted.

Upon the death of the mice injected with the splenic material, the culture was again tested for capsular swelling. When positive reactions were consistently obtained, the cultures were put under vacuum and stored at 4°C.

2. *Action on horse blood agar.* Stock cultures and antitoxin-treated cultures were plated and any variations noted. The stock culture plate served as the control.

3. *Growth in serum broth.* Cultures were grown in serum broth for varying periods of time at 37°C and room temperature (1 to 72 hours), the macroscopic and microscopic conditions of the growth were noted. Microscopic examination was made primarily to ascertain the presence or absence of capsules.

4. *Capsular swelling preparations.*

a. 1 mm loop of culture.

† 200 to 500 units U.S.P.H.S. or 10,000 to 25,000 original neutralizing units.

b. 5 mm loop of antiserum.
c. 5 mm loop of methylene blue.
d. Incubation at 52° for varying periods of time (½ to 24 hrs).
e. Control with saline or with heterologous serum for the detection of cross reactions.

Results. The results of these experiments may best be shown by a comparison with beta hemolytic streptococci not so treated. The treated organisms generally show encapsulation which is readily accentuated by the presence of specific antiserum. The virulence of the treated organism is increased and the hemolytic zone around the colonies is altered. These results are shown in Table I.

Summary. A method of obtaining virulent, encapsulated group A beta hemolytic streptococci, sensitive to the reaction of capsular swelling with homologous antisera, has been described. Essentially this consists of the injection of living streptococci into mice simultaneously treated with unrefined scarlet fever antitoxin.

TABLE I.

Untreated	Treated with Antitoxin
1. Action on horse blood agar.	
a. Hemolytic zone sharply defined and transparent.	*a.* Hemolytic zone frequently not sharply defined and is generally translucent (18 to 24 hours).
b. No pigment was ever noted on base of colony.	*b.* Base of colony frequently showing rose to deep rose shades.
c. Smooth or rough colonies.	*c.* Smooth colonies.
2. Growth in broth.	
a. Often flocculent with sediment.	*a.* Invariably of even turbidity.
b. Capsule not visible in ordinary wet methylene blue preparation.	*b.* Capsule frequently visible in wet methylene blue preparation.
c. Soon loses capsules in broth as shown by dry capsule stains.	*c.* Capsules observed with capsule stains after 48 hours in many cases.
3. Capsular swelling.	
a. Negative with specific antiserum.	*a.* Positive with antiserum. This reaction is unmistakable.
4. Agglutination.	
a. Organisms readily agglutinated with homologous serum using rapid slide agglutination test.	*a.* To date these organisms have proved inagglutinable with homologous serum, using rapid slide test.
5. Virulence for mice.	
a. May or may not be virulent.	*a.* Virulent (10^{-4} to 10^{-9}).
b. If virulent, 10 lethal doses generally kill in 16 to 48 hr.	*b.* 10 lethal doses kill in 5 to 12 hr.
c. Many mouse passages are necessary to build virulent strains.	*c.* With strains so far employed a relatively few passages (1 to 10) are required to obtain the desired virulence.