

18.2 / 1918 d
P 345

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS ISOLATED FROM EPIDEMIC POLIOMYELITIS BY IMMUNE SERUM

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Recent investigations on the bacteriology of poliomyelitis have shown quite constantly in the aetia of infection and in the infected tissues of epidemic poliomyelitis a pleomorphic streptococcus or micrococcus which, soon after isolation, has tended to localize electively in the central nervous system of animals and to produce paralysis. These properties are soon lost on cultivation. The importance of immunologic studies was recognized early and Sept. 11, 1916, immunization experiments were begun by the injection of a monkey with the pleomorphic streptococcus in order to protect it against virus should it recover from the effects of the injection.¹ Since then, horses and monkeys have been immunized and their serum tested for immune bodies (chiefly agglutinins), for neutralizing and protecting power over virus, and for curative effects on experimental poliomyelitis in monkeys. The agglutinating power of the serum of patients and monkeys that have recovered from poliomyelitis, together with numerous normal controls, and its curative effect on poliomyelitis in man has been studied. Summaries of these studies have already been published.^{2,3} In this paper we wish to record in greater detail the experiments and results obtained.

TECHNIC

In the immunization of horses increasing doses of the pleomorphic streptococcus were injected intravenously on 3 consecutive days, with an interval of a week between each series from November 2 to May 1. The bacteria used for the injections were grown in dextrose broth or ascites dextrose broth for 24 hours, then centrifugalized out and suspended in salt solution.

Horse 1 was injected from Nov. 2, 1916, to May 1, 1917. At first strains from human poliomyelitis (heated to 60 C.) were injected, and for a short time both human and monkey strains were used. For many weeks, however, live cultures of strains from experimental poliomyelitis in monkeys, were injected exclusively. Test bleedings were made November 2, 4 and 22, December 22, January 8 and 30, March 3, April 3, and May 14 and 16. The serum obtained

TABLE 17
THE DEVELOPMENT OF AGGLUTININS FOR THE PLEOMORPHIC STREPTOCOCCUS IN THE
SERUM OF MONKEYS WITH PARALYZED VIRUS

Strain	Dilutions of Serum	Serum from										
		Monkey 147			Monkey 148			Monkey 150				
		Normal		Immune	Normal		Immune	Normal		Immune		
		4/18	4/20	5/2	5/14	4/18	4/20	4/20	4/18	4/20	5/2	5/14
729.9 (Cord)	1:1	-	-	-	0	-	-	-	-	-	-	+++
	1:10	0	0	0	+	+	+	+	+	+	+	+++
	1:50	0	0	0	0	0	0	0	0	0	0	0
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0
729 (Tonsil pus)	1:1	-	-	-	0	-	-	-	-	-	-	+
	1:10	0	0	0	+	+	+	+	+	+	+	0
	1:50	0	0	0	0	0	0	0	0	0	0	0
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0
899 (Pons)	1:1	-	-	-	++	-	-	-	-	-	-	+++
	1:10	+	0	0	++	+	+	+	+	+	+	+++
	1:50	0	0	0	+	+	+	+	+	+	+	++
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0
M145.3 (Cord)	1:1	-	-	-	0c	-	-	-	-	-	-	+c
	1:10	0	0	0	0	0	0	++	0	0	0	++
	1:50	0	0	0	0	0	0	+	0	0	0	0
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0
M148.2 (Cord)	1:1	-	-	-	+	-	-	-	-	-	-	++
	1:10	0	0	0	++	0	0	++	0	0	0	++
	1:50	0	0	0	0	0	0	+	0	0	0	0
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0
6224.7 (Pneumo- cocci) Control	1:1	-	-	-	0	-	-	-	-	-	-	0
	1:10	0	0	0	0	0	0	0	0	0	0	0
	1:50	0	0	0	0	0	0	0	0	0	0	0
	1:250	0	0	0	0	0	0	0	0	0	0	0
	1:1250	0	0	0	0	0	0	0	0	0	0	0
	1:6250	0	0	0	0	0	0	0	0	0	0	0

in the other bleedings except that in Monkey 148 there was a distinct increased agglutinating power of the serum on April 27 over all the strains. This increase, however, was not as marked as on April 29. There was no demonstrable diminution in the agglutinating power of the serum due to age.

The agglutinin content of the serum was increased in all the monkeys that developed typical attacks of poliomyelitis. This was shown toward all the strains except in 2 instances. The increase in agglutinating power by the immune serum was no greater toward the homologous strain (Monkey 148) than toward the heterogeneous strains (729, 899 and Monkey 145). The serum of Monkey 148, however,

agglutinated the strain from this monkey slightly more markedly than did that of Monkeys 147 and 150. There was no increase in agglutination of the control pneumococcus strain. The cultures from the brain of Monkey 148 showed, in addition to the pleomorphic streptococcus, a hemolytic streptococcus which was not agglutinated by the serum from any of the bleedings of this monkey.

SUMMARY

The pleomorphic streptococcus isolated from the tonsil and central nervous system of human poliomyelitis and from the central nervous system of monkeys paralyzed with virus has marked antigenic properties.

The strains from both human and monkey poliomyelitis are cross-agglutinated in high dilution by the serum from horses hyperimmunized with human and monkey strains respectively, and in lower dilution by the serum of persons who have had poliomyelitis. Moreover the serum of monkeys acquires specific agglutinating power over these strains as they develop poliomyelitis following injection of virus. This agglutinating power of the serum following poliomyelitic attacks has been shown to persist for months, and hence cannot be regarded as due to mobilization of preformed antibodies or to nonspecific changes in the serum incident to fever etc. at the time of the attack. Streptococci and pneumococci from sources other than poliomyelitis are with few exceptions not agglutinated more by the antipoliomyelitis serums than by normal horse serum. A few of a large number of strains approached in agglutinability the pleomorphic streptococcus.

Normal human and normal monkey serum has little or no agglutinating power over the poliomyelitis strains or over control strains. The agglutinin content toward these strains of serum of persons and monkeys suffering from other streptococcus infections was no higher than of the respective normal serums. Poliomyelitic human and monkey serums showed no increase in agglutinating power over streptococci from sources other than poliomyelitis. Antipneumococcus, antimeningococcus and antistreptococcus horse serums do not agglutinate the poliomyelitic strains more than normal horse serum.

Judging by the results with normal horse serum the pleomorphic streptococcus is more easily agglutinated than green-producing streptococci and pneumococci from a wide range of sources. A method has thus been found which proves that the streptococcus, found so con-

stantly in poliomyelitis, is immunologically quite distinct when first isolated. There is a marked difference in the degree with which the various strains retain their specific agglutinability. Anaerobic cultivation tends to preserve this property; aerobic cultivation tends to destroy it. It may be lost by either method without noticeable changes in morphology or cultural characteristics, but usually these changes occur simultaneously. Some strains retain the specific agglutinating condition through many culture generations. In some instances it may be lost suddenly even during one subculture. The specific agglutinating condition was preserved for months in the dried brain substance of human cases and in brain substance in sealed pipets of animals showing paralysis.

By means of agglutination experiments it has been possible to differentiate the pleomorphic streptococcus from green-producing streptococci isolated occasionally from the central nervous system of uninoculated and inoculated animals. The latter may be regarded as antemortem or postmortem invaders.

The results support the view that the elective localizing power of the pleomorphic streptococcus as first demonstrated by Rosenow, Towne and Wheeler⁴ has significance and that it in some way bears etiologic relationship to epidemic poliomyelitis.

1. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: Observations on Immunity of Monkeys to Experimental Poliomyelitis. *Jour. Am. Med. Assn.*, 1917, 68, pp. 280-282.

2. Rosenow, E. C.: The Production of an Antipoliomyelitis Serum in Horses by Inoculations of the Pleomorphic Streptococcus from Poliomyelitis. *Jour. Am. Med. Assn.*, 1917, 69, pp. 261-265.

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4. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: The Etiology of Epidemic Poliomyelitis. Preliminary Note. *Jour. Am. Med. Assn.*, 1916, 67, pp. 1202-1205.