

chronic non-streptococcic infections, from quiescent or recovering cases of atrophic arthritis, or from hypertrophic arthritis.

3. Intravenous streptococcic vaccine therapy does not influence the bactericidal property of blood while the agglutinating titer may be greatly increased.

4. There is no correlation between streptococcic bactericidal property, agglutinating titer and sedimentation rate in the cases so far examined.

5. Serum alone usually shows little or no bactericidal power against *Streptococcus viridans*, irrespective of its agglutinating titer. In exceptional cases there may be definite inhibitory action.

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#### Importance of Continuous Agitation in Bactericidal Tests with Streptococci.

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Although the advantages of continuous agitation in phagocytosis experiments had been noted by earlier investigators,<sup>1, 2, 3</sup> Robertson and Sia<sup>4</sup> were apparently the first to use the rotatory-oscillation method extensively. All have observed that constant mixing during incubation promotes bactericidal action and produces more constant results than stationary incubation or intermittent agitation. Most of the previous work has been done with cultures of pneumococci and we were faced with the necessity of checking these factors with the streptococcus with which we were working.

An agitating machine utilizing the same principle as that in the machine used by Fenn<sup>2</sup> and later in the improved machine of Robertson and Sia<sup>4</sup> was designed and built for us by Mr. Wm. H. Hamilton, E. E. It consists of 3 brass flanges mounted rigidly on a motor driven shaft. Two of the flanges act as tube holders, having 5/8 in. perforations bored at uniform intervals near the periphery, while the third flange acts as a guard. The flanges will hold 18 tubes at

<sup>1</sup> Rosenow, E. C., *J. Infect. Dis.*, 1906, **3**, 683.

<sup>2</sup> Fenn, W. O., *J. Gen. Physiol.*, 1920, **3**, 439.

<sup>3</sup> Kite, G. L., and Wherry, W. B., *J. Infect. Dis.*, 1915, **16**, 109.

<sup>4</sup> Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **39**, 219.

one time and the angle of the tubes can be altered by placing one end of the tube in the second or third perforation from that opposite the one containing the other end of the tube. The tubes are held in place by a rubber band which encircles them. A constant speed electric fan motor furnishes the power which is transmitted by pulley through a short belt (rubber band) and a worm and circular gear.

The rotating rack revolves at a constant speed of 18 r.p.m. and the mixtures shift from the base of the tube to the top against a paraffined rubber stopper and back again during each revolution. The chief advantages of this design are the mechanical simplicity, constant speed and small space occupied by the machine. It fits conveniently on the top shelf of a small incubator and requires no more attention than occasional oiling of the motor (Fig. 1).

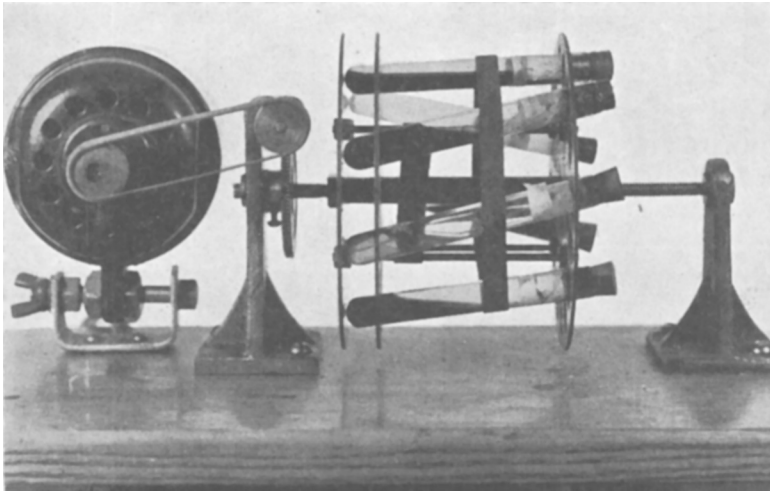


FIG. 1.

Tests were performed with defibrinated blood and washed suspensions of 24-hour cultures of *Streptococcus viridans*, with and without agitation in the machine. The mixtures were made in the proportion of 3 cc. of freshly drawn, defibrinated blood and 1 cc. of gelatin-Locke's solution containing about 10,000 bacteria. A 0.5 cc. sample of this mixture was plated with 10 cc. of dextrose agar immediately and again after 4 hours of incubation. The results are indicated in Table I. In the stationary tubes an actual reduction in the number of bacteria as indicated by the colony count was noted in only 2 cases of the 19 tested. In every case a much greater killing effect was obtained in the mixtures that were rotated in the machine.

TABLE I.  
Bactericidal Effect With and Without Rotatory Agitation.

Case No.	Colony Increase or Decrease in %	
	Stationary	Rotatory Agitation
1 (F.W.)	+100	-100
2 (K.S.)	+1220	+59
3 (Z.W.)	+290	-78
4 (T.S.)	+1330	-98
5 (W.F.)	+1330	+1140
6 (A.H.)	+1000	+44
7 (D.C.)	+2000	+100
8 (I.H.)	+540	-99
9 (A.F.)	+2000	+690
10 (M.S.)	+2000	+260
11 (M.K.)	-75	-100
12 (W.H.)	+44	-75
13 (R.H.)	+322	-100
14 (M.S.)	+270	-100
15 (M.B.)	+110	-100
16 (G.M.)	+51	-100
17 (C.C.)	+127	+80
18 (A.R.)	-68	-100
19 (A.A.)	+200	+122

Similar differences were noted in 7 tests in which, instead of incubating in stationary tubes after the initial thorough mixing, the tubes were mixed by inverting several times by hand at 15-minute intervals and allowed to settle in racks in the interim (Table II).

TABLE II.  
Bactericidal Effect with Intermittent Shaking and Rotatory Agitation.

Case No.	Colony Increase or Decrease in %	
	Intermittent Shaking	Rotatory Agitation
1 (F.L.)	+60	-100
2 (D.P.)	+100	-100
3 (G.E.)	-12	-100
4 (E.M.)	+300	-99
5 (J.E.)	-56	-100
6 (Q.L.)	+2000	+43
7 (M.S.)	+1000	-99

In every instance the colony count was less in mixtures rotated constantly in the agitating machine.