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Electrophoretic potential and virulence of diphtheria bacilli.

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Measurements of electrophoretic velocities on strains of pneumococci and other organisms indicate that many vital phenomena are closely associated with the electrokinetic P. D. between the organism and its menstruum.

It has become evident from the work of Falk and his associates[1] that there are significant parallelisms, direct or inverse, between electrophoretic potential, virulence, agglutinability and other characteristics of bacteria. For pneumococci of the types 1, 2, 3, 4 it has been found that the P. D. is higher the greater the virulence for white mice and vice versa.

The work here reported was undertaken to determine whether or not P. D. would parallel virulence of various strains of diphtheria bacilli as well as to obtain comparable data in regard to P. D. on avirulent or pseudodiphtheria bacilli.

The cataphoretic potentials on corynebacteria were determined by the method described and used by Falk, Gussin and Jacobson.[1] For reasons discussed by them results are expressed in terms of observed velocities (μ/sec) instead of expressing the electrical P. D. at the interface between the bacterium and the menstruum in millivolts.

P. D. in observed velocities (μ/sec) may be converted into millivolts by multiplying by the factor 1.3. Ten measurements of velocity (5 with one and 5 with reversed orientation of the electrical field) were made at stationary layers V_s133, 497, in the same medium in which the bacteria were grown. The same methods were used in measuring velocities of cells in distilled water.

The electrokinetic P. D. on 65 strains of corynebacteria parallel the virulence (toxigenicity) of these bacteria. Measurements of potential on 48 hr. veal broth cultures of toxigenic strains give

[1] Falk, I. S., Gussin, H. A., and Jacobson, M. A., *J. Inf. Dis.*, 1925, xxxvii, 481, 495, 499; Falk, I. S., and Jacobson, M. A., *ibid.*, 507; xxxvii, 182, 188.

potential differences of 4.2 to 1.0 (μ/sec) with an average of 3.4 (μ/sec). When these strains are grown in veal peptone broth (48 hour cultures) the P. D. falls, giving values from 3.0 to 0.8 (μ/sec).

Measurements of cataphoretic potentials on avirulent (non-toxigenic) strains which ferment saccharose quite uniformly give very much higher values (P. D. 12.1 (μ/sec)). Non-fermenting avirulent cultures gave potential differences of 7.9 to 10.0 (μ/sec). Strains with a P. D. of intermediate values, 4.4 to 6.2 (μ/sec), are according to morphological and biochemical characteristics typical diphtheria bacilli but are not virulent. These differences are not due to changes in pH of the menstrua as the same potentials were observed in sugar free buffered broth (pH 7.2).

Distilled water suspensions of corynebacteria washed 3 times in distilled water gave the following potentials: all avirulent strains P. D. = 20.0 — 34.0 (μ/sec) with an average potential of 26.0 (μ/sec); all virulent strains range in electrophoretic velocities (P. D. μ/sec) from 2.6 to 11.3 (μ/sec) with an average P. D. of 7.0 (μ/sec).

Apparently no parallelism exists between the minimal fatal dose of washed bacilli suspended in distilled water and their P. D. in the same menstruum. The lowest values observed in broth cultures were found on the Park No. 8 strain of C. diphtheria. The degree of toxicity parallels P. D., *i. e.*, low potential is a concomitant of high toxicity. Salted out, purified toxic materials depress P. D. in a quantitative manner. The power of virulent strains

TABLE I.
Electrophoretic Potentials on Corynebacteria.

Cultures	pH average	48 hr. broth cultures (pH 7.2)			Dist'd H_2O suspensions P.D. (μ/sec) average
		P.D. (μ/sec) average	Sugar free buffered (pH 7)		
			pH average	P.D. (μ/sec) average	
Virulent (40 strains)	6.2	3.4	7.2	3.3	7.0
Non-ferm.	7.0	8.1	7.4	9.1	25.0
Aviru- Dext. ferm	5.8	5.5	7.2	8.0	31.00
lent Sucr. ferm. (25 strains)	5.8	12.0	7.2	9.0	34.00

to elaborate toxin diminishes with increases in P. D. and is enhanced when the P. D. is decreased with suitable reagents. If P. D. and M. L. D. of broth cultures are determined daily for 12 days a direct parallelism is shown to exist.

Rapid identification of virulent diphtheria bacilli can be effected by determinations of P. D., especially of distilled water suspensions prepared from 18 hour glycerin agar cultures.

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Influence of anti-serum and of animal passage upon virulence and electrophoresis of pneumococci.

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In earlier publications[1] we discussed at length the parallel relations between the virulence and the electrophoretic potentials of pneumococci. We reported that variant strains (Blake) of type I pneumococci, which differ in their virulence for mice, also differ in a parallel manner in their electrophoretic potentials.

In the studies reported here we have undertaken to determine whether "rough" colony varieties could be produced from the Blake Type I pneumococcus (designated as A) and its variants (designated as B and C respectively). These variants are derivatives of the A strain, and were obtained by Blake and Trask by growth in the presence of specific anti-serum. We have also undertaken a series of experiments to determine changes in virulence and P. D. upon successive passage through white mice of cultures of significantly different virulence and P. D.

We have found that the sequence of decreasing virulence for white mice, decreasing P. D. and increasing agglutinability is: A, B, C. The A, B and C strains form "smooth" colonies on peptone, serum or blood agar plates. Strains which give "rough"

[1] Falk, I. S., Gussin, H. A., and Jacobson, M. A., *J. Infect. Dis.*, 1925, xxxvii, 481; Falk, I. S., Jacobson, M. A., and Gussin, H. A., *ibid*, 495, 499; Falk, I. S., and Jacobson, M. A., *ibid.*, 507; 1926, xxxviii, 182, 188.