ELECTROPHORESIS OF DIPHTHERIA BACILLI

II. MICRO-ELECTROPHORESIS AND THE DIFFERENTIATION OF VIRULENT AND NON-VIRULENT DIPHTHERIA BACILLI

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The experiments thus far reported[1] have shown that there exist roughly parallel differences in P.D. between the toxigenic and non-toxigenic corynebacteria. The electrophoretic mobility varies inversely with toxigenicity, i.e., low potential is a concomitant of high toxigenicity. The studies reported here were designed to attempt an application of electrophoretic measurements to the determination of "virulence" on cultures submitted to the Bureau of Laboratories and Research in the Chicago Department of Health. It was our intent first to determine how closely "virulence" determinations made by electrophoretic measurements would conform to guinea pig tests, and, second, if the results were satisfactory to attempt the development of an electrophoretic technique that would be simple and rapid as well as reliable.

Before undertaking these routine tests it was necessary to find a broth medium which would permit maximum toxin production in the shortest time and which would not contain substances that would interfere with or cause equivocal mobility readings. Cystine was tried with the results that have been described in the preceding paper. The following media were also tried: veal infusion + 2 per cent Merck's peptone without NaCl; plain veal infusion; veal infusion + Fairchild peptone without salt; veal

[1] Cf. the preceding paper, in this JOURNAL, by Jensen and Falk.

TABLE 1

Electrophoretic mobilities: μ per second

CULTURE	GLASS (48-HOUR PEPTONE MERCK)	QUARTZ (48-HOUR PEPTONE MERCK)	QUARTZ (24-HOUR VEAL)	GLASS (48-HOUR VEAL)	GLASS (48-HOUR PEPTONE BUFFERED)	GLASS (48-HOUR PEPTONE CYSTINE BUFFERED)	VIRULENCE (BY GUINEA PIG TEST)
1	5.1	13.0	8.5	6.2	6.8		+
2	4.0	10.0		4.0	4.5	5.6	+
3	4.5	8.0		3.4	4.0	4.0	+
4	5.6	9.0	8.0	3.4		4.2	+
5				5.6	4.5	4.2	+
6	4.8	7.5		7.5	lost		+
7				6.6	8.5	4.8	+
8	3.0	9.0		2.8	4.4		+
9				4.8	3.7	4.0	+
10	4.2	6.8	7.7	2.1	3.4	4.0	+
11				3.7		4.2	−
12	3.4	10.0	7.4	4.8	4.2	6.1	−
13	6.8	11.3	9.0	7.5		6.1	+
14	4.5	8.5	7.4	3.6	4.2	4.7	+
15	5.2	9.0	10.0	6.2	6.8	4.2	+
16	4.0	6.8		3.4	4.0	4.7	+
17	3.9	8.0	7.0	7.5		4.7	+
18				3.4		4.1	+
19	5.2	8.5	7.9	3.5		5.1	+
20	4.9	6.2	6.4	6.4		5.2	+
21				4.8	4.5	6.1	+
22	3.4	10.0	10.0	10.0	4.5	5.2	+
23	4.2	8.5		6.8	3.4	3.4	+
24	6.2	7.7		3.4		4.2	+
25					6.8	6.8	−
26					4.2	6.6	+
27					4.4	4.4	+
28					4.2	4.2	+
29					3.7	3.7	+
30					3.7	2.8	+
31					4.2	3.7	+
32						4.4	+
33						5.6	+
Park No. 8	3.0	8.0	7.4	2.4	5.5	6.5	+

infusion + 2 per cent Difco peptone buffered with Na_2HPO_4; the same medium with cystine; veal infusion + 2 per cent Merck's peptone with cystine and veal infusion + 2 per cent Witte's peptone without salt. All the media were adjusted to pH 7.4.

Of these media, the veal 2 per cent Witte's peptone gave fairly constant results and was used in the final determinations.

Table 1 shows values obtained in casting about for a suitable medium to be used as a standard for this technique. Only a

TABLE 2

Electrophoretic mobilities in distilled water suspensions (24 hour glycerol agar cultures)

CULTURE	QUARTZ CELL P.D.	GLASS CELL	VIRULENCE
	μ per second		
1	17.0	28.0	+ (mixed)
2	17.0	17.0	+
3	17.0	19.0	+
4	17.0	19.0	+
5	34.0	22.6	+
6	15.0	28.0	+
7	34.0	28.0	+ (mixed)
8	17.0	17.0	+
9	17.0	28.0	+
10	17.0	17.0	+
11	17.0	28.0	+
12	18.0	22.6	+
13	17.0	22.6	+
14	17.0	17.0	+
15	16.0	22.6	+
16	13.0	17.0	+
17	34.0	34.0	+
18	17.0	22.6	+
19	17.0	17.0	+
20	20.0		+
21	17.0	17.0	+
22	18.0		+
23	17.0	19.0	+
24	16.0	17.0	+
25		24.0	−
26		20.0	+
27		18.8	+
28			+
29		17.0	+
30		17.7	+

few of the tests are listed. Determinations made with a quartz cataphoresis cell were also made along with the tests in a glass apparatus. The measurements in the quartz apparatus did not

TABLE 3

Identification of unknown corynebacteria

CULTURE	P.D. (48-HOUR 2 PER CENT WITTE'S PEPTONE BROTH)	VIRULENCE BY ELECTROPHORESIS	VIRULENCE ACCORDING TO GUINEA PIG TESTS
	μ per second		
1	4.8	+	+
2	4.8	+	+
3	4.8	+	+
4	4.2	+	+
5	4.0	+	+
6	4.0	+	+
7	4.5	+	+
8	4.0	+	+
9	4.0	+	+
10	4.0	+	+
11	5.2	−	−
12	5.7	−	−
13	4.8	+	+
14	4.5	+	+
15	4.8	+	+
16	4.5	+	+
17	4.7	+	+
18	6.0	+	+
19	4.5	+	+
20	4.7	+	+
21	4.8	+	+
22	4.8	+	+
23	4.0	+	+
24	4.0	+	+
25	11.3	−	−
26	4.8	+	+
27	4.4	+	+
28	4.2	+	+
29	4.0	+	+
30	4.0	+	+
31	3.7	+	+
32	4.5	+	+
33	4.8	+	+
34	4.0	+	+
35	11.3	−	−
36	11.3	−	−
37	4.2	+	+
38	8.0	−	−
39	4.0	+	+
40	4.0	+	+
41	4.0	+	+

TABLE 3—*Continued*

CULTURE	P.D. (48-HOUR 2 PER CENT WITTE'S PEPTONE BROTH)	VIRULENCE BY ELECTROPHORESIS	VIRULENCE ACCORDING TO GUINEA PIG TESTS
	μ per second		
42	4.0	+	+
43	6.8	−	−
44	4.0	+	+
45	4.2	+	+
46	4.8	+	+
47	4.2	+	+
48	4.8	+	−
49	4.2	+	−
50	4.8	+	+
51	3.7	+	+
52	4.5	+	+
53	3.4	+	+
54	4.8	+	+
55	3.7	+	+
56	4.8	+	+
57	4.8	+	−
58	4.8	+	+
59	4.2	+	+
60	3.4	+	+
61	4.8	+	+
62	3.4	+	+
63	3.4	+	+
64	4.5	+	+
65	4.2	+	+
66	4.4	+	+
67	4.4	+	+
68	4.4	+	+
69	4.2	+	+
70	4.4	+	+
71	11.3	−	−
72	5.0	+	+
73	4.0	+	+
74	4.8	+	+
75	4.8	+	+
76	4.8	+	+
77	3.2	+	+
78	4.8	+	+
79	4.4	+	+
80	4.4	+	+
81	4.4	+	+
82	4.8	+	+
83	4.0	+	+

TABLE 3—*Continued*

CULTURE	P.D. (48-HOUR 2 PER CENT WITTE'S PEPTONE BROTH)	VIRULENCE BY ELECTROPHORESIS	VIRULENCE ACCORDING TO GUINEA PIG TESTS
	μ per second		
84	No culture		
85	4.0	+	+
86	4.8	+	+
91	6.8	−	−
92	4.8	+	+
93	4.8	+	−
94	4.8	+	+
95	3.8	+	+
96	3.8	+	+
97	4.2	+	+
98	4.0	+	+
99	4.2	+	+
100			
101	4.0	+	+
102	4.0	+	+
103	6.0	−	−
104	4.4	+	+
105	11.3	−	−
106	3.8	+	+
107	3.2	+	+
108	3.8	+	+
109	4.2	+	+
110	11.3	−	−
111	6.8	−	−

parallel those made in the glass cell and the use of the quartz apparatus was discontinued.

In table 2 are listed the measurements obtained with distilled water suspensions of eighteen-hour glycerol agar (0.5 per cent beef serum) slants. For reasons which we have not discovered, freshly isolated bacilli have higher mobilities in distilled water than have strains which have been cultivated in the laboratory for six months or more.

In this work it was found that pseudo-diphtheria bacilli were often introduced in the transfers of several colonies from Loeffler's medium. These mixed cultures in distilled water gave equivocal mobilities. When each colony is transferred to a separate single tube, these results have not been noted except in a few instances in the present series.

Using the large apparatus, mentioned in the preceding paper, for electrophoresis determinations, P.D. (μ per second) 5.0 was taken as the criterion for differentiating toxigenic from non-toxigenic bacilli.[2] All forty-eight-hour broth cultures with a P.D. (μ per second) higher than 5.0 were considered non-toxigenic; those lower than P.D. (μ per second) 5.0 were called toxigenic.

In table 3 are listed the results of potential measurements with the diagnoses from them, and the results of virulence tests conducted by the guinea pig method, injecting subcutaneously 0.5 to 2.0 cc. of a salt solution suspension from the original Loeffler's serum culture. The results of each test were unknown to the individuals performing the other until after the completion of all of the cataphoresis experiments.

In this series of 105 unknown cultures of corynebacteria the following findings were obtained: *a*, 88 were *virulent* by both electrophoresis and by guinea pig tests; *b*, 13 were *non-virulent* by both tests; *c*, 4 were *virulent* by electrophoresis and *non-virulent* by guinea pig tests.

An electrophoretic mobility of 5.0 μ per second had been taken as the dividing line between the strains that were to be called "virulent" and those that were to be called "non-virulent." It is significant to note that of the four cultures on which the electrophoretic and the guinea pig tests disagreed, three had given mobilities of 4.8 μ per second (and were just barely in the "virulent" category by this test), and the fourth had given a mobility of 4.2 μ per second. There were no reports available on guinea pig retests. Thus, we may say that the electrophoretic mobility method erred only four times in 105 and in each case on the side of sanitary safety by calling a strain "virulent" which by a guinea pig test was "non-virulent."

Many of these cultures had been taken from cases of scarlet fever and ear infections and, as the mobilities show, are all low grade toxin producers.

[2] We have already called attention (in the preceding paper) to the objections against the assumption of a discontinuity in virulence. Yet such an assumption must be made if the diagnostic laboratory wishes to say that a culture is either "virulent" or "non-virulent."