Months	Temperatures at which spores were heated. Degrees Centigrade					entigrade.
heating.	100	107	115	118	121	Total
1	1532	521	1039	52	432	3576
2	68	130	312	5	57	572
3	160	124	310	8	49	651
4	126	53	220		42	441
5	48	33	121	1	25	228
6	35	11	49	ī	18	114
7-9	39	36	98		24	197
10-12	9	18	41		12	80
13-18	39	16	62	1	27	144
19-21	3	3	2			8
22 - 24	15	6	21	1	8	52
25 - 27	9	4	21	_	5	39
28-30	5	4	8		3	20
31 - 33	2	4	5		_	11
34-36	1	2	7	1	1	12
37-39	1 1	3	9		2	15
40-42	4	ĩ	6		1	12
43-45	l ī		4		Ī	6
46 - 48	2		8		2	12
49-51			6		2	8
$52 \cdot 54$	1		1 I	_		2
55-57		2	2		1	5
58-60	_	1	5	_	2	8
61-63		_	1			1
64-66	_		2			2
67						_
68						—
69	_	1	1			2
70						<u> </u>
71	1		1		—	2
72	1					1
73						
No. positive	2103	972	2363	70	714	6222
No. ĥeated	3802	2905	14270	490	7954	29421

TABLE I.Time of Germination in Months after Spores of Clostridium Botulinum wereHeated in Oil-stratified Broth in Sealed Tubes.

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The Adaptation in Vitro of Diphtheria Bacillus to Specific Antitoxin.

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Observations on the transmutation of toxic and virulent strains of B. *diphtheriae* into atoxic and non-virulent varieties by means of cultivation in a medium containing either normal serum or specific

antitoxin have been reported by Bernhardt,¹ Levinthal² and, after the completion of the present work, by Becker.³ In this paper, which deals with the same problem, the relation between toxin production and virulence of the adapted diphtheria strain was given more attention than has been accorded to it in the past. At the same time the flocculating properties of the atoxic filtrate of an adapted culture when brought in contact with the specific antitoxic serum, were studied in view of the doubt which has recently been expressed in the literature on the specific nature of the flocculation occurring in neutral mixtures of bacterial toxins with their respective antitoxins (Schultz⁴).

Two diphtheria strains, the Park-Williams No. 8 and a recently isolated strain D X, which differed widely under normal standard conditions in toxigenicity and virulence, were employed. The P. W. strain produced a powerful toxin with an m.f.d. of 0.002, while the filtrate of the D X strain obtained under the same conditions yielded a low grade toxin with an m.f.d. of 0.02 (30 cc. of sugar-free veal infusion broth with 0.2% dextrose incolated with one loop of a 24-hour Loeffler slant culture and incubated for 5 days at 35-36° C.). On the contrary, the P. W. strain was fatal for guinea pigs only in amounts as large as 1/4 Loeffler slant, while the D X strain was highly virulent, 1/20 slant killing a guinea pig within 31 hours. The antiserum was a nonpreserved diphtheria antitoxic horse serum with a titer of 350 units per cc. Normal horse and tetanus antitoxic horse sera were run along for purposes of control. The inhibitory action of the 3 sterile sera on the growth of the 2 diphtheria strains was determined in preliminary tests. While the antidiphtheric serum showed little or no inhibitory effect on the growth of the P. W. strain, and even in larger amounts inhibited but slightly the D X strain, inhibition of growth of both strains was quite apparent with the 2 other control sera in much smaller doses.

The process of adaptation was carried out as follows: Three sets of broth-serum mixtures were prepared by adding to a constant amount, 5 cc. of broth, 0.3, 0.5 and 1.0 cc. of each of the 3 sera. The two diphtheria strains were cultivated in parallel sets in the mentioned broth-serum mixtures by keeping the cultures for 2 successive passages in the same serum concentration, transfers being made from each tube at the end of 48 hours incubation. Thus after a total of 6 passages the 2 strains grew well in a medium containing 1.0 cc. of antidiphtheric serum in 5 cc. of broth. Daily observations on the activity of growth and on the morphological appearance of the bacilli showed no marked deviation from the

nonadapted cultures. At this stage of the adaptation the 2 specifically adapted diphtheria strains were examined under standard conditions for toxin production, virulence and fermentation reactions, together with the parent cultures which had been carried on simultaneously in broth alone, and with the organisms cultivated in the control serum-broth mixtures. It was found that the 2 diphtheria strains cultivated in the specific antitoxic medium had undergone a profound change in their toxigenic power. This change may be taken to indicate a complete loss of toxin production in view of the fact that the intracutaneous injection into the guinea pigs of 0.1 cc. of a 1:10 dilution, and the subcutaneous injection of amounts up to 2 cc. of the undiluted filtrates, failed to elicit any suggestion of either a local or systemic reaction. On the other hand, the toxigenicity of the 2 strains cultivated in the presence of the control sera had in each instance remained practically unaltered. Subcutaneous inoculations of the organisms into guinea pigs revealed the remarkable fact that all the strains, including the 2 specifically adapted cultures, had not dropped in virulence. Fermentation tests with saccharose, maltose, dextrose and dextrin showed all strains to react true to type.

The atoxic filtrate of the P. W. strain, if given in a single subcutaneous dose of 2 cc., failed to induce the formation of antitoxin in guinea pigs within the period of 3 weeks, as measured by intracutaneous tests. Finally, flocculation reactions were carried out by combining the atoxic filtrates of the 2 adapted strains with diphtheria antitoxic serum according to the method of Ramon. The results of these tests, while not quite constant, demonstrated that the atoxic filtrates flocculated much less than the corresponding potent control toxins. The remarkable fact should be emphasized, however, that some definite flocculation did occur with these atoxic and non-antigenic filtrates.

A continuation of the adaptive process beyond the period indicated led to somewhat irregular results with both the experimental and the control cultures. Further experiments are necessary to determine more accurately whether the atypical properties of the diphtheria bacillus acquired during the exposure to specific antitoxin are permanent or only transitory. The evidence so far seems to point to the latter.

¹ Bernhardt, G., Z. f. Hyg. u. Inf. Kr., 1916, lxxvii, 179.

² Levinthal, W., Z. f. Hyg. u. Inf. Kr., 1926, evi, 679.

³ Becker, Z. f. Immun. Forschg., 1927, lii, 402.

⁴ Schultz, E. W., Immun. Forschg. u. Exp. Therap., 1928, in press.