

# THE PROPHYLACTIC USE OF PERTUSSIS VACCINE CONTROLLED BY THE COMPLEMENT FIXATION TEST \*

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During the past five years numerous clinical reports have been made on the prophylactic and curative value of pertussis vaccine. Graham,<sup>1</sup> Ladd<sup>2</sup> and Bamberger<sup>3</sup> reported favorably on its curative power in small doses. Hess<sup>4</sup> was fortunate in being able to observe the effect of vaccines during an epidemic of whooping cough in an institution; he was skeptical of its curative power, but was favorably impressed by its prophylactic value, even though 10 per cent. of his treated patients developed pertussis. Hartshorn and Moeller,<sup>5</sup> after reviewing the literature and reporting cases of their own, thought the vaccine worthy of further trial, but did not recommend its general use. Abt<sup>6</sup> says that "the possibility of immunizing by vaccination is still an open question for pertussis." Luttinger,<sup>7</sup> after an extensive experience with the whooping-cough clinic of the New York City Health Department, concludes that pertussis vaccine in large doses (that is, one-half billion, one billion and two billion bacteria) is of value in preventing whooping cough. In his most recent report<sup>8</sup> he says that "his results would warrant the routine administration of pertussis vaccine for both curative and prophylactic purposes." Von Sholly, Blum and Smith,<sup>9</sup> using the same material as Luttinger, conclude that "more observations and more critical observations with controls for comparison must be made before the case can be made out for the curative and prophylactic value of pertussis vaccine."

It was thought that this question could be attacked from a new angle. The observations on the results of vaccines made thus far have been chiefly clinical. If vaccines are of value it would be natural to

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1. Graham, E. E.: *AM. JOUR. DIS. CHILD.*, 1912, **3**, 41.

2. Ladd: *Arch. Pediat.*, 1912, **29**, 581.

3. Bamberger: *AM. JOUR. DIS. CHILD.*, 1913, **5**, 33.

4. Hess, A. F.: *Jour. Am. Med. Assn.*, 1914, **63**, 1007.

5. Hartshorn and Moeller: *Arch. Pediat.*, 1914, **31**, 586.

6. Abt: *Arch. Pediat.*, 1916, **33**, 881.

7. Luttinger: *New York Med. Jour.*, 1915, **101**, 1043.

8. Luttinger: *Jour. Am. Med. Assn.*, 1917, **68**, 1461.

9. Von Sholly, Blum and Smith: *Jour. Am. Med. Assn.*, 1917, **68**, 1451.

expect that after their administration specific antibodies would be present in the blood. We have two methods for determining the presence of specific antibodies — the agglutination and complement fixation tests. The agglutination test in pertussis has been tried by many workers, Shiga,<sup>10</sup> Wollstein,<sup>11</sup> Povitzsky and Worth<sup>12</sup> and others, and all agree that it is far from satisfactory. The complement fixation reaction, however, has been more successful. Bordet and Gengou,<sup>13</sup> using the bacillus discovered by them as antigen, found a positive complement fixation test in all cases of pertussis. Bächer and Menschikoff<sup>14</sup> found positive complement fixation in pertussis only after the injection of vaccine, but clinically noticed no benefit from the use of the vaccine. Shiga, Imai and Eguchi<sup>10</sup> were able to differentiate between *B. influenza* and the Bordet-Gengou bacillus by means of complement fixation. Wollstein<sup>11</sup> in nine cases of pertussis was unable to make the complement fixation test react positively. Olmstead and Povitzsky<sup>15</sup> found that "the separation by morphologic and cultural characteristics of the typical Bordet-Gengou bacillus from atypical strains of the Bordet-Gengou bacillus and from the influenza bacillus has been confirmed by complement fixation tests." Winholt<sup>16</sup> found a positive complement fixation obtainable two weeks, but stronger about eight to ten weeks, after the onset of the disease, and that when the influenza bacillus was used as antigen with serum of pertussis patients, no complement fixation occurs. Friedlander,<sup>17</sup> by a slight modification of technic, obtained positive complement fixation in all cases of pertussis even in the early catarrhal stages.

It was, therefore, proposed to select a number of healthy children who, as far as could be determined, had never had whooping cough, and vaccinate them with different pertussis vaccines, in order to study the effect of such treatment on antibody formation, by means of the complement fixation test.

#### TECHNIC

Two types of vaccines were employed:<sup>18</sup> a commercial vaccine, purchased in the open market, and a vaccine made by the ordinary method of heating the bacteria for one hour at 60 C. The latter was standardized, in the usual manner, by counting against the red blood corpuscles of normal blood. Two

10. Shiga, Imai and Eguchi: *Centralbl. f. Bakt.*, abt. 1, 1912.

11. Wollstein: *Jour. Exper. Med.*, 1909, **11**, 41.

12. Povitzsky and Worth: *Arch. Int. Med.*, 1916, **17**, 279.

13. Bordet and Gengou: *Ann. d. l'Inst. Pasteur*, 1906, **20**, 218.

14. Bächer and Menschikoff: *Centralbl. f. Bakt.*, abt. 1, 1912, **61**, 218.

15. Olmstead and Povitzsky: *Jour. Med. Research*, 1916, **33**, 379.

16. Winholt: *Jour. Infect. Dis.*, 1915, **16**, 397.

17. Friedlander and Wagner: *AM. JOUR. DIS. CHILD.*, 1915, **8**, 135.

18. I am indebted to Dr. W. P. Larson for valuable assistance with the laboratory work.

different strains of Bordet-Gengou bacillus were used, one obtained from the New York City Department of Health and the other from a commercial biological house.

The technic of the complement fixation tests was that ordinarily used. About 1 c.c. of the patient's blood was drawn and allowed to coagulate; the serum was separated in the centrifuge and heated to 56 C. for ten minutes (we find that ten minutes is sufficient to inactivate the serum and there is less danger of impairing its antibody content); in the tests 1 to 2 drops of undiluted serum from a capillary pipet (of which about 20 drops make 1 c.c.) was taken. The amboceptor consisted of antishoop rabbit serum (1:40). The antigen was prepared by scraping, in distilled water, a forty-eight hour growth of *Bacillus pertussis* on blood agar and shaking for twelve hours, after which it was incubated at 48 C. for twenty-four hours. The antigen was standardized against the blood serum of a known case of pertussis.

The attempt was made to immunize rabbits by injecting prophylactic doses of vaccines and, at the same time, inoculating control rabbits with living Bordet-Gengou bacilli. A comparison of the antibody production of living and dead bacilli was rendered impossible by the fact that many rabbits gave a positive complement fixation test before treatment was begun. The tests had, therefore, to be made entirely on humans. For this purpose fairly normal and healthy children, ranging from 6 months to 3 years, were chosen from the inmates of Lymanhurst, City Children's Hospital.

In all, seventeen patients were treated; these were divided into three series. In the first series of four cases, three were given vaccine prepared by us and one a commercial vaccine, once a week for four weeks; the first two doses were one hundred million bacilli, and the last two doses two hundred million. Blood for the complement fixation test was taken before each injection, and again two weeks after the last injection. All tests were absolutely negative. In the second series of four cases, all were given our vaccine, two hundred million being given once a week for five weeks. Complement fixation tests performed before each injection and one performed two weeks after the last injection were, likewise, all negative. In the third and last series, the dosage recommended by the New York City Health Department was used; one half billion, one billion and two billion bacteria were given at two-day intervals. There were nine cases; of these, five received our vaccine and four received commercial vaccine. Of the five who received freshly prepared vaccine, three reacted positively and two were negative. Of the four cases receiving commercial vaccine, one reacted positively and three negatively. In this last series of nine cases the ages ranged from 6 months to 3 years. The ages of those giving positive reaction were 12, 24, 26 and 36 months, respectively; of those giving negative reactions, 6, 14, 14½, 16 and 20 months.

## SUMMARY AND CONCLUSIONS.

Our observations in seventeen cases would theoretically justify the prophylactic vaccination against pertussis.

In none of the cases treated with the smaller doses could any antibodies be demonstrated. Whereas, large doses, such as recommended by the New York City Health Department, showed antibodies in 44 per cent. of the cases. The freshly prepared vaccines were, apparently, more effective than the stock vaccines, as 60 per cent. of the patients treated gave positive fixation reaction. From this, it may be concluded that it is possible to immunize children against pertussis if sufficiently large doses of freshly prepared vaccine be used.

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