The Known and Unknown of Bacillus Pertussis Vaccine*

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DANISH State Serum Institute investigators and clinicians have made valuable contributions on *B. pertussis* vaccine since 1916. Miller [1] says:

> The strains are kept on Bordet-Gengou medium until inoculated on the "vaccine medium" (3 parts nutrient agar (calf), 2 parts potato-glycerin agar, and 2 parts defibrinated horse blood). The 3 day growth is washed into 1 per cent formalin in physiological sodium chloride. After formalization for 1 week the suspension is centrifuged, resuspended in 0.5 per cent phenol in physiological sodium chloride and standardized to 10,000 million bacteria per c.c.

For nearly 20 years a standard technic has been in use—the total dosage of 2.2 c.c. is divided into 3 injections (0.5, 0.7 and 1.0 c.c.), given at intervals of 3 or 4 days. Madsen,[2, 3] found this ineffective as a curative agent; given as a prophylactic, 1 to 3 months before exposure, it failed to prevent the disease in 364 nonimmunes. The course was more frequently mild, however, and the percentage of deaths was lower when injections were completed a few weeks before symptoms appeared. Discussing F. McDonald's [4] summary on *B. pertussis* vaccine, R. Smith [5] cautions against hasty conclusions on pertussis immunization. He says:

> Pertussis is such a dread disease that physicians and parents easily take "the will for the deed" so far as proof in relation to prevention is concerned. Enthusiasm has waxed and waned many times. . . . One is certainly justified in using vaccine in an attempt to produce immunity against whooping cough, but one must be honest with himself and with his patients in acknowledging that proof of its protective efficacy is lacking. One must guard against drawing conclusions from a few cases, from clinical impressions, or from uncontrolled statistical reports.

When whooping cough occurs during the first few years of life, its course is often influenced by age, previous health, nutritional state, stability of the nervous system, hygienic care (aseptic nursing), climate and season. In the vaccinated and nonvaccinated the duration and severity often vary greatly. In a crucial study of immunization only nonimmunes between 8 months and 3 years of age should be used. Children should be excluded if they ever had any persistent cough. The very young may not yet possess the power to elaborate immunity from injected antigen, regardless of its potency and dosage; children over 3 may require a larger dosage of antigen, or may already be immune. Because the communicability index for pertussis is about 75 per cent, appreciably more than 25 per cent of the vaccinated should escape the disease

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when they are definitely exposed to infection. Exposure to infection is more likely to be early, intimate, repeated and prolonged when it occurs within the family. The best controls are, therefore, the nonimmune (nonvaccinated) siblings. Exposure occurs more frequently outside, but doubt may exist when vaccinated nonimmunes escape such transient exposures. Did the patient disseminate bacilli at the time of contact? did the vaccinated child aspirate bacilli? or, was the child immune before vaccination? Diagnosis should be verified by cough plates, repeated white cell and differential white cell blood counts. Of importance is the duration of the cough before exposure of the injected child occurs—also the time interval between vaccination and exposure. Because specific serologic tests are not an index of immunity, and without a reliable skin test,[6] the ultimate test of immunity is exposure to infection.

Between 1928 and 1932, 394 selected young nonimmunes, average age about 14 months, were injected with an especially prepared *B. pertussis* vaccine.[7, 8] Each child was injected with a total of 7 to 8 c.c. (approximately 70 to 80,000 million bacteria). Thirty intimate (familial) exposures to the disease in siblings have occurred; and 169 of the vaccinated children have been exposed (?) a total of 360 times in 7 years. No child injected with this Evanston vaccine has contracted typical whooping cough. A frail girl of 2 years, injected soon after recovery from measles, developed a very mild cough (1 cough plate positive, blood findings repeatedly normal) within 3 months after she had been injected with a total of 7 c.c.; her brother (control) was convalescing from typical pertussis at the time. In 1933, Macdonald and Macdonald[9] reported important inoculation and exposure experiments on humans.

Two commercial laboratories were authorized by Northwestern University Medical School to make this vaccine according to detailed specifications. Freshly isolated, hemolytic strains are sent to them monthly; fresh, defibrinated human blood is used in the Bordet medium; the 3 day growth is scraped off; it is not washed; 0.5 per cent phenol in physiologic sodium chloride is used as preservative and diluent; the sterile product is standardized to contain approximately 10,000 million bacteria per c.c.; it is refrigerated until transported. Jobber, druggist, and physician are cautioned to keep it cold. Table I is a summary of vaccinations with approved commercial vaccines since 1932.

Thirty-three physicians in 19 states have reported a total of 2,945 vaccinations with approved commercial vaccines. The average age of their patients was well over 3 years. Seven children of 3 physicians, and a nurse, were willfully, intimately, and repeatedly exposed to the disease without contracting it. Only a few of the physicians ascertained the instances of exposure, but all reported their failures. A total of 130 known exposures and 21 failures were reported. Most failures occurred in children well over 3 years of age.

The prompt decrease in the incidence of typhoid fever in the Army and Navy, coincident with compulsory antityphoid vaccination in 1912, is accepted as evidence of effective specific immunization. Although typhoid vaccine has been perfected since then, the dosage augmented, and general hygienic measures have improved, typhoid fever still occurs in some of those exposed after vaccination. An authority recently estimated such failures at about 10 per cent. At first, toxin-antitoxin immunization against diphtheria failed to protect about 30 per cent of the injected children. Soon after scarlet

fever immunization was introduced, the number of injections was increased to 5, and the strength of the toxin appreciably increased. With the hope of decreasing the percentage of failures in pertussis immunization, children over 3 years will probably require a total of 10 c.c. or more of the approved commercial vaccine. One, 2, 2 c.c. would then be given in each arm, in 3 consecutive weeks.

Little is known about the factors which may cause failure—the antigen may be impotent when injected, or the individual may not possess the power to develop immunity. Little is known about the stability of the fraction which confers immunity. Endotoxin, prepared according to Besredka's method as described by Bordet [10] was found to contain living *B. pertussis*. Mishulow, Mowry, and Scott [11] obtained a toxic filtrate from bacilli grown on horse-blood chocolate-agar with 1 per cent horse-serum beef-heart broth added. They produced the Schwartzman phenomenon with it. Gundel, Keller, and Schlüter [6] prepared an endotoxin which was very toxic for rabbits and mice; skin tests with dilutions of it showed no specificity; nor was its necrotic action neutralized by the addition of convalescent pertussis serum. Regarding Krueger's [12] endo-antigen, McDonald says: "If the filtrate is water-clear, is one certain that 'cell metabolites' are the only masses filtered out?" Miller's recent complement fixation tests on rabbits, within a few weeks after undenatured endo-antigen was injected, seem to compare favorably with parallel tests on rabbits injected with the Danish vaccine. The relative impotency of the vaccine might be attributed, at least in part, to denaturation effected by the 1 per cent formalin. Truschina, Pechletzkaja and Murawjewa [13] maintain that their bouillon culture filtrate purified by Huntoon's method, contains soluble toxin which possesses specific antigenic properties. Dilutions of it produced positive skin tests more frequently in the nonimmune than in the immune.

No one has shown that immunization is possible with vaccine made from old stock strains, grown without blood. Leslie and Gardner[14] maintain that "Phase I" strains yield the best antigen. Until it is known with certainty whether some strains yield more potent antigen than others, it is advisable to use recently isolated, hemolytic strains. They may probably be used as long as they fulfil the requirements of Leslie and Gardner's Phase I strains. It is of economic importance to learn whether other bloods, *e.g.* sheep, in the culture medium for general use by health departments, orphanages and foundling homes, will yield a safe and potent vaccine. The Michigan State Health Department vaccine, made with sheep's blood, is washed, that of the New York City Health Department is not washed. The work of Kendrick and Eldering [15] and that of Park and Mishulow should determine, within a few years, whether safe and potent vaccine can be prepared without human blood.

The Helber counting chamber, with the vaccine diluted with Callison's fluid, probably yields more constant checks than the Wright or Harrison methods. Uniformity of the vaccine is important, but methods applicable for washed vaccines (Hopkins's tube) yield different counts when compared with unwashed vaccines of identical opacity. The Nephelometer has advantages after an accurate standard has been established, but the latter should not change color in the course of time. The Danish method of carefully matching the density of each new lot of vaccine with a well guarded standard tube of vaccine is simple, quick, and relatively accurate. It is our method of choice.

TABLE I

SUMMARY OF VACCINATIONS WITH 8 C.C. OF APPROVED COMMERCIAL VACCINE

	Number of Injections	Known Exposures	Failures	Age	Interval Between Injection and Exposure	Per Cent Protection
Private Patients (1932 to Sept. 1, 1935)	458	Familial 13 Casual 27	Gilroy Gilroy Kelly Mathews Pick	4 yrs. 7 yrs. 3 yrs. 4 yrs. 6 yrs.	6 mos. 6 mos. 2 yrs. 1 yr. 20 mos.	87.5
Evanston Health Department (1934 to Sept. 1, 1935)	604	Familial 14 Casual 50	Johnson Keefer Spencer Spencer	9 mos. 3 yrs. 2½ yrs. 4 yrs.	8 mos. 8 mos. 5 mos. 5 mos.	93.4
2 Orphanages	242	23	Trush	9 mos.	8 mos.	95.0
Total	1,304	127	10	Av. 3¾ yrs.	Av. 8 mos.	Av. 92.0

Although some children may develop immunity within a shorter period of time, our records show that most children require somewhat more than 3 months for complete immunization. Lymphocytosis, quite like that of pertussis, often occurs within a month after vaccine injection. To give the 3 bilateral injections at shorter intervals (*e.g.*, the 8 c.c. completed within a week) might hasten the immunity response. Work along this line is in progress.

Because whooping cough is a disease of early life, and as most of the deaths occur during the first 2 years, it is desirable to immunize as early as possible. Four hundred (" Cradle ") infants, less than 6 weeks of age, have been injected with a total of 6 c.c. of the approved commercial vaccines. Since December, 1934, 168 have been given a total of 8 c.c. They withstand the injections remarkably well. There have been 9 subsequent exposures. Four of the children escaped, the other 5 contracted mild pertussis. It therefore seems that the best age is between the 6th and 8th month of life.

Warmth accelerates chemical change. Interrupted refrigeration, as during transit, a month on the druggist's shelf, or a few weeks in the physician's bag, acts deleteriously. Mishulow, Oldenbusch, and Scholl [16] elicited complement-fixation reactions with the serum of rabbits, injected with *B. pertussis* vaccine that had been stored for several years at 8 to 10° C. Uninterrupted refrigeration of biological products is a desideratum.

Physicians inquire about the best sequence for immunization procedures and the importance of spacing. Fishbein [17] says:

> Perhaps the best plan would be to allow 4 months to intervene between successive immunization procedures. Because whooping cough causes more deaths in children under 2 years of age than diphtheria, measles, and scarlet fever combined, it is prudent to immunize first against whooping cough—preferably during the second half year of life. Four months later a single alum toxoid injection against diphtheria may be given. Four or more months after, when the Schick test is done, preferably in the spring or autumn, the smallpox vaccination may be done.

Very little is known about the effect of other diseases on the immunity response from injected pertussis vaccine. Two failures occurred in children who were injected with the approved vaccine soon after recovery from measles; another occurred in a girl of 6 who was injected soon after recovery from mumps; 20 months later she contracted typical pertussis. Her injected brother, who did not have mumps, escaped pertussis, although intimately exposed to her for weeks.

SUMMARY

Bacillus pertussis vaccine, like typhoid vaccine, is an immunizing, not a curative, agent. A time interval of several months is required for immunization to be complete. About 10 per cent of the children injected with a total of 8 c.c. of the approved commercial vaccine contracted pertussis when subsequently exposed to infection. Some of the factors which might interfere with the immunity response are controllable.

REFERENCES

1. Miller, J. Experimental Observations on the Antigenic Potency of H. pertussis Extracts. *J. Immunol.*, 26:247, 1934.

2. Madsen, T. Whooping Cough; Its Bacteriology, Diagnosis, Prevention and Treatment. *Boston M. & S. J.*, 192:50, 1925.

3. Madsen, T. Vaccination Against Whooping Cough. *J.A.M.A.*, 101:187, 1933.

4. McDonald, F. Whooping Cough; With Particular Reference to Prophylaxis and Treatment with Vaccines. *New England J. Med.*, 213:198, 1935.

5. Smith, R. "Summary" of the Symposium on the Control of Communicable Diseases. *New England J. Med.*, 213:211, 1935.

6. Gundel, M., Keller, W., and Schlüter, W. Serologic Diagnosis and Specific Treatment of Pertussis. *Ztschr. f. Kinderh.*, 57, 89, 1935.

7. Sauer, L. Whooping Cough; A Study in Immunization. *J.A.M.A.*, 100:239, 1933.

8. Sauer, L. Immunization with Bacillus Pertussis Vaccine. *J.A.M.A.*, 101:1449–1451, 1933.

9. Macdonald, H., and Macdonald, E. Experimental Pertussis. *J. Infect. Dis.*, 53:328, 1933.

10. Bordet, J., and Genou, O. L'Endotoxine Coqueluche. *Ann. d'l Inst. Pasteur*, 23:415, 1909.

11. Mishulow, L., Mowry, I., and Scott, E. Pertussis Toxic Filtrates and Toxin-vaccines. *J. Immunol.*, 19:227, 1930.

12. Krueger, A. Method for Preparation of Bacterial Antigens. *J. Infect. Dis.*, 53:237, 1933.

13. Truschina, E., Pechletzkaja, W., and Murawjewa, O. Das Toxin der Keuchhustenmikrobe. *Ztschr. f. Immunitätsforsch.*, 83:124, 1934.

14. Leslie, P., and Gardner, A. The Phases of Hemophilus Pertussis. *J. Hyg.*, 31:423, 1931.

15. Kendrick, P., and Eldering, G. Significance of Bacteriological Methods in Diagnosis and Control of Whooping Cough. *A.J.P.H.*, 25:147, 1935.

16. Mishulow, L., Oldenbusch, C., and Scholl, M. Potency of Stored Pertussis Vaccines. *J. Infect. Dis.*, 41:169, 1927.

17. Fishbein, M. *Handbook of Therapy*, American Medical Association, Ed. 10, 1935, p. 60.