Neonatal Response to DTP Vaccines

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IMMUNIZATION and vaccination during the first, second, or third month of life has been advocated by health authorities to provide protection during the period of greatest vulnerability to infection (*1–4*). As infants are susceptible to pertussis from birth, and fatality is highest during the first 6 months of life, delay in pertussis immunization until 3 to 6 months of age fails to prevent most of the deaths from this disease. In addition, the advent of routine poliomyelitis vaccination requires establishing optimum immunization schedules for diseases for which specific prevention is now available. General concern over the ability of the infant to respond during the first month of life to a variety of antigens, singly or in combination, has deterred early immunization procedures. The tendency to delay immunization has also resulted from unwillingness to expose the newborn to local and systemic reactions that may accompany injection.

Antigens that will stimulate maximum protection in the newborn with a minimum of side reactions are diligently sought. The choice of antigen is complicated by the variation in potency of available preparations (*5*). Our study measures response to combined immunization initiated within the first 2 weeks of life, compares the response to aluminum phosphate and aluminum hydroxide adsorbed antigens, and observes local and systemic reactions following the use of each of these preparations.

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Method of Study

A group of 112 maternity cases in the seventh and eighth month of pregnancy was selected for study from prenatal clinics of the Los Angeles City Health Department. These patients were selected on the basis of their willingness to cooperate, freedom from disease, stabilized marriage, and stable residence pattern.

Patients in the study were delivered at the Los Angeles County General Hospital. At term or at delivery a 10-ml. sample of blood was collected from the mother. Cord blood specimens were also requested. These specimens were followed by 5-ml. specimens of whole blood collected from the infants at intervals of approximately 1 month, 6 months, and 1 year following completion of diphtheria-tetanus toxoid and pertussis vaccine (DTP) inoculations. The Los Angeles City Health Department laboratory obtained agglutination titers and performed antibody titrations.

Pertussis agglutination titers, based upon the ability of the patient's serum to agglutinate a standard number of *Haemophilus pertussis* organisms, were obtained according to standard methods (*6*), except that the organisms were incubated overnight at 37° C. and not at room temperature. The titer represents the highest serum dilution showing a 2-plus reaction.

Diphtheria antitoxin titration was performed according to a modification of the method of Frobisher (*7*). The titration was based on the standardization of a diphtheria toxin, the end reaction in the presence of a known amount of

antitoxin producing a zone of erythema approximately 1 cm. in diameter in the skin of a guinea pig. A dilution of serum that gave the same reaction as the standard antitoxin to the same amount of toxin was recorded as the same amount of antitoxin as the standard. The response to tetanus toxoid was not determined in this study.

As soon as possible following discharge from the hospital, the infants received the first of three intramuscular (lateral gluteal) 0.5-ml. injections of commercially prepared DTP. Aluminum phosphate adsorbed antigens and aluminum hydroxide adsorbed antigens were used in alternate infants. Each 0.5 ml. dose contained the equivalent of 30 billion *H. pertussis* organisms. All of the initial injections were given within the first 14 days after birth except for one set of twins who were not available for immunization until the 19th day. The first injection was given in the home. The second and third doses were scheduled at intervals of 4 weeks. Followup home visits were made by public health nurses or by physicians to obtain case histories, to observe reactions, and to schedule clinic visits for subsequent injections and collection of specimens. One child health conference session was reserved weekly at the health center for the study cases. Every effort was made to adhere as closely as possible to the schedule for inoculations and collection of specimens. If the patient failed to appear at the clinic as scheduled, the injections were given and the blood specimens collected in the home within 10 days.

Classification of local reactions was based upon the degree of erythema and induration at the site of injection. Local reaction was recorded as mild, moderate, or severe according to the following definitions:

Mild: Erythema of less than 3 cm. in diameter and/or induration persisting for not more than 2 days, or small persistent nodule.

Moderate: Erythema of 3 cm. or more in diameter with induration persisting for more than 2 days.

Severe: Induration progressing to necrosis or accompanied by a wide area of erythema.

Systemic reactions were classified as:

Mild: Temperature not more than 100° F., restlessness, drowsiness, or irritability of less than 12 hours.

Moderate: Temperature up to 101° F., symptoms of illness persisting for 12 hours or more.

Severe: Temperature above 101° F., in the presence of symptoms and signs of illness.

Results

From the 112 pregnant women who entered the study, 93 newborn infants were available for followup. Of these, 78 completed the series of inoculations and submitted at least one postvaccination blood specimen. Second specimens were obtained from 72 infants and third specimens from 54. The median age at the time of collection of each of the 3 specimens was 3.2 months, 8.5 months, and 15 months.

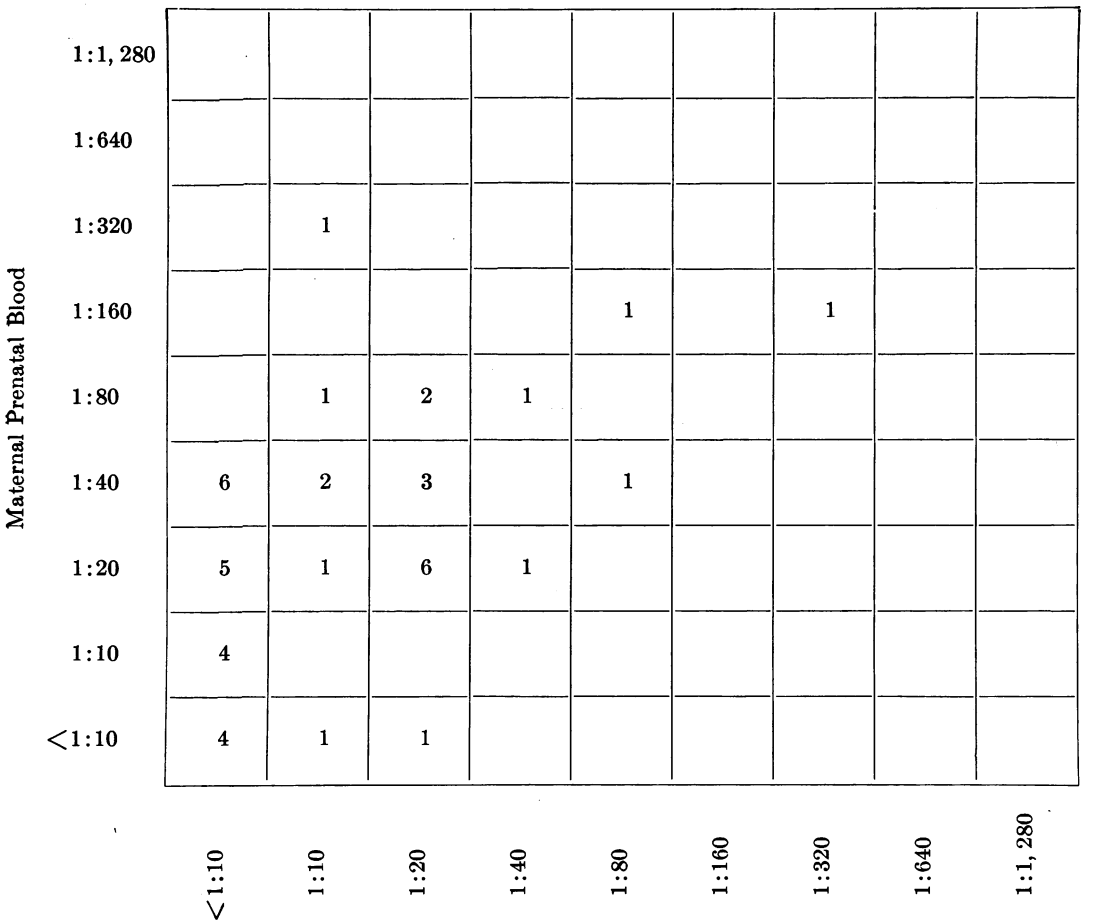
Response to Pertussis Vaccine

The distribution of "prevaccination" titers (as reflected by maternal and cord blood specimens) and of postvaccination titers for infants

Table 1. Pertussis agglutination titers

Titer	All mothers	Mothers of infants in study	Cord	Infant postvaccination		
				1 month	6 months	1 year
1:1,280	0	0	0	1	0	1
1:640	0	0	0	5	1	2
1:320	0	0	1	3	9	2
1:160	6	5	0	14	9	8
1:80	6	5	2	11	12	10
1:40	8	7	2	7	4	4
1:20	17	15	12	7	10	7
1:10	22	18	6	5	6	5
<1:10	53	43	19	25	21	15
Total	112	93	42	78	72	54

Figure 1. Pertussis agglutination titers, maternal and cord blood



is shown in table 1. Of the 93 mothers with infants in the study, 61 (65.6 percent) had titers of 1 : 10 or less. Only 5 had titers greater than 1 : 80. Cord blood specimens were submitted for testing following 42 deliveries. The quantitative distribution of maternal prenatal and the corresponding cord blood pertussis agglutination titers is shown in figure 1. Higher titers were observed in the corresponding maternal specimens. Titers of 1 : 10 or less were obtained in 10 of the maternal and in 25 of the cord blood specimens.

The agglutination titers at the time of the first postvaccination bleeding (1 month following third DTP injection), according to the antigen used, are shown in table 2. Higher agglutination levels were observed among the 41 infants who received the aluminum phosphate adsorbed antigen. The median titer among infants receiving the aluminum phosphate antigen was 1 : 80 as compared with a median titer of 1 : 10 among 37 infants receiving the aluminum hydroxide adsorbed antigen.

One month postvaccination titers were determined in 30 of the infants for whom the agglutination level of cord blood was observed at birth (fig. 2). Fifteen of these infants received aluminum phosphate and 15 aluminum hydroxide adsorbed antigen. A comparison of specimens tested 1 month after completion of vaccination with the cord blood specimens shows that 11 infants who received aluminum phosphate and 2 who received aluminum hydroxide adsorbed antigen had an increase in the pertussis agglutination titer to 1 : 80 or greater.

The ability to maintain the agglutination

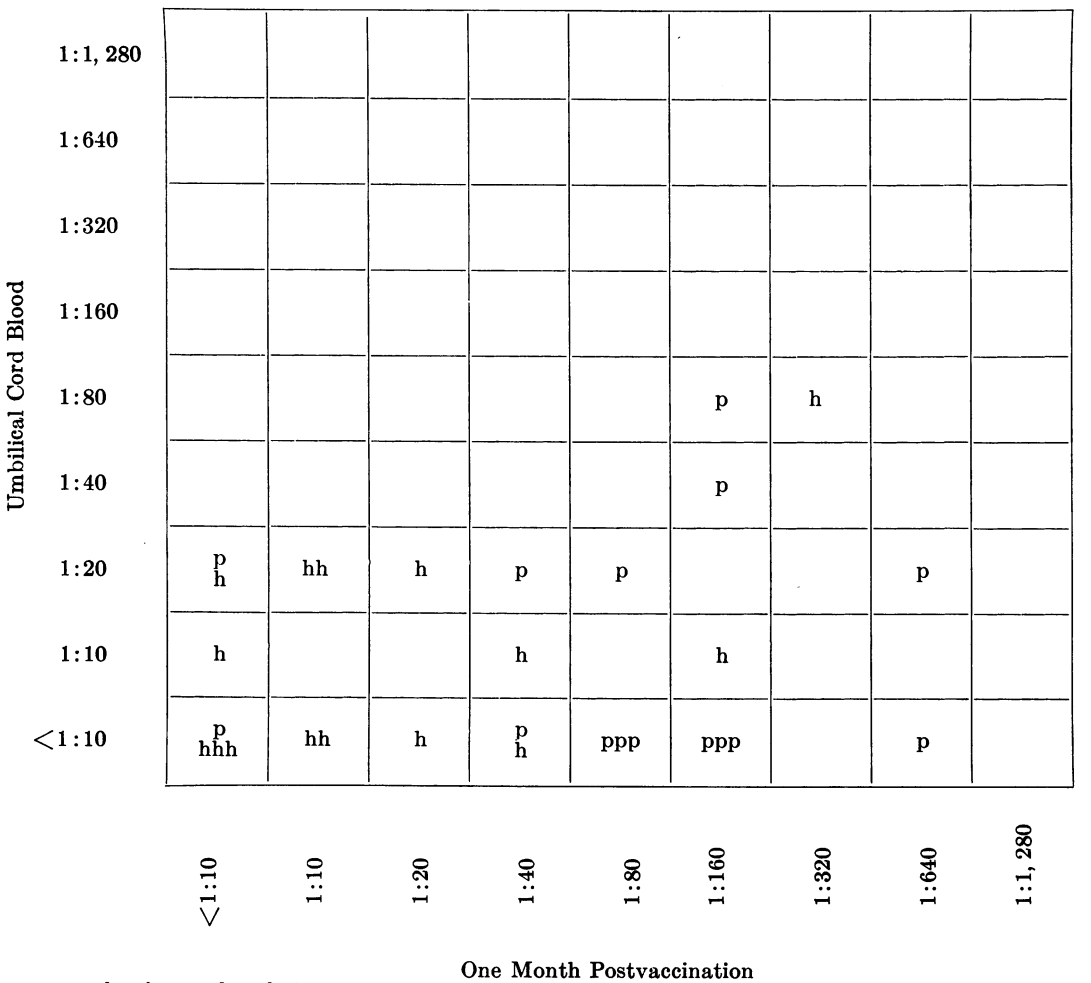
titer is observed by comparing the titer at the third postvaccination bleeding (1 year after completion of vaccination) with the titers on first postvaccination bleeding (fig. 3). Among the 54 infants from whom 1-month and 1-year postvaccination blood specimens were obtained, 28 received the aluminum phosphate and 26 the aluminum hydroxide adsorbed antigen. Among those receiving aluminum phosphate adsorbed antigen 19 had titers of 1:80 or higher 1 month following vaccination. Of these, 15 had titers of 1:80 or more 1 year following vaccination. Among the infants receiving aluminum hydroxide who were successfully followed for 1 year, 3 had titers of 1:80 or more 1 month following vaccination. One of these

Table 2. Pertussis agglutination titers 1 month following vaccination, by type of antigen

Titer [1]	Aluminum phosphate	Aluminum hydroxide
1:1,280	1	0
1:640	5	0
1:320	2	1
1:160	12	2
1:80	9	2
1:40	2	5
1:20	2	5
1:10	0	5
<1:10	8	17
Total	41	37

[1] Median for aluminum phosphate is <1:80; aluminum hydroxide is >1:10.

Figure 2. Pertussis agglutination titers of cord blood and 1-month postvaccination blood specimens, by type of antigen



had a 1:80 titer and two had 1:10 titer or less 1 year later. Of the 23 infants injected with aluminum hydroxide who had titers less than 1:80 one month following vaccination, 5 had titers of 1:80 or more one year later. Observation of the serum agglutination titers 1 month and 1 year after vaccination indicates that once agglutinins are stimulated, the aluminum phosphate adsorbed antigen was the better of the two in maintaining the titer for 1 year.

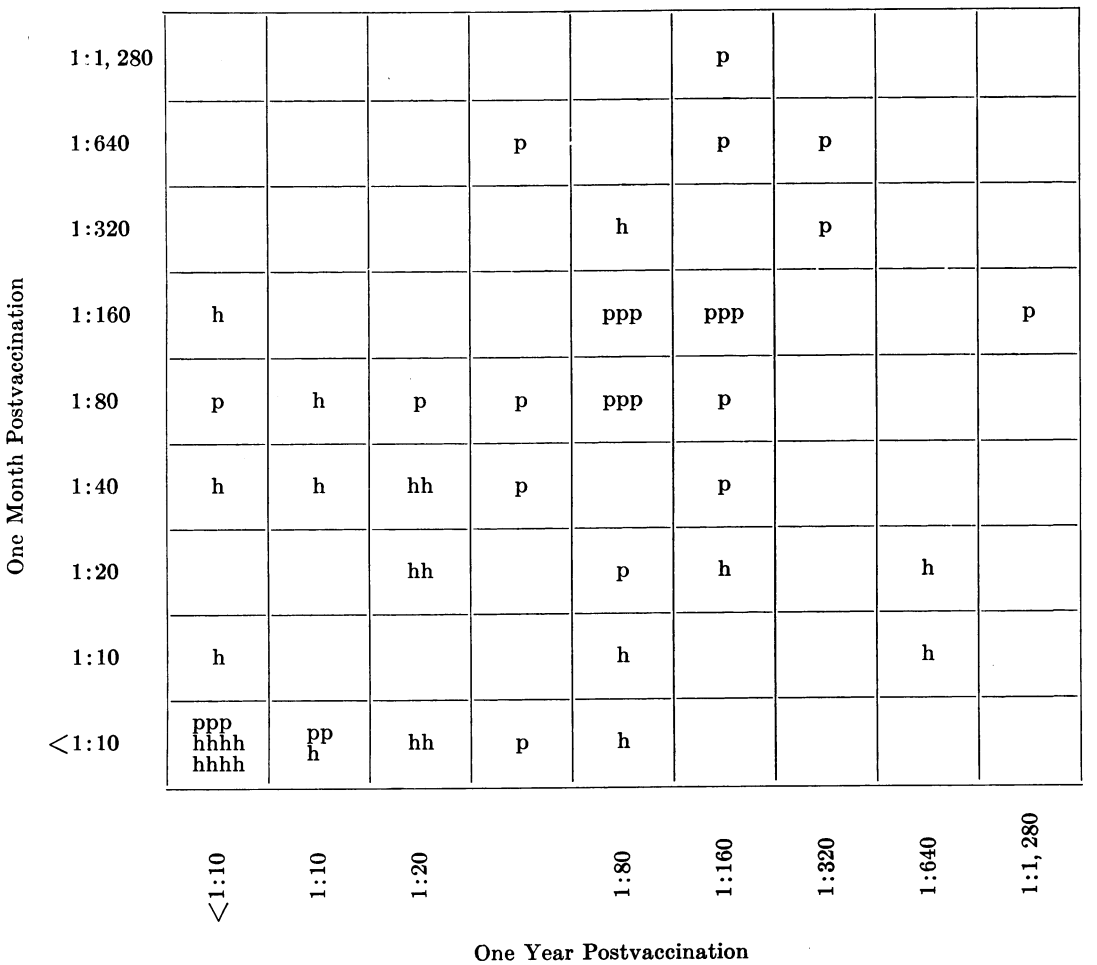
Response to Diphtheria Toxoid

Closer agreement was observed between maternal and cord blood diphtheria antitoxin levels (fig. 4) than in pertussis agglutinins (fig. 1). In 9 of 42 cases there was no demonstrable diphtheria antitoxin (less than 0.01 units per ml.) in maternal and cord blood. Both maternal and cord blood revealed 0.01 units per ml. in 7 cases, 0.1 unit in 12 cases, and 1.0 unit in 2 cases.

Among the 30 infants with a cord blood and 1 postinoculation specimen, 18 showed increases in antitoxin level by the first month after completion of the injections, 9 remained the same, and 3 had a lower level than was observed in the cord blood (fig. 5). The results according to antigen used are as follows:

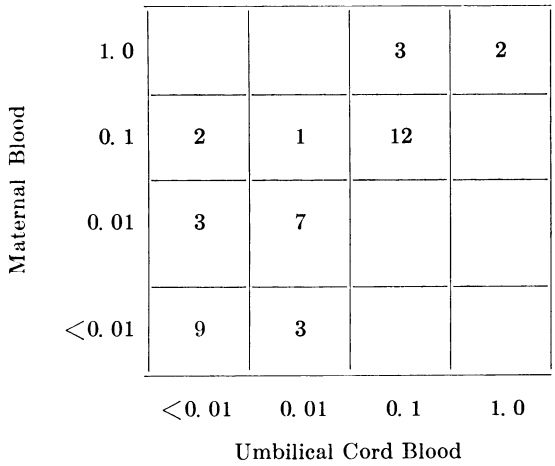
	Phosphate	*Hydroxide*	*Total*
Titer increase	9	9	18
Titer same	4	5	9
Titer decrease	2	1	3
Total	15	15	30

Figure 3. Pertussis agglutination titers 1 month and 1 year following vaccination, by type of antigen



p=aluminum phosphate
h=aluminum hydroxide

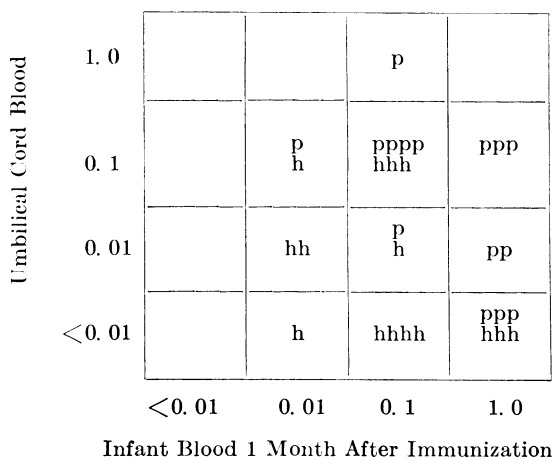
Figure 4. Diphtheria antitoxin units per milliliter, maternal and cord blood.



Although higher titers were observed 1 month following inoculation in the group receiving the aluminum phosphate antigen, these infants also had higher titers in the preinoculation cord blood specimens (fig. 5). There were no differences in the antigens when response was based upon increase in titer.

Titration of diphtheria antitoxin 1 year after completion of the DTP injection can be compared with the levels observed at 1 month following the third injection in 54 infants from whom 1-month and 1-year postvaccination

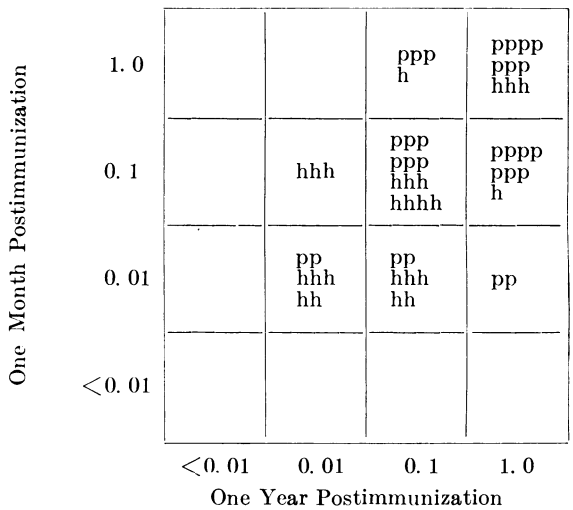
Figure 5. Diphtheria antitoxin units per milliliter, cord blood and infant blood 1 month following immunization.



p=aluminum phosphate
h=aluminum hydroxide

specimens were obtained (fig. 6). Twenty-five of these infants had received the aluminum hydroxide and 29 the aluminum phosphate adsorbed antigen. Thirty infants maintained the same level of antitoxin units per ml., equally distributed between the two antigens. An increase at 1 year was noted in 17 of the 54 infants, including 6 of the 25 who had received aluminum hydroxide adsorbed antigen and 11 of the 29 receiving the aluminum phosphate antigen. The antitoxin level was lower at 1

Figure 6. Diphtheria antitoxin units per milliliter, 1 month and 1 year following immunization.



p=aluminum phosphate
h=aluminum hydroxide

year in 7 of the infants, 4 of whom had received aluminum hydroxide antigen and 3 aluminum phosphate antigen. No differences were observed in the ability of the two antigens to maintain antitoxin levels of 1.0, 0.1, or 0.01 units per ml. at 1 year following completion of immunization.

Combined Protection

Table 3 presents the combined results of pertussis agglutination and diphtheria antitoxin titration 1 month after the third inoculation. These data may be observed at any agglutination and antitoxin level for pertussis and diphtheria. Selecting as a criterion a titer of 1:160 or more for pertussis protection and at least 0.1 antitoxin unit per ml. (AU/ml.) for

Table 3. Pertussis agglutination and diphtheria antitoxin titration 1 month following completion of DTP inoculations, by type of antigen

Pertussis titration	Diphtheria antitoxin units per ml.									
	Aluminum phosphate					Aluminum hydroxide				
	<0. 01	0. 01	0. 1	1. 0	Total	<0. 01	0. 01	0. 1	1. 0	Total
1:1,280				1	1					0
1:640		1	1	3	5					0
1:320			8	1	9				1	1
1:160		1	6	5	12			2		2
1:80			4	5	9		1	1		2
1:40		1		1	2		2	2	1	5
1:20			1	1	2		2	2	1	5
1:10					0		2	2	2	6
<1:10		3	5		8		7	8	4	19
Total	0	6	25	17	48	0	14	17	9	40

diphtheria protection, the following results are obtained.

	PHOSPHATE		HYDROXIDE	
	Number	*Percent*	*Number*	*Percent*
Failure	23	48. 0	37	92. 5
Success	25	52. 0	3	7. 5
Total	48	100. 0	40	100. 0

Selecting a titer of 1 : 80 or more for pertussis and at least 0.01 AU/ml. for diphtheria as a criterion for protection, the differences in the percent of successes and failures for the two products are greater.

	PHOSPHATE		HYDROXIDE	
	Number	*Percent*	*Number*	*Percent*
Failure	12	25. 0	35	87. 5
Success	36	75. 0	5	12. 5
Total	48	100. 0	40	100. 0

Differences in the antigens with respect to combined diphtheria and pertussis protection 1 year following the inoculations may be derived from table 4. Requiring agglutination at 1 : 160 dilution and 0.1 AU/ml. as a criterion, 35.5 percent of the infants receiving the aluminum phosphate and 11 percent of those receiving aluminum hydroxide were protected 1 year after the last inoculation. At the levels of 1 : 80 agglutination and 0.01 AU/ml., 58 percent of the infants injected with aluminum phosphate antigen, and 22 percent injected with aluminum hydroxide were protected at 1 year.

Reactions Following Injections

Infants were observed for local and systemic reactions following 283 of the injections. Local reactions ranging from mild to severe were observed following 55 of the injections (19 percent). Systemic reactions ranging from mild to moderate, according to the criteria used, followed 33 injections (11 percent). Local reactions occurred among 33 (23 percent) of the 147 who received aluminum phosphate adsorbed antigen and among 22 (16.2 percent) of 136 receiving aluminum hydroxide adsorbed antigen injections. Of the 30 systemic reactions, 12 followed the aluminum hydroxide, and 18 the aluminum phosphate antigen. The differences observed are not statistically significant.

Discussion

The data indicate that of the two antigens used, the aluminum phosphate adsorbed product is superior in combined diphtheria and pertussis neonatal immunization. The possibility that factors other than antigenicity could have influenced the results must be considered. Although losses from the study impose important limitations, the losses were equally divided according to the antigen used. Of 21 infants

Table 4. Pertussis agglutination and diphtheria antitoxin titration 1 year following completion of inoculations, by type of antigen

Pertussis titration	Diphtheria antitoxin units per ml.									
	Aluminum phosphate					Aluminum hydroxide				
	<0.01	0.01	0.1	1.0	Total	<0.01	0.01	0.1	1.0	Total
1:1,280				1	1					0
1:640					0		1	1		2
1:320				2	2					0
1:160			2	6	8			1		1
1:80			5	2	7				3	3
1:40			3	3	6					0
1:20				1	1		1	4	1	6
1:10		1	1		2		2			2
<1:10		1	3		4		4	9		13
Total	0	2	14	15	31	0	8	15	4	27

lost to the study before the third postvaccination specimen, 11 were in the aluminum phosphate group and 10 in the aluminum hydroxide group.

While the two antigens appeared comparable with respect to the diphtheria toxoid component, when viewed in the light of ability to meet the criteria set on the basis of antitoxin units per milliliter and agglutination titers, the aluminum phosphate adsorbed antigen was better than the aluminum hydroxide adsorbed antigen. In view of the multiplicity of factors that govern the occurrence of disease, including dosage of infection, specific immunity, and other resistance factors, it is difficult to establish arbitrary pertussis agglutination and diphtheria antitoxin levels above which a person is immune and below which he is susceptible. By utilizing the data in tables 3 and 4, however, one may compare the response to the antigens according to any desired combinations of pertussis agglutination and diphtheria antitoxin levels.

In general, the infants tolerated the inoculations very well. Many of the mild systemic reactions occurring within a few hours of injection were reported by the parent. Possibly, some of these were over-reported. Some of the local reactions observed by physicians and nurses, however, were unnoticed by the parent. Sauer observed a lower frequency of systemic and local reactions following aluminum phosphate adsorbed antigens than were observed following the use of alum precipitated antigens (*8*). In our experience local and systemic reactions are more frequently discovered when infants are systematically observed by physicians or nurses than when the frequency of reaction is based on reports by the parent.

The results of the study support neonatal inoculation as a safe, effective, acceptable procedure. The type of antigen used appears to be of importance in view of the variations observed in response to available preparations. Furthermore, the loss of immunity as indicated by the agglutination and antitoxin levels 1 year after the third inoculation reemphasizes the need for a fourth injection or booster rose at approximately 1 year of age or earlier.

Summary

A group of infants was inoculated during the neonatal period with aluminum phosphate adsorbed or aluminum hydroxide adsorbed diphtheria-tetanus toxoid and pertussis vaccine, combined. The infants were observed up to 1 year following completion of the series of three inoculations.

Based on diphtheria antitoxin titrations, responses to the aluminum phosphate and aluminum hydroxide adsorbed antigens were comparable.

Based on pertussis agglutination titers 1 month and 1 year following vaccination, the aluminum phosphate was better than the alumi-

num hydroxide adsorbed preparation. Better combined protection against diphtheria and pertussis followed inoculation with the aluminum phosphate adsorbed antigens.

No differences in local or systemic reactions following the two types of antigens were observed.

The data confirm the desirability of a fourth dose or booster dose at 1 year of age when inoculation is begun during the neonatal period.

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Conference on Training

Sixty-five representatives of professional and educational groups will attend a national conference on public health training to be held in Washington, D. C., July 28–30, 1958, under the sponsorship of the Public Health Service.

Dr. Berwyn F. Mattison, executive secretary of the American Public Health Association, will be chairman of the meeting. Dr. Malcolm H. Merrill, director of the California Department of Public Health, will summarize conference conclusions.

The meeting will review responsibilities of local, State, and Federal agencies for the training of public health personnel. The Health Amendments Act of 1956 requires that a national conference be held to assist the Surgeon General of the Public Health Service in evaluating the public health traineeship program authorized by the act. The act also requires the Surgeon General to make an evaluation report to Congress by January 1, 1959.

The purpose of the traineeship program is to help alleviate the acute shortage of trained personnel in public health agencies. Under the act, the Public Health Service awards traineeships to individuals and makes training grants to schools offering graduate courses in public health. From August 1956 through April 1958, almost 1,000 persons received graduate training in public health through this program.