Responses of Infants to DTP-P Vaccine Used in Nine Injection Schedules

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STUDIES of immune response to the individual components of a multiple vaccine containing poliomyelitis antigens combined with the widely used triple combination of diphtheria and tetanus toxoids and pertussis vaccine (DTP-Polio) soon followed the demonstration of the effectiveness of poliomyelitis vaccine in the 1954 field trial (*1*, *2*). Results of experiments in guinea pigs and monkeys (*3*) encouraged Brown and Kendrick (*4*) to study this multiple preparation in children. In general, they obtained good response to the individual antigens; they noted particularly the suppressive effect of maternal antibody on the infant's response, especially to the poliomyelitis components.

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Meanwhile, Batson and associates (*5*) reported satisfactory response of infants to poliomyelitis vaccine when it was mixed with DTP in a syringe immediately before injection. Schuchardt and associates (*6*) obtained a good response in monkeys to the individual components of DTP-Polio; they found some indication of an increased response to poliomyelitis antigen with the combined preparation. Wilson and associates (*7*) studied a group of infants in Canada, most of whom were under 5 months of age at the time a primary course of quadruple vaccine was initiated, with a recall dose 1 year later. Most of the infants responded well to all three types of poliovirus and in all but one of those who failed to respond, the initial level of maternal antibody was high. Diphtheria and tetanus antitoxin titers were also measured and found satisfactory.

In several studies of DTP-Polio, Barrett and associates (*8–10*) analyzed their findings in detail. In their last report (*10*) these authors studied particularly the age factor in the response of infants 1 day old to 7 months of age at the start of immunization, but considered that four injections with combined antigen at monthly intervals overcame such interference encountered in infants started as early as 3 months of age. Even with four injections, a booster dose after about 6 months was considered important for reinforcing basic immunity.

The possible relationship of a DTP-Polio vaccine to the development of an efficient immunization procedure in young infants has stimulated recent studies by a number of other authors (*11–18*), some of them being specifically

concerned with the age factor. Some of the technical details of preparation and standardization of DTP-Polio vaccine as well as its efficiency have been considered by a number of authors, including Cohen and associates (*19*) and Beale and Ungar (*20*).

The study reported here was started in 1960 with the specific objective of comparing the primary and secondary responses of infants to the individual components of DTP-Polio vaccine used in nine selected injection schedules.

Study Plan

In some of the studies cited above, maternal antibodies were shown to interfere with the active immunization of infants. This observation, which was particularly clear with poliomyelitis vaccine, is of special importance in relation to the recommendation of the 1964 report of the Committee on the Control of Infectious Diseases, American Academy of Pediatrics, that primary immunization with DTP-Polio be started as early as 8 weeks of age. At present a relatively high level of maternal poliomyelitis antibody is almost assured, as a result of widespread use of polio vaccine in pregnant women. In fact, an unpublished survey in 1958 by two of us (Volk and Brown) showed that 177 of 186 post partum serum specimens from mothers had antibodies to at least one type of poliomyelitis virus and 73 (39 percent) were positive for all three types. Twenty-three percent of these women had not been vaccinated and many others received only one or two doses. In the infant, the progressive disappearance of maternal antibody is conditioned by the initial level at birth and may not be complete in occasional infants before 10 or 12 months of age. Data relating to this phenomenon have been discussed by several authors, particularly Gitlin and associates (*21*).

Our study was designed to test the previous observation of the suppressive effect of maternal antibody on active immunization and, if confirmed, to determine if such suppression could be minimized by use of some particular schedule.

In the selection of the area and population for the investigation, we were fortunate in the unique situation provided by Saginaw County, Mich. In this area, during the past 20 years, studies have been in progress on the immune response to single and multiple antigens. At the time our study was under consideration, Volk and associates were completing a study which concerned the responses of a population previously injected with different combinations of antigens (*22*). The organization used by these authors, including a staff experienced in the required field and laboratory procedures, was made available for this new study of quadruple vaccine, DTP-Polio. In obtaining infants for the study, the public health nurses of the Saginaw County Department of Health, under the direction of Mrs. Maude Gilbert, made house visits, explained the objectives of the plan, and obtained participation of the mothers. The Sisters of the Guadalupe Center of Saginaw cooperated with the county public health nurses.

Injections were made and blood samples were taken by two physicians: Dr. Rebii Hankan of the resident staff of Saginaw County Hospital, and Dr. Henry T. Forsythe, a practicing physician.

The observations of the suppressive effect of maternal antibody on an active primary response to injected antigen dictated the selection of groups of infants of different ages, from 3 to 7 months, in which varying amounts of maternal antibody would be assured. In the selection of defined schedules it was kept in mind that the age of the infant not only at the time of the first injection but also at the time of completion of immunization might be critical. Also, increasing the interval between injections might influence the response either by reason of the lengthened interval or because of the increased age when the series of injections was completed.

All the schedules selected for study, with the exception of B, called for a primary series of three injections. In terms of the month-age at which each injection was given the schedules were as follows: A, 3–4–5; B, 3–4–5–7; C, 3–5–7; D, 4–5–6; E, 4–6–8; F, 5–6–7; G, 5–7–9; I, 6–7–8; J, 7–8–9.

A booster (reinforcing, recall) dose was given to all infants 1 year after completion of the primary series, as described below.

Description of vaccine. The combined DTP-

Polio vaccine used in the study was "Quadrigen," supplied in two lots (Nos. 060111 H and 070697 B) by Parke, Davis & Co. The schedule recommended by the manufacturer was three injections of 0.5 ml. at monthly intervals, followed by an additional injection after 6 to 12 months. The potencies of the several components were supplied by the manufacturer, who reported that 0.5 ml. of lot 060111 H injected into guinea pigs produced 4 units of diphtheria and 6 units of tetanus antitoxin per ml. of serum. The potency of the pertussis component was 16.06 protective units per total human dose. The poliomyelitis ratios in comparison with the National Institutes of Health standard were: type 1, 1.84; type 2, 1.95; type 3, 2.32. Injection of 0.5 ml. of lot 070697 B into guinea pigs elicited production of 2 units of diphtheria and 4 units of tetanus antitoxin. The potency of the pertussis component was 16.56 mouse protective units per total human dose. The poliomyelitis ratios in comparison with the NIH standard were: type 1, 2.3; type 2, 5.57; type 3, 5.54.

The potencies were determined by the standard animal tests prescribed by the National Institutes of Health and, when released, each lot exceeded all requirements for potency and safety.

Potency of the pertussis component. To determine whether there was a loss of pertussis potency, tests were made by the Michigan Department of Health Laboratories, according to methods outlined by the National Institutes of Health. One lot was tested 2 months after the stated expiration date and during the first 3 months of the study. The potency was only 5.4 mouse protective units per total human immunizing dose although originally it had exceeded the NIH requirement of 12 units. Because of this low potency a second lot was supplied by the manufacturer; it was found to be even lower (0.72 unit) in potency.

Within 6 months of the start of the study it was recognized that pertussis antigen in a combined DTP-Polio vaccine containing phemerol deteriorated (*23*, *24*). The DTP-Polio was withdrawn from the market, and no new lots were available in the United States. In order to complete the study, therefore, we continued to use the two lots with which we had started, recognizing that the pertussis antigen was of relatively low antigenicity.

Vaccine dosage. For each injection of the primary series, 0.5 ml. of Quadrigen was given intramuscularly, and for the booster injection, only 0.2 ml. The booster dose was smaller than usually employed and smaller than recommended by the manufacturer.

Reactions. Although the evaluation of clinical reactions was not a main objective of this study, all mothers were instructed to notify the clinic nurse immediately following any unusual reaction. Further, each mother was consulted at each clinic visit concerning the behavior of the child after the preceding injection. No untoward reactions were reported.

Blood samples. Blood samples were taken by the femoral route from each infant before the first injection, 2 weeks after the primary series of three injections, 12 months after the primary series (prebooster), and 2 weeks postbooster. In addition, samples were taken from some of the infants 6 months after the last primary injection to compare antibody titers at this time with those found after 12 months.

The blood samples were allowed to clot at room temperature and then mailed to Ann Arbor.

Antibody titrations. In the virus laboratory of the department of epidemiology, School of Public Health, University of Michigan, Ann Arbor, the blood samples were centrifuged and the clear serums stored at 4° to 6° C. until tested.

Neutralizing antibodies for the three immunologic types of poliomyelitis virus were determined in Ann Arbor, with the technical assistance of Mrs. Florence Malone, using the metabolic inhibition test in plastic panels of monkey kidney tissue cultures (*25*). The titers were expressed as the reciprocal of the highest original dilution of serum that neutralized 100 $TCID_{50}$ of the respective viruses. Appropriate controls were incorporated into each test.

The serums were then sent to the Michigan Department of Health laboratories, Lansing, where diphtheria, tetanus, and pertussis antibody titrations were performed, with the technical assistance of Miss Frances Angela. The methods for antitoxin titrations were described in an earlier publication (*22*). Pertussis re-

sponse was measured by specific agglutination, as described by Kendrick and associates (*26*).

Nearly 500 infants had been included in the study, but because of breakage, insufficient volumes, and toxicity of some serum specimens, testing for antibodies to all components of the vaccine was possible with only 373 infants. The following data on primary response represent only these children.

Primary Response

Poliomyelitis. The individual preprimary and postprimary antibody titers of each child in the nine groups against the three types of poliomyelitis are shown in figures 1, 2, and 3. These data show that many infants had maternally acquired antibodies in high titer before vaccination at the age of 3 or 4 months. At progressively older ages more infants were found with either low or nondetectable titers, reflecting passive antibody decay. The infants with high prevaccine titers responded poorly to vaccine; in fact the titers in most infants who had high prevaccine titers either remained the same or actually decreased after injection of Quadrigen. This confirms previous observations of the suppressive effect of maternal antibodies on active immunization with this vaccine. This finding was most frequent when prevaccine titers were 16 or greater and they occurred in all groups regardless of the age of the infants, indicating that the level of maternal antibody rather than age itself was the predetermining factor in the response. On the other hand, when prevaccine titers were less than 16 a significantly greater percentage of children responded with increased antibody titers. Furthermore, the postprimary geometric mean antibody titers of these infants with low preprimary antibodies were higher in each group than were those of children starting with titers of 16 or greater. Thus there was a clearly demonstrable inverse relationship between the height of the mean antibody titers before and after primary vaccination.

Group B was the only group in which four injections of vaccine were given during the primary series. In terms of both percent responding and geometric mean titers following vaccination, these children appeared to be better immunized than any of those receiving only three injections, with the exception of those in group G, 5–7–9. However, even the additional dose of vaccine was unsuccessful in overcoming the suppressive effect of maternal antibodies if the titers were 16 or greater.

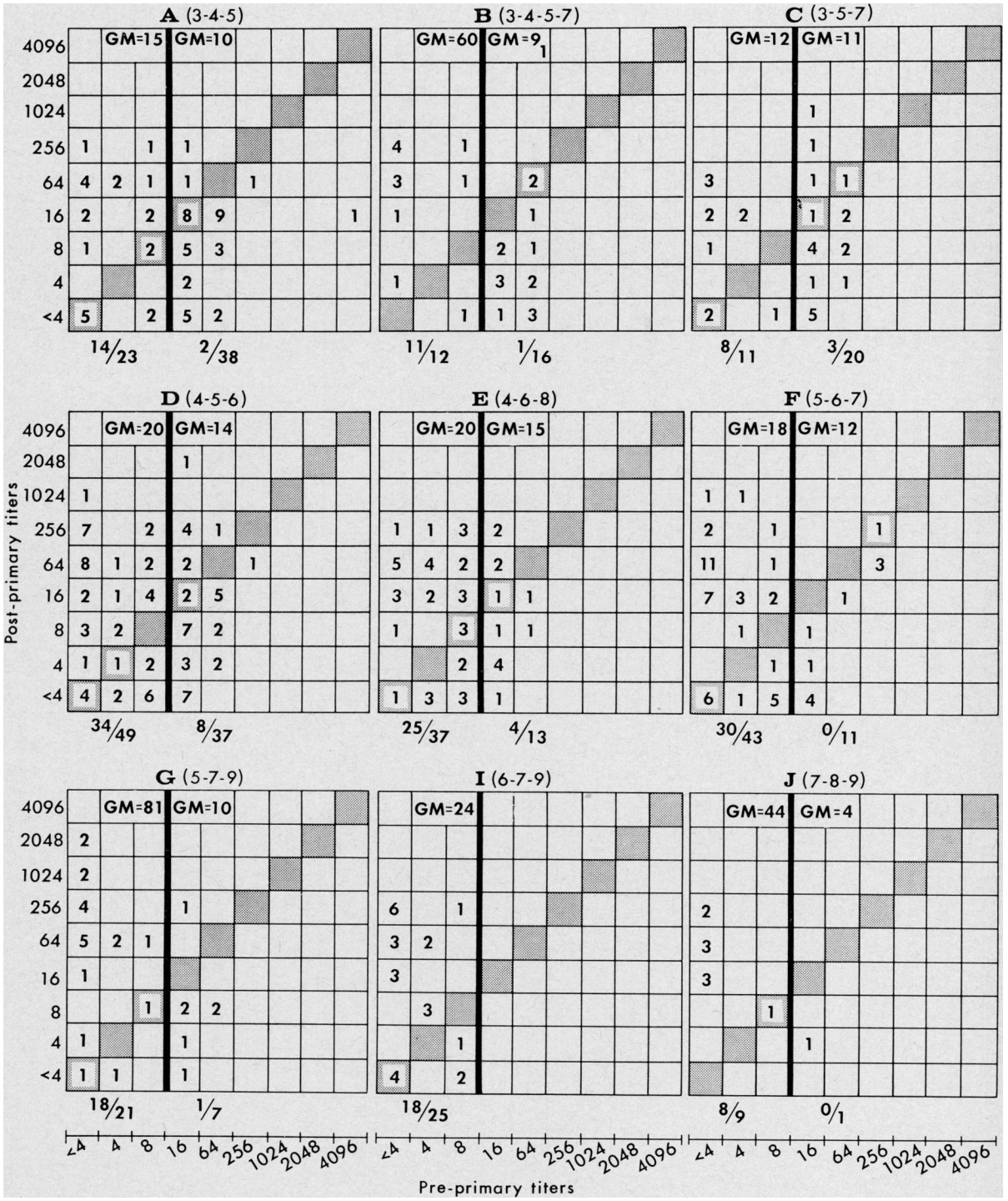
A comparison of the responses of infants receiving vaccine on a 2-month schedule with those on a 1-month schedule can be made for groups in which the first injections were given at 3, 4, and 5 months of age, respectively. In terms of the percent responding, there was an apparent advantage for the 2-month interval when preprimary titers were less than 16 for children started at the age of 3 months (group C compared with group A). This, however, may well be due to the older age of group C children at the time of the last two injections (5 and 7 months) rather than to the spacing of injections. In like manner, more of those started at 5 months of age on the bimonthly schedule responded to types 1 and 3 than did those on a monthly schedule. Almost all groups of infants with high prevaccine titers (16 or greater) showed a distinctly better percentage of responders following the 2-month than following the 1-month schedule of inoculation, and even here the suppressive effect of maternal antibodies was clearly evident.

When analyzed in terms of geometric mean antibody titers, similar results were observed; the majority of those with low preprimary titers showed higher means following the bimonthly schedules. However, with titers of 16 or greater there were no advantages seen with either interval, except for the type 3 response of those in the bimonthly series.

A comparison of the antibody responses to the three types of virus shows clearly that the type 1 component was the least effective antigen in this particular vaccine. All data, however considered, show that primary responses in infants with little or no prevaccine antibody are dramatically superior to those in infants with prevaccine antibody, and this desirable situation is most likely to be found in children 5 or more months of age.

Diphtheria and tetanus. Since the responses to the diphtheria and tetanus components were similar they are described together. In table 1 the geometric mean preprimary and post-

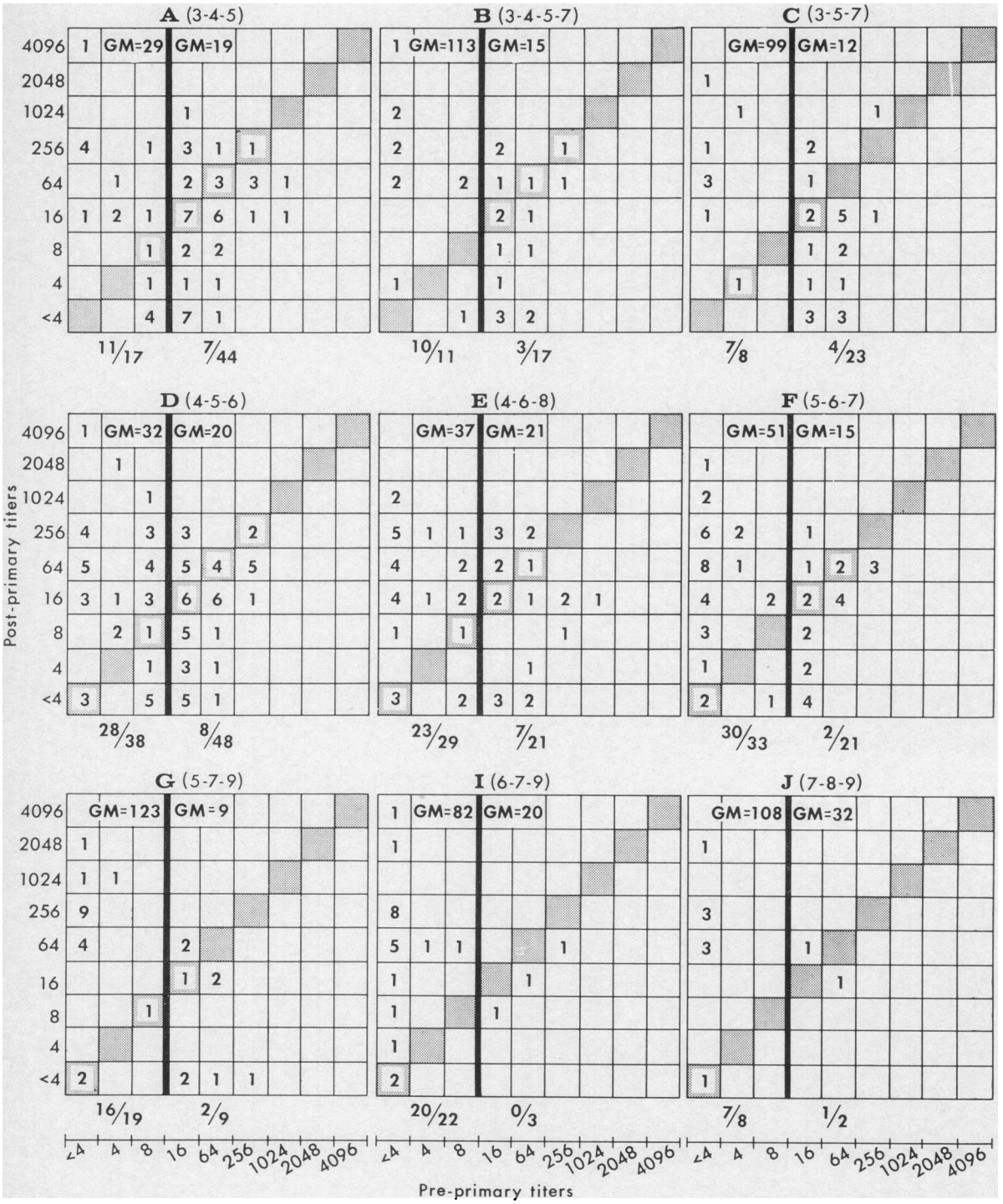
Figure 1. Type 1 poliomyelitis antibody titers in nine groups of infants before and after primary vaccination with DTP–P, by group and month-age at time of injection



NOTE: Ratio—Number with increased titer / Total with preprimary <16 or ≥ 16

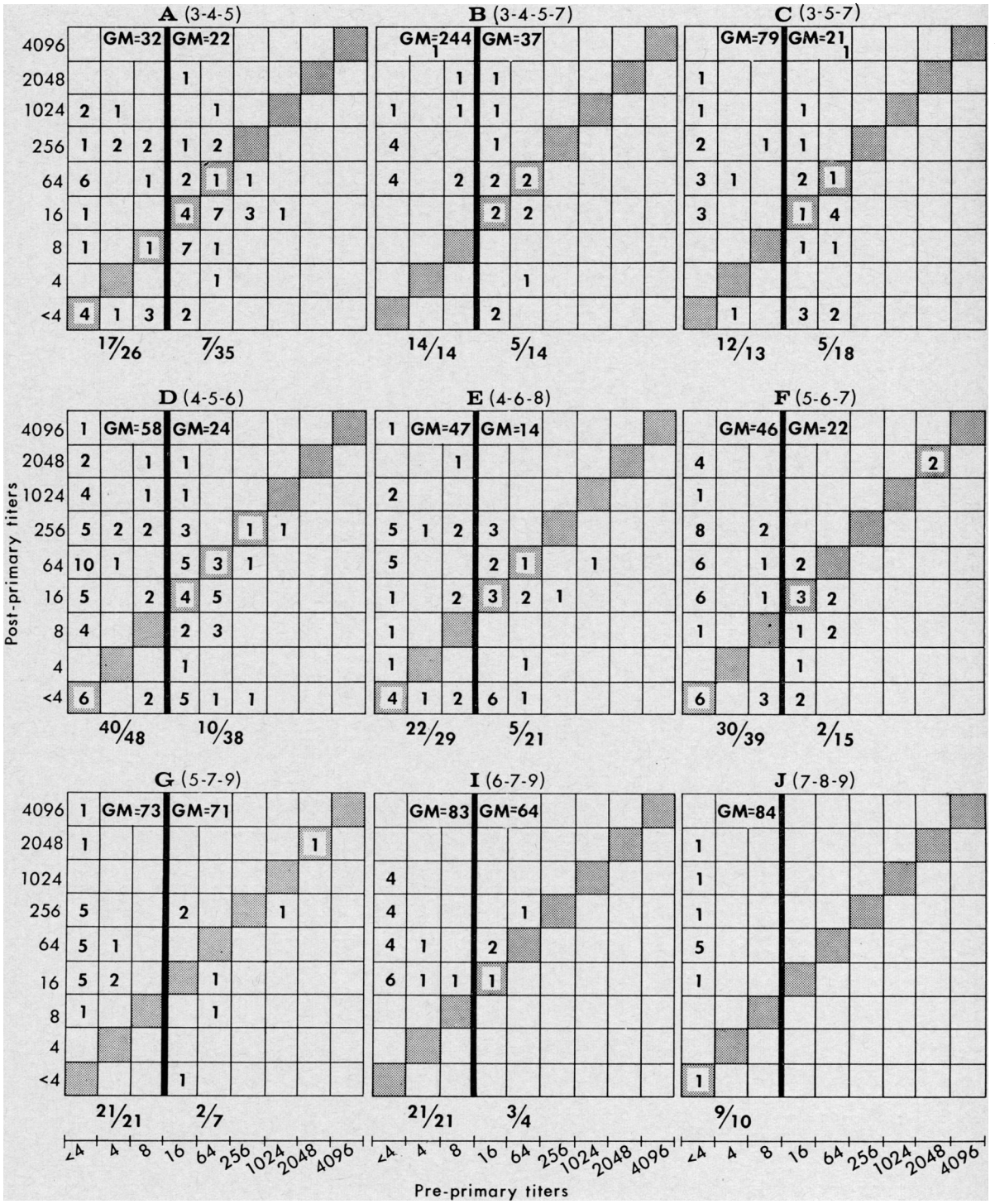
GM—postprimary geometric mean.

Figure 2. Type 2 poliomyelitis antibody titers in nine groups of infants before and after primary vaccination with DTP–P, by group and month-age at time of injection



NOTE: See figure 1.

Figure 3. Type 3 poliomyelitis antibody titers in nine groups of infants before and after primary vaccination with DTP–P, by group and month-age at time of injection



NOTE: See figure 1.

primary antitoxin titers, in terms of units per ml. of serum, are grouped according to immunization schedule. It is readily apparent from the low titers before vaccination that few infants had demonstrable maternal antibodies. A comparison of 1- and 2-month injection schedules (group A with C, D with E, and F with G), shows that the mean titers in the groups of children who were injected on a bimonthly schedule were higher than in the groups on a monthly schedule. The differences are all significant ($P=<0.01$), except in the F and G diphtheria schedules where $P=0.13$. Also, within the range tested, the older the children were at the time immunization was begun, the higher the geometric mean titers.

For this comparison only the means of groups A, D, F, I, and J could be compared because only in these groups were the children all immunized on a monthly schedule. For diphtheria, the mean titers varied from 3.7 units in the A group of children injected on a 3–4–5-month schedule, to 9.3 units per ml. in the J group injected on a 7–8–9-month schedule. For the tetanus component, the mean titers of these same two groups varied from 13.5 to 29.1 units. The mean titers in groups A (3–4–5) and D (4–5–6) were approximately the same. Group J (7–8–9) contained only 10 children, so there may be some reservation regarding the significance of these mean titers. In group B, the only one to receive four injections (3–4–5–7), and in contrast to the higher poliomyelitis titers, the mean titers were lower than those of group C which had received only three injections.

Because the children receiving vaccine on a bimonthly schedule were necessarily older at completion of the primary series of injections than those started at the same time but injected on a monthly schedule, it was important to compare the findings in groups of infants who were the same age at completion of primary vaccination. In figure 4, the postprimary geometric mean titers were plotted for all the groups according to the month-age at which the third and final primary injection was given, whether on a monthly or bimonthly schedule.

The mean tetanus antitoxin titers were relatively close for both schedules for all three ages of completion, 7, 8, and 9 months, respectively. The mean titers of diphtheria antitoxin also were relatively close for the two schedules among infants completing their primary series at 7 and 8 months, respectively; the higher titer by the monthly schedule for the children completing the series at 9 months is based on only 10 infants and the interpretation is held in question. For those children who completed the series of three primary injections at 5 and 6 months, respectively, all by the

Table 1. Geometric mean titers in infants for diphtheria and tetanus antitoxin and pertussis agglutinin, before and after primary administration of DTP-Polio vaccine

Group schedule (month-age)	Number infants	Geometric mean titers					
		Units of antitoxin				Pertussis agglutinin	
		Diphtheria		Tetanus			
		Pre-primary	Post-primary	Pre-primary	Post-primary	Pre-primary	Post-primary
A, 3–4–5	61	0.0016	3.7	0.0006	13.5	6.0	15.6
B, 3–4–5–7	28	.0007	4.5	.0005	20.0	5.7	25.6
C, 3–5–7	31	.0014	6.0	.0007	24.0	7.3	24.5
D, 4–5–6	86	.0017	3.3	.0010	13.6	5.9	14.7
E, 4–6–8	50	.0008	5.8	.0007	22.6	6.7	19.5
F, 5–6–7	54	.0012	4.7	.0008	21.3	6.7	24.9
G, 5–7–9	28	.0014	6.1	.0006	29.2	6.4	29.7
I, 6–7–8	25	.0010	6.3	.0007	21.2	5.9	32.9
J, 7–8–9	10	.0008	9.3	.0005	29.1	7.1	26.4

NOTE: Titers are expressed as reciprocals.

monthly interval schedule, the geometric mean titers were consistently low for both diphtheria and tetanus antitoxin.

The numbers of infants responding to primary vaccination with given titers are presented in tables 2 and 3 for the different schedules. All the children developed presumably protective diphtheria or tetanus antitoxin titers (0.01–0.05 unit per ml. of serum) after the primary series, irrespective of the injection schedule used. The lowest postprimary diphtheria antitoxin titer observed in the children in any

Figure 4. Comparison of geometric mean diphtheria and tetanus antitoxin responses of infants who received third DTP–P injection monthly or bimonthly at same age

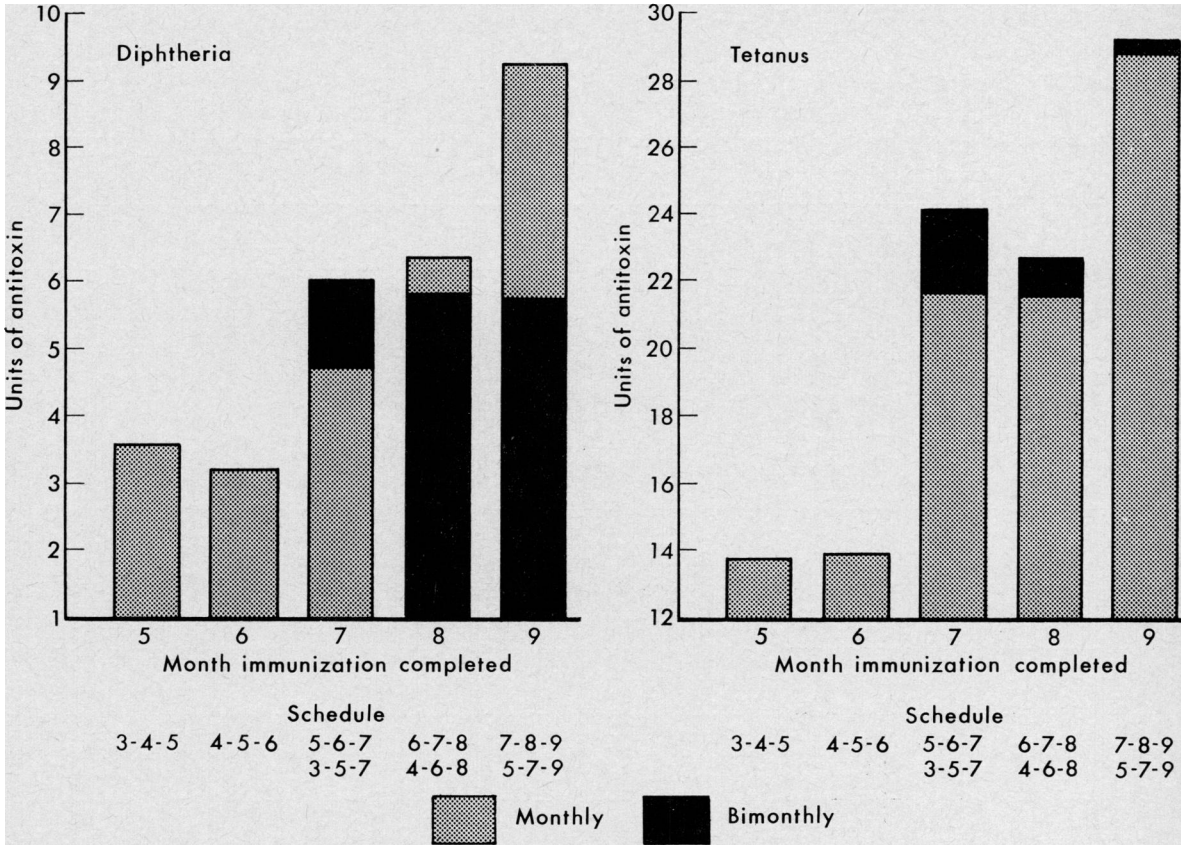


Table 2. Distribution of diphtheria antitoxin titers in infants following primary administration of DTP-Polio vaccine

Schedule	Units of antitoxin											Total infants
	0.125	0.20	0.60	1.0	3.0	5.0	7.5	10.0	15.0	20.0	30.0	
A	------	1	2	1	31	14	8	2	2	------	------	61
B	------	------	------	2	10	7	5	3	1	------	------	28
C	------	------	------	------	9	8	6	3	4	1	------	31
D	1	1	4	3	47	17	7	4	2	------	------	86
E	------	------	------	------	17	8	9	14	1	1	------	50
F	------	------	------	1	30	8	5	3	3	3	1	54
G	------	------	------	1	8	6	6	1	3	1	2	28
I	------	------	------	------	8	4	4	6	2	------	1	25
J	------	------	------	------	1	2	1	2	2	2	------	10

NOTE: Titers are expressed as reciprocals.

group was 0.125 unit and the lowest tetanus antitoxin titer, 0.6 unit. The postprimary diphtheria and tetanus antitoxin titers, arranged according to magnitude, showed more variation in titer following the monthly schedules of groups A and D than in the corresponding bimonthly groups C and E. In addition, the lowest titers were also observed in the monthly groups A and D.

The diphtheria and tetanus titers of the children in one representative group (D) are presented in table 4 according to their preprimary titers, which are assumed to be a measure of maternal antibody. Although less clearly indicated than by the poliomyelitis results, the tetanus antitoxin responses suggest that maternal antibody affected antitoxin production. This effect was less apparent in the diphtheria responses. Groups A, C, and F also included a few children whose serum contained high

Table 3. Distribution of tetanus antitoxin titers in infants following primary administration of DTP-Polio vaccine

Schedule	Units of antitoxin														Total infants
	0.6	1.0	3.0	5.0	7.5	10	15	20	30	40	60	80	100	120	
A	-----	-----	7	4	7	6	14	5	13	2	3	-----	-----	-----	61
B	-----	-----	-----	-----	2	-----	14	2	6	2	2	-----	-----	-----	28
C	-----	-----	-----	-----	2	1	9	1	12	2	3	1	-----	-----	31
D	1	2	4	5	11	8	23	5	22	2	3	-----	-----	-----	86
E	-----	-----	-----	2	6	3	5	5	17	6	4	2	-----	-----	50
F	-----	-----	1	-----	4	4	14	4	17	5	4	1	-----	-----	54
G	-----	-----	-----	-----	1	2	5	2	7	4	4	2	1	-----	28
I	1	-----	1	1	1	-----	4	4	6	1	5	-----	-----	1	25
J	-----	-----	-----	-----	-----	-----	3	1	3	1	1	-----	1	-----	10

NOTE: Titers are expressed as reciprocals.

Table 4. Effect of pre-existing antibody on diphtheria and tetanus antitoxin responses of infants in group D (4–5–6 month-age schedule)

Diphtheria				Tetanus			
Preprimary titer (units)	Number infants	Postprimary titer (units)	Number infants	Preprimary titer (units)	Number infants	Postprimary titer (units)	Number infants
0.6	1	0. 2	1	3. 0	2	1. 0	1
.125	2	3. 0	2			3. 0	1
.05	4	3. 0	2	. 6	1	7. 5	1
		5. 0	2	. 2	1	1. 0	1
.03	6	3. 0	3	. 125	3	. 6	1
		7. 5	1			3. 0	1
		10. 0	1			10. 0	1
		15. 0	1	. 03	1	15. 0	1
.01	7	3. 0	4	. 01	1	10. 0	1
		5. 0	2	. 005	2	15. 0	2
		10. 0	1	<. 005	75	3. 0	2
.005	9	1. 0	1			5. 0	5
		3. 0	7			7. 5	10
		10. 0	1			10. 0	6
<.005	57	. 125	1			15. 0	20
		. 60	4			20. 0	5
		1. 0	2			30. 0	22
		3. 0	29			40. 0	2
		5. 0	13			60. 0	3
		7. 5	6				
		10. 0	1				
		15. 0	1				

NOTE: Titers are expressed as reciprocals.

titers of maternal antibody. The responses in these few children were generally weaker than in others in the group.

Pertussis. As mentioned previously, the pertussis component of the quadruple antigen was weak when measured by animal potency tests. Also, while a good antigen would be expected to stimulate a postprimary agglutinin titer of 320 or higher in a large proportion of an injected group, the antigen used in this study produced much lower titers. The analysis of data, therefore, is necessarily based on results obtained with a weak antigen.

Even with this antigen, however, certain facts are apparent from the data in table 1. As in the response to the diphtheria and tetanus antigens, the geometric mean pertussis agglutinin titers in those groups injected on a bimonthly schedule were higher than in those injected on a monthly schedule (C compared with A, E with D, and G with F). However, these differences were not statistically significant; in fact, the probability of chance occurrence is greater than 1 in 10. The children starting their injection schedule when 5, 6, or 7 months old had higher postprimary titers than those started when 3 or 4 months old. The mean titer following schedule B (3–4–5–7) is approximately the same as in the three-injection schedules C (3–5–7) and F (5–6–7), suggesting that in this instance the age at completion of primary vaccination may have been of greater importance than the additional injection.

Preprimary titers did not exceed 80 in any of the children studied. In the three children with a titer of 80, the postprimary titers were <10, 20, and 320. In the four children with a preprimary titer of 40, the postprimary titers were 20, 40, 80, and 160, respectively. It is difficult to decide from these limited data whether or not the presence of maternal antibody had an influence on agglutinin production.

Six-month postprimary titers. In a number of children from each of the groups except B, determinations were made of the level of antibody to each of the six antigens 6 months after the primary injections had been completed. The low geometric mean titers for the three poliomyelitis antigens were so similar to those subsequently found after 12 months that the results are not given in detail.

The 6-month geometric mean titers for diphtheria and tetanus had declined appreciably but were still relatively high in all groups; the lowest titer observed was 0.05 unit of diphtheria antitoxin in two of the children and 0.125 unit of tetanus antitoxin per ml. of serum in one of the infants. The pertussis agglutinin titers also had declined from the postprimary levels in all groups except A and E, in which the titers had remained the same.

Secondary Response

Booster or reinforcing injections of 0.2 ml. of DTP-Polio were given 1 year following completion of the primary series. Antibody titra-

Table 5. Geometric mean poliomyelitis antibody titers before and after booster injection of DTP-Polio vaccine and percentage of infants responding with increased titers

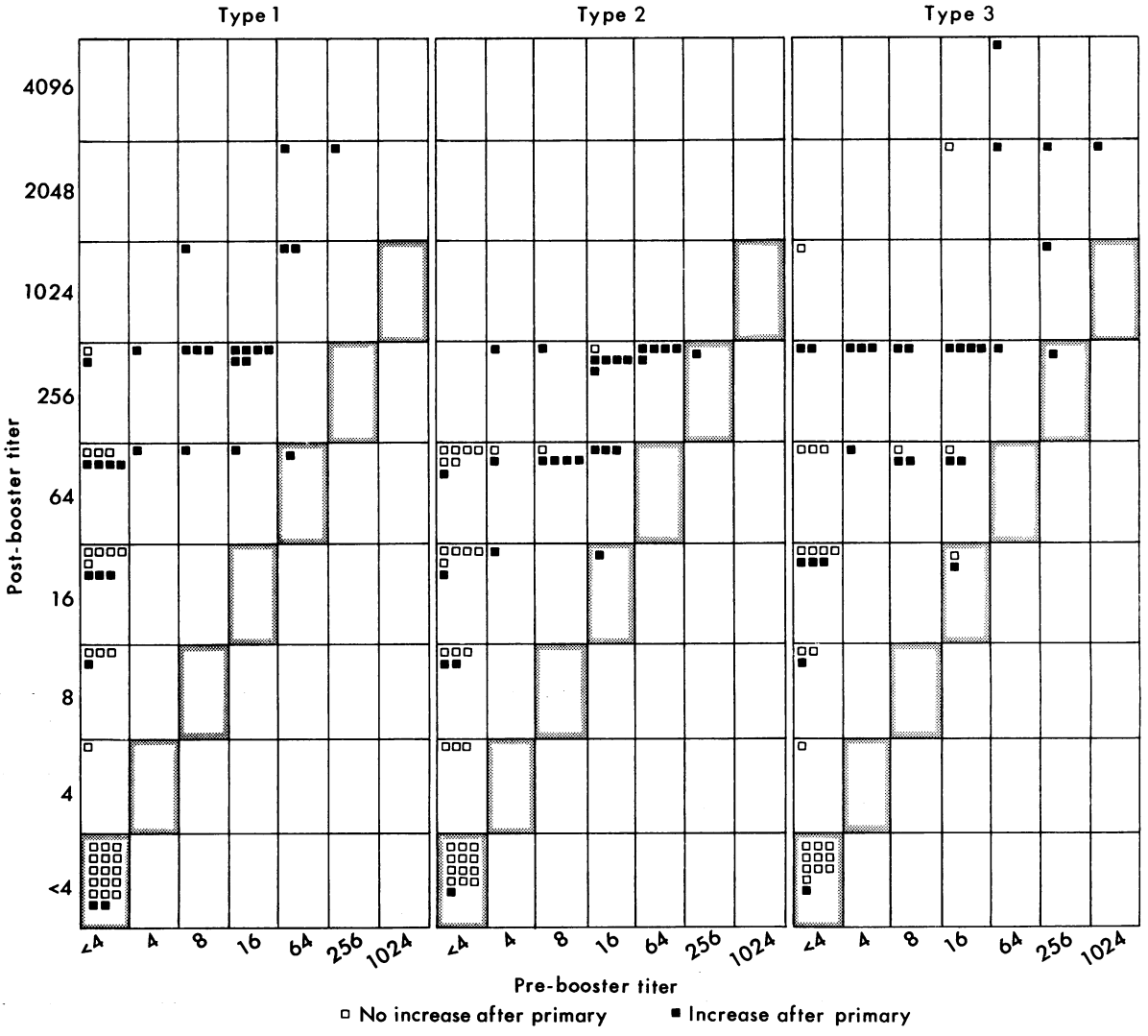
Group schedule (month-age)	Number infants	Type 1			Type 2			Type 3		
		Pre-booster	Post-booster	Percent	Pre-booster	Post-booster	Percent	Pre-booster	Post-booster	Percent
A, 3–4–5	44	3	25	61	3	25	64	4	56	77
B, 3–4–5–7	8	6	13	50	3	19	75	5	38	75
C, 3–5–7	24	3	30	70	3	41	74	7	64	75
D, 4–5–6	60	4	27	69	5	25	75	6	47	77
E, 4–6–8	33	6	43	81	4	35	76	6	77	76
F, 5–6–7	35	4	27	71	3	36	88	5	91	91
G, 5–7–9	21	11	84	65	8	49	71	9	141	90
I, 6–7–8	16	6	56	75	10	54	75	7	95	87
J, 7–8–9	6	3	40	83	3	90	83	9	72	66

NOTE: Titers are expressed as reciprocals.

tions on serums collected before and after this stimulus have been completed on 247 of the original 373 infants.

Poliomyelitis. The geometric mean titers for poliomyelitis antibodies before and after secondary stimulation are presented in table 5. From the very low mean titers shown prior to this injection, many of the children had no detectable antibodies this long after primary. The booster injection, however, resulted in a demonstrable increase in mean titer for most groups. The percent responding demonstrated that many infants who had failed to respond to the primary series did so as a result of the secondary stimulus. The majority of unsatisfactory responses to booster were in children who had failed to show an increase in antibodies after the primary series. This fact is illustrated in figure 5, which presents the individual responses of infants in group D (4–5–6) to booster according to their original primary response. Most of those who had failed to respond to primary had no detectable titers before booster, approximately half of these failed

Figure 5. Poliomyelitis antibody titers of group D infants before and after booster injection of DTP–P, according to their primary responses



completely to respond to secondary stimulation and those that did attained low titers. On the other hand, of those infants who had responded to primary with increased titers, most still had antibodies 1 year later and, with few exceptions, they reacted to the additional stimulus with the development of higher titers of antibody.

Thus the effect of maternal antibodies in suppressing active immunization following primary vaccination is also reflected in an unsatisfactory reaction to the secondary stimulus a year later. All evidence points strongly to the advantage of delaying primary immunization with inactivated poliomyelitis vaccine until later in infancy.

The mean secondary antibody titers in groups which had received their primary stimulus at 2-month intervals were, without exception, higher than those originally injected on a 1-month schedule (table 5). However, the actual percentage of those responding was not superior to those injected every month. Interestingly, the booster response of group B infants who had received four injections of vaccine during the primary, which resulted in good antibody titer, was not as great as in other groups. The number of those studied, however, was small and therefore too much significance cannot be applied to this observation.

Diphtheria and tetanus. Geometric mean prebooster and postbooster diphtheria and tetanus antitoxin titers for the nine schedules are given in table 6. Groups B and J had only eight and six children, respectively, and the mean values may not be reliable. As expected, children in all schedules showed lower titers at 12 months than at 6 months postprimary.

In all of the groups only three children had prebooster diphtheria antitoxin titers as low as 0.01 unit and 16 had titers of 0.03 to 0.05 unit per ml. of serum. The lowest prebooster level of tetanus antitoxin was 0.125 unit, and this was observed in only one child.

The postbooster diphtheria data show that, in contrast to the results after the primary injections, no essential differences existed between the mean antitoxin titers of the groups of children whose monthly immunization schedule was started at different ages. In fact, there was little difference between the mean postbooster titers of any of the groups, confirming the importance of the secondary stimulus in compensating for weaker primary responses.

The mean postbooster tetanus antitoxin titers were likewise not essentially different among the various groups of children. However, the mean titers of the groups of children previously immunized on a monthly schedule were slightly lower than the mean titers of comparable groups immunized on a bimonthly schedule.

Pertussis. The mean pertussis agglutinin titers were still low even after the booster injections. The pattern of responses was not essen-

Table 6. Geometric mean prebooster and postbooster diphtheria antitoxin and tetanus antitoxin titers for each immunization schedule

Group schedule (month-age)	Number infants	Geometric mean titers			
		Diphtheria antitoxin		Tetanus antitoxin	
		Prebooster	Postbooster	Prebooster	Postbooster
A, 3–4–5	44	0.33	12.4	1.8	34.1
B, 3–4–5–7	8	.46	15.9	1.8	27.5
C, 3–5–7	24	.19	11.8	1.2	37.2
D, 4–5–6	59	.25	10.9	2.0	30.0
E, 4–6–8	32	.38	14.8	1.8	37.2
F, 5–6–7	35	.36	[1] 12.2	2.2	33.0
G, 5–7–9	21	.31	12.0	2.4	48.0
I, 6–7–8	15	.46	12.5	2.0	33.3
J, 7–8–9	6	.55	21.8	2.9	41.6

[1] Only 34 infants tested.

NOTE: Titers are expressed as reciprocals.

tially different from that observed after primary immunization. Although lower than would be expected from a good pertussis component, the postbooster titers were consistently higher than prebooster (12 months postprimary), and also higher than 2 weeks postprimary (table 7). As with tetanus and poliomyelitis results, the bimonthly primary schedule was associated with higher postbooster titers than was the monthly schedule for the children whose primary series were begun at the age of 3 months (C and A). Little difference between postbooster titers of other comparable groups was observed.

Discussion

The data obtained in this study, although lending some support to the factor of immunologic immaturity of the very young infant, emphasize particularly the suppressive effect of maternally transmitted antibodies on the active response to vaccine.

The most noticeable suppressive effect on the response to active immunization was directed against poliomyelitis because of the high levels of specific maternal antibodies. This confirms the previous observations of two of us (Brown and Kendrick) and of others, and this suppressive effect should be a basic consideration in the planning of injection schedules by all investigators and administrators. The titer of the maternal antibodies appears to be the critical factor, since small amounts do not interfere. As most pregnant women are vaccinated against poliomyelitis, augmenting any naturally acquired immunity already present, the young infant is likely to have passive antibodies in high titer for these antigens at the time of injection. Conversely, most adults have not had a recent stimulus with diphtheria, tetanus, or pertussis antigens, and therefore antibodies to these components are usually at a low level or absent in the circulating blood of infants. Nevertheless, some suppressive effect of maternal antibodies was seen in the response to tetanus toxoid and possibly to the pertussis antigen in a few infants. Should the immune status of the adult population be changed, this problem could be intensified.

In addition to the starting age, the age at which the primary injection series is finished is of obvious importance. The antibody-forming mechanism is more developed and the amount of maternal antibody is less in the older infant. Thus, in this study, the higher titers obtained in groups of infants starting at the same age but with a 2-month interval between injections as compared with 1 month may have been related primarily to the older age at completion of the injection series and not alone to the longer interval per se. In addition to the higher titers,

Table 7. Prebooster and postbooster pertussis agglutinin geometric mean titers correlated with primary vaccination titers

Group schedule (month-age)	Primary series of injections					Booster injection		
	Number infants	Pre-primary	Post-primary, 2 weeks	Postprimary, 6 months		Number infants	Pre-booster[1]	Postbooster, 2 weeks
				Number	Titer			
A, 3–4–5	61	6. 0	15. 6	27	15. 0	44	6. 8 (43)	23. 4
B, 3–4–5–7	28	5. 7	25. 6	0		8	7. 7	36. 7
C, 3–5–7	31	7. 3	24. 5	16	15. 4	24	10. 0	33. 3
D, 4–5–6	86	5. 9	14. 7	28	9. 3	59	7. 4	24. 0
E, 4–6–8	50	6. 7	19. 5	17	20. 0	32	8. 9	24. 8
F, 5–6–7	54	6. 7	24. 9	18	14. 1	35	10. 0 (33)	37. 6 (34)
G, 5–7–9	28	6. 4	29. 7	16	15. 4	21	10. 7	38. 7
I, 6–7–8	25	5. 9	32. 9	8	20. 0	15	12. 1	50. 3
J, 7–8–9	10	7. 1	26. 4	5	17. 4	6	12. 6	25. 2

[1] Prebooster was postprimary 12 months.

NOTE: Figures in parentheses indicate the number of infants tested if different from the totals in column 7. Titers are expressed as reciprocals.

there was a greater uniformity of primary response to the diphtheria and tetanus components of the vaccine following the bimonthly schedule. Less variation occurred in titer in those infants injected on a bimonthly schedule than in those on a monthly schedule. Another distinct advantage of the 2-month schedule was seen in the postbooster mean poliomyelitis titers. All groups that had received their primary series at 2-month intervals had, without exception, higher postbooster titers against all three types of virus than did their corresponding monthly groups.

The booster injections given 12 months after the primary series resulted in geometric mean antibody titers for diphtheria, tetanus, and pertussis that were higher than after the primary in all groups. Higher mean poliomyelitis titers were also observed for most injection schedules with several exceptions, which were probably related to the fact that more children in these particular groups had failed to respond properly to the primary series because of maternal antibody suppression, making the so-called booster in fact the primary stimulus for many.

Any attempt to interpret the serologic data of this study in terms of actual protection against disease is difficult, especially with such relatively small numbers of subjects. Certainly the antitoxin titers attained against diphtheria and tetanus were far in excess of levels usually assumed to be protective. In order to relate actively acquired poliomyelitis neutralizing antibodies to protection it is tempting to associate higher titers with stronger immunity. However, the only factual evidence relating titers to resistance against disease is that of the field trial of 1954 (*2*), which showed clearly that even minimum titers of 1:4 were statistically significant in terms of protection.

Attention has already been called to the relatively low potency of the pertussis component of the vaccine used. However, there is no basis for believing that this should invalidate the observations on the efficacy of different immunization schedules. As with the other antigenic components there is evidence as to the importance of the age factor in the character of the pertussis antibody response of the infant even though only weak reactions were elicited. It may be relevant that in a study of infants, each injected with DTP vaccine at 1, 5, and 9 weeks of age, Di Sant'Agnese (*27*) referred to a "ceiling" of about 60 percent of infants who could develop a pertussis agglutinin titer around 400, either after the primary or secondary stimulus.

There is a growing conviction among investigators that the agglutinin response is not a direct measure of protective potency. This idea is supported by an increasing body of experimental data that indicate that agglutinins and protective antibodies are stimulated by separate antigens. In this connection, Butler and associates (*17*), in a comparison of two vaccines, observed the better protection rate following home exposure among those children injected with the vaccine that stimulated the lower level of agglutinins. A recent report by Munoz and Hestekin (*28*) adds evidence on this point. When, however, as in the study reported here the pertussis antigen is a suspension of whole organisms, there is good reason to suppose that even though the protective status of the child cannot be expressed quantitatively in terms of agglutinin titer, the protective and agglutinin responses develop in parallel. Also, it is recalled that mouse protection tests indicated a low potency for this antigen. Taking all into consideration, we believe the level of protection in the infants of this study was relatively low. In the one attack of whooping cough that occurred during the study, the agglutinin titer 8 months after the primary series and 2 months before onset of disease was negative at a 1:10 dilution. Two months after onset the titer was between 80 and 160.

Perhaps the more important fact revealed by this study was not the degree of protection attained in the children who responded to the vaccine but rather the indication by actual serologic measurements that many infants were probably *unprotected* during the critical period between primary and secondary stimulation and in some cases even following booster doses. Defining a vaccine failure as an instance in which the postvaccine antibody titer was the same or less than the preprimary titer, 50 percent of the infants failed to respond to types 1 and 2 poliomyelitis and 39 percent to type 3. Of those infants sampled 6 months after primary, 60, 67, and 62 percent had no detectable

antibodies to types 1, 2, and 3, respectively, and 30, 25, and 20 percent failed to respond to booster stimulation.

It is possible that the experimental booster dose of 0.2 ml. may have been too small for optimum response to the poliomyelitis antigens. However, the majority of these failures were observed in children with maternal antibodies at the time of their first injection. Similar failures were reported by Jenkins (*29*) and by Perkins and associates (*30*) when vaccine was given to infants at the age of 2 months. In like manner, 35 percent of the subjects failed to respond to primary stimulation with the pertussis antigen and 21 percent failed after booster, but it must be kept in mind that this particular component of the vaccine was shown to be poor. There were even three failures in response to the diphtheria component (one after primary and two after booster), and four to tetanus (two each after primary and booster), and all occurred in infants with high titers of maternal antibody. These observations concur with those of Bell (*31*) who, on the basis of a Schick test 1 year after immunization, observed that children who received two injections of diphtheria toxoid between 2 and 5 months of age had twice as many failures to immunization as children who received at least one of the injections at 6 to 23 months of age. The response of young infants to active immunization has been reviewed recently by Evans and Smith (*32*).

Summary and Conclusions

Three hundred and seventy-three infants were injected with a commercial vaccine containing antigens of diphtheria, tetanus, pertussis, and poliomyelitis.

Nine variations in the injection schedule were employed to allow comparison of serologic responses according to the starting ages, the length of interval between injections, and the ages at completion of schedule.

The diphtheria and tetanus antibody responses were generally high in all groups of infants following both primary and secondary inoculation and were judged to be far in excess of the levels usually required for protection.

The pertussis component of the vaccine was of relatively low potency; 35 percent of the infants failed to respond to primary stimulation and 21 percent failed after the booster.

Fifty percent of the infants failed to respond to primary stimulation with types 1 and 2 poliomyelitis and 39 percent to type 3; 30, 25, and 20 percent respectively failed to respond to the three types after booster.

The majority of vaccine failures occurred in infants with high titers of maternal antibody at the time of the first injection. This suppression of active immunization occurred most frequently in children started at the age of 3 or 4 months and was most noticeable in the response to poliomyelitis components of the vaccine. Infants aged 5 months or older at the time of immunization showed the best response.

A comparison of the data from the groups of children on monthly and bimonthly injection schedules showed that for any given starting age those on the bimonthly schedule had: (*a*) higher geometric mean postprimary antibody titers toward all the components of the vaccine, (*b*) greater uniformity in postprimary diphtheria and tetanus antitoxin titers among the children within the group, and (*c*) higher mean postbooster tetanus, pertussis, and poliomyelitis titers.

If poliomyelitis immunization is started too early, many failures may be expected and the risk of disease following exposure will exist during the interval between the primary series and the "booster" injection. Even after the booster, more than one-fifth of all infants in this study could have been considered susceptible to poliomyelitis.

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Changing Mortality Trends

The crude death rate in the United States, after a long period of rapid and substantial decline, has remained somewhat stationary during the past 10 years or so. In 46 States and the District of Columbia the crude death rates have leveled off or begun to increase. Similar trends have been observed in a number of other countries.

For almost every U.S. age group from infancy to late middle or old age, for whites as well as for nonwhites, for females as well as for males, changes have occurred in mortality trends. Beginning about 1950, the rate of decrease in infant mortality dropped to about one-third of the rate of decline in the preceding 17-year period. Death rates for certain subpopulations in the older ages are now stationary or increasing.

U.S. males continue to have less favorable mortality rates than U.S. females. Moreover, the mortality rate for females is still declining whereas the rate for males is decreasing more slowly or not at all. The death rate for males is higher than the corresponding one for females in almost every disease category. Even for diabetes and malignant neoplasms, males no longer have lower death rates. The diabetes death rate for females in the 45- to 54-year age group appears to be leveling off while the death rate for males is turning upward. Also, while the death rates for diseases of the respiratory system excluding influenza and pneumonia are rising at an accelerated pace in the older ages, these rates are particularly high among males.

A recent publication of the National Center for Health Statistics, "The Change in Mortality Trend in the United States" (PHS Publication No. 1,000, Series 3, No. 1), presents details on these trends, analyzes their probable causes, and discusses the future outlook.

About 1938, antimicrobial therapy began to prevent deaths from diseases of infectious origin. Improved sanitation, immunization, and new therapeutic procedures all but eliminated a number of these diseases as causes of death. The impetus imparted by this reduction in disease, however, has gradually diminished as the proportion of deaths from infective diseases decreased. For some time now, the current mortality trends of chronic diseases and accidents and other violence—"which constitute the core of mortality in the present population"—have exerted a more significant check on the downward trend of the death rate.

A comparison of U.S. mortality rates for 1960 with the lowest rates achieved by any country of low mortality in 1959 or 1960 suggests that some depression of the U.S. rate may be possible. If U.S. mortality had been as low, the crude death rate for the United States would have been 7.3 per 1,000 population compared with the recorded U.S. rate of 9.5 in 1960; there would have been some 397,000 fewer deaths that year in the United States.

In view of the mortality experience for the past decade, it does not seem likely that the death rate for the United States will soon approach the levels already attained by various other countries.