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Antibody
maternal antibody
newborn
parainfluenza type 3 vaccine

Parainfluenza Virus Immunization

II. The Influence of Age and Maternal Antibody upon Successful Immunization with an Alum-Adsorbed Parainfluenza Type 3 Vaccine

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Extract

A newly developed inactivated, alum-adsorbed parainfluenza vaccine was evaluated for its effectiveness in evoking antibody responses in young infants. Immunization consisted of three monthly doses of 0.5 ml each. Four age groups were selected for initiation of immunization; 2 days, 6 weeks, 12 weeks and 24 weeks of age.

Successful immunization was achieved when immunization was begun at 24 weeks of age (7 of 8 infants with 4–16 fold increase in antibody titer). Younger infants were successfully immunized if the pre-immunization serum antibody titer was less than 1:64 (12/15 [80 %] infants with pre-immunization titers of 1:32 or less developed 4–32 fold increase in antibody titer) (fig. 6).

Maternal antibody may interfere with immunization with parainfluenza vaccine in infants below the age of 6 months. Some infants, regardless of age, are successfully immunized if the titer of maternal antibody is sufficiently low.

Speculation

It is possible that, using a sufficiently potent antigen, parainfluenza virus immunization may be successful regardless of age.

In a previous report, the antigenicity of parainfluenza type 3 virus vaccines was described [4]. The vaccinees were children between the ages of two and nine years, most of whom had pre-vaccine serum antibody titers indicative of prior natural infection. Of the five children with pre-vaccine titers of less than 1:8 serum dilution, all responded to immunization with four-fold or greater antibody increases. This degree of successful immunization encouraged us to employ one of the vaccines in young infants. The results of the latter trial form the basis for this report.

Materials and Methods

Vaccines: The formalin-inactivated, concentrated, parainfluenza 3 alum-precipitated vaccine (P3-178A) has been described in detail elsewhere [4, 6].

Influenza virus vaccine was obtained commercially[1]. It contains the following concentrations of inactivated antigens:

Asian	200 CCA μ/ml
PR-8	100 CCA μ/ml
Ann Arbor (1/57) . .	100 CCA μ/ml
Great Lakes.	100 CCA μ/ml

The vaccine was diluted with sterile 0.85 % saline in order to achieve a final concentration of 15 CCA μ/0.1 ml.

[1] Eli Lilly and Company.

Serologic Methods: The parainfluenza hemagglutination-inhibition antibody (HIA) technique has been described previously [4]. It was modified by receptor destroying enzyme (RDE) treatment of serum [1].

Population: All vaccinees were healthy infants born at Fitzsimons General Hospital and attending the Well-Baby Clinic. The immunization program was described in detail to the parents and volunteers solicited. Signed consent forms were obtained in each instance. Infants were assigned alternately to an influenza (control) or parainfluenza group although excessive numbers of volunteers were encountered at some age levels and assigned to either the control or parainfluenza group. All infants were free of acute or chronic infections. Newborn infants of 40-week gestation were observed for 24 or more hours in the nursery prior to initiation of the immunization program.

Immunization Program (table I)

Age groups: Four age levels were selected for immunization trial: 1. the newborn period (2 days of life); 2. six weeks; 3. twelve weeks; and 4) twenty-four weeks of life.

Influenza immunization: All vaccinees in this group received two 0.1 ml intradermal injections of polyvalent influenza vaccine containing 15 CCA units per 0.1 ml dose. The doses were administered two months apart (table I).

Parainfluenza immunization: All vaccinees in this group received three 1.0 ml intramuscular injections of P3-17-8A at monthly intervals.

Blood specimens: Blood specimens were collected by sterile venipuncture just prior to the first dose of vaccine (day 0) and 30 days after the last dose of vaccine in the primary series (day 90). For the newborn group, cord serum was utilized as the initial specimen and maternal venous blood collected within two days of delivery for comparison (table II).

'Booster' dose schedule: All vaccinees in the newborn, 6-week, and 12-week groups received a 1.0 ml intramuscular dose of parainfluenza vaccine when they attained the age of 6 months *regardless* of whether they received influenza or parainfluenza vaccine in the primary series. Blood specimens were collected prior to and thirty days following this 'booster' dose (table I).

Table I. Immunization schedule for control and parainfluenza vaccinees

Day of study	Control group			Study group	
	Serum	Influenza vaccine	Parainfluenza vaccine	Serum	Parainfluenza vaccine
0	×	×		×	×
30					×
60		×			×
90	×			×	
6 m.[1]	×		×	×	×
7 m.[1]	×			×	

[1] Applies only to newborn, 6-week, and 12-week vacinees.

Results

A Comparison of Maternal and Cord Serum Parainfluenza 3 HIA titers: Twenty-one of 27 cord sera had higher titers of antibody than their corresponding maternal sera (table II).

Table II. Degree of difference observed between maternal and cord serum parainfluenza 3 HIA titers

	Degree of difference (cord titer/maternal titer)					
	−2×	0	+2×	+4×	+8×	Total
Number	1	5	15	5	1	27

Results of neonatal immunization: The groups receiving parainfluenza or influenza vaccine were almost identical in respect to the degree of HIA decrease during the first 90 days of life (table III). Only one of the 15 infants in the parainfluenza group experienced a rise in antibody titer (from 8 to 64) and no increases were observed among 12 influenza vaccinees.

Similarly, if one compares the initial, pre-vaccine serum (cord serum) with that obtained just prior to the booster dose at 6 months of life, no differences are noted in antibody decline for the two groups (table III). The same infant who experienced an eight-fold rise in the 90 days' serum maintained a titer at 6 months which was two-fold higher than the cord level.

Unexpected turnover at Lowry Air Force Base and Fitzsimons General Hospital resulted in the loss of some subjects to the study. Thus, of the original 27 vaccinees in the newborn group (15 parainfluenza; 12 influenza) only 17 were available for booster immunization (10 parainfluenza; 7 influenza). One month following administration of the booster dose of parainfluenza vaccine, two of the 10 parainfluenza vaccinees had a two-fold rise in antibody. All of the influenza group and 8 of 10 of the parainfluenza group either had stationary titers or further decreased their titers two-fold. It may be significant that 5 of the 7 influenza

Table III. Comparison of serum parainfluenza 3 HIA titers for 'newborn' vaccinees prior to and at 30, 180 and 210 (post-booster) days after primary immunization

Days post-immunization	Vaccine group	Total no. infants	Degree of antibody change —64×	—32×	—16×	—8×	—4×	—2×	0	+2×	+8×
30	P3 17 8A	15			1	10	2	1			1
	influenza	12			1	8	3				
180	P3 17 8A	10	1	4	3		1			1	
	Influenza	7	1	4	2						
210	P3 17 8A	10						2	6	2	
(post-booster)	Influenza	7						1	6		

vaccinees tested at 30 days following the booster dose had no detectable antibody (fig. 1).

Results in the 6-week old immunization group: A marked disparity in the degree of antibody change following primary immunization was noted for the parainfluenza and influenza groups. Six of the 15 parainfluenza vaccinees either maintained stationary titers or increased their titers from 2 to 32-fold (table IV). All 11 of the influenza vaccinees decreased their titers from two-fold to eight-fold. The same trend is observable if one compares the pre-vaccine serum HIA titers with that serum obtained just prior to the booster dose; 5 of 13 parainfluenza vaccinees either dropped their titer slightly, maintained it or, in one instance, increased the titer 32-fold (table IV). All 9 of the influenza vaccinees decreased their titer from 4 to 16-fold. If one compares pre-booster and 30-day post-booster sera, 6 of 13 parainfluenza vaccinees had a two to four-fold increase in titer whereas in only one of 9 influenza vaccinees was a twofold rise observed (table IV). Two-fold increases are considered since infants in this group would normally be expected to *decrease* their titers in the interval between serum specimens. Fig. 2 details the absolute titers observed in the four serum specimens. Eight of the 9 influenza vaccinees had titers of 8 to 16, 30 days following the booster injection. In contrast, 5 of the 13 parainfluenza vaccinees had titers of 64 or greater.

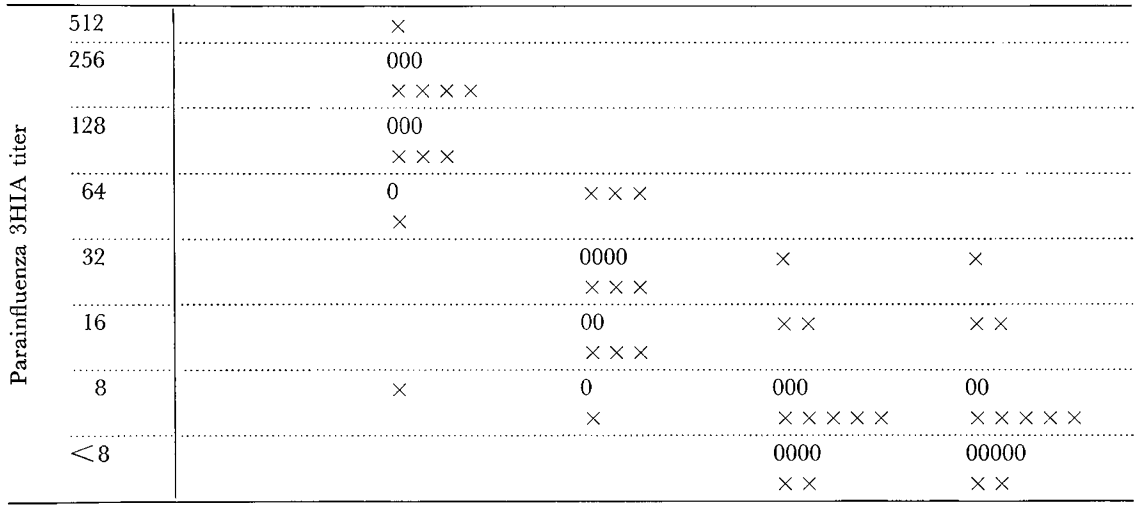


Fig. 1. Absolute parainfluenza 3 HIA titers in sera obtained during the course of immunization with parainfluenza or influenza vaccine (newborn group). × = Parainfluenza vaccinees, 0 = Influenza vaccinees.

Table IV. Comparison of serum parainfluenza 3 HIA titers for 6-week-old vaccinees prior to and 30, 180 and 210 (post-booster) days following primary immunization

Days post-immunization	Vaccine group	Total No. infants	Degree of antibody change							
			—16×	—8×	—4×	—2×	0	+2×	+4×	+32×
30	P3 17 8A	15		2	6	1	3	1	1	1
	Influenza	11		3	4	4				
180	P3 17 8A	13	3	2	3	2	2			1
	Influenza	9	2	3	4					
210	P3 17 8A	13				3	4	4	2	
(post booster)	Influenza	9				3	5	1		

Results of 12-week old immunization group: The range of antibody change following primary immunization in the 12-week old vaccinees was not different for the parainfluenza and influenza groups (table V). Two of the 10 parainfluenza vaccinees did increase their titer substantially (one fourfold and one 64-fold), whereas none of the influenza vaccinees demonstrated this degree of antibody rise. Similarly, comparing pre-vaccine sera with sera just prior to the booster dose of vaccine, little difference can be observed between the two groups except for the same two individuals observed to have marked antibody increase following primary immunization (table V). An interesting discrepancy between the two vaccine groups was observed following booster immunization. The influenza group split into two distinct categories: 11 of the 15 infants either maintained or decreased their titers two-fold, whereas the remaining 4 had four to eight-fold rise in antibody titer. All of the parainfluenza vaccinees either maintained or increased their titer two to four-fold (table V). The resultant final titers are demonstrated in fig. 3. Ten of the 15 influenza vaccinees had decreased their titer to undetectable levels 30 days following the booster dose. All of the 9 parainfluenza vaccinees had detectable antibody at a level of 1:8 or higher, and four were 1:32 or greater.

Results in the 6-month old immunization group: A clear-cut difference in the antibody response of the parainfluenza and influenza groups was observed for the

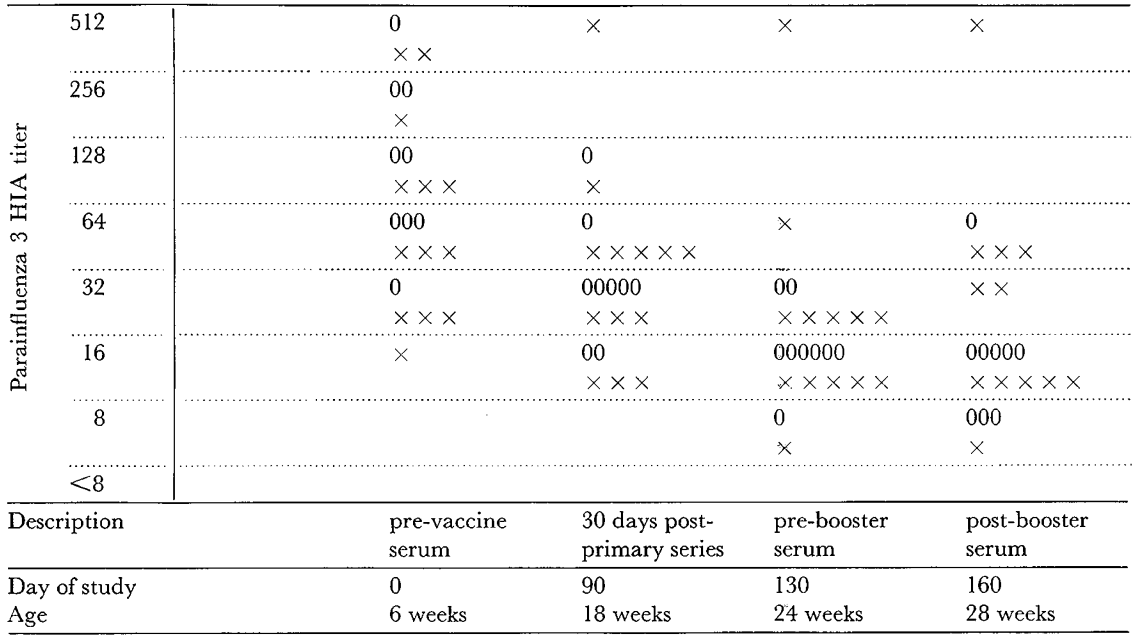


Fig. 2. Absolute parainfluenza 3 HIA titers in sera obtained during the course of immunization with parainfluenza or influenza vaccine (6 weeks group). × = Parainfluenza vaccinees, 0 = Influenza vaccinees.

Table V. Comparison of serum parainfluenza 3 HIA titers for '12-week-old 'vaccinees prior to and 30 days after primary immunization

Days post-immunization	Vaccine group	Total No. infants	Degree of antibody change —16×	—8×	—4×	—2×	0	+2×	+4×	+8×	+64×
30	P3 17 8A	10		1	3	2	1	1	1		1
	Influenza	20		1	7	8	1	3			
180	P3 17 8A	9	1		4	1	1	1			1
	Influenza	15		2	6	3	4				
210	P3 17 8A	9					4	4	1		
	Influenza	15				4	7		3	1	

6-month old vaccinees (table VI and fig. 4). Seven of the 8 parainfluenza recipients had four-fold to 16-fold increases in antibody titer whereas only one of 7 influenza vaccinees demonstrated a 16-fold rise. The remaining 6 influenza vaccinees either maintained their very low titers or decreased them to undetectable levels. Fig. 4 demonstrates the distribution of absolute titers following primary immunization in this group. No booster doses have yet been given to this group.

Table VI. Comparison of serum parainfluenza 3 HIA titers for '6 month' vaccinees prior to and 30 days after primary immunization

Vaccine Group	Total	Degree of antibody change —2×	0	+2×	+4×	+8×	+16×
P3-17-8A	8	1			5	1	1
Influenza	7	4	2				1

Composite data

By utilizing all of the control children and only the prevaccine sera of the parainfluenza group it is possible to construct a chart for the change in parainfluenza 3 HIA with increasing age (fig. 5). It is apparent that a steady decline in parainfluenza serum HIA levels occurs over the first seven months of life with the majority of children losing all detectable titers by the seventh month. A few infants became infected by the eighth month. Comparison of pre-vaccine and post-

Parainfluenza 3 HIA titer					
1024		×	×	×	
512	×			0	
256				0	
128		0		0	
				× ×	
64	0	00			
	×	× ×			
32	0000000	0000	0	0	
	× × × ×	× × ×	×	×	
16	000	00000	00		
	×	× × ×	× × ×	× × ×	
8	000	000	000000	0	
	× ×		× × × ×	× ×	
<8	0	0	000000	0000000000	
Description	pre-vaccine serum	30 days post-primary series	pre-booster serum	post-booster serum	
Day of study	0	90	145	149	
Age	12 weeks	24 weeks	32 weeks	36 weeks	

Fig. 3. Absolute parainfluenza 3 HIA titers in sera obtained during the course of immunization with parainfluenza or influenza vaccine (12 weeks). × = Parainfluenza, 0 = Influenza.

Parainfluenza 3 HIA titer		
128		0 × ×
64		× ×
32	×	× ×
16	00 × ×	×
8	0000 × × ×	00
<8	00 × ×	00000 ×
Description	pre-vaccine serum	30 days after immunization
Day of study	0	90
Age	6 months	9 months

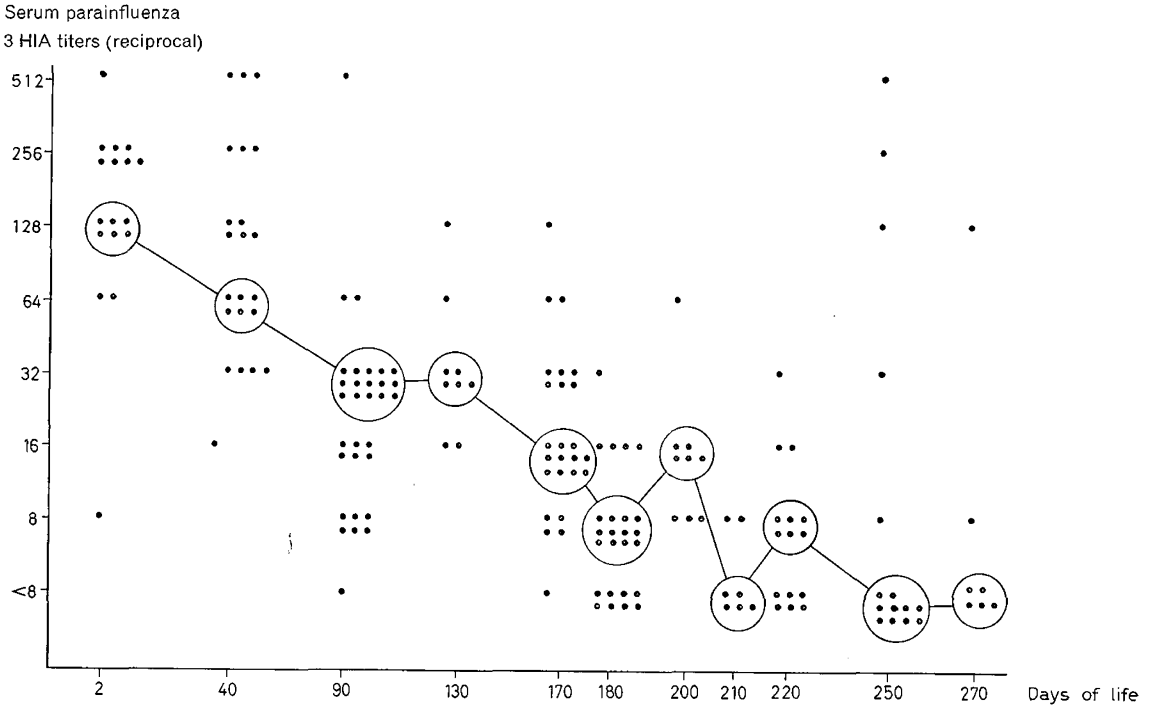
Fig. 4. Serum parainfluenza 3 HIA titers pre- and 30 days post-immunization (6-month-old vaccinees). × = parainfluenza vaccine, 0 = influenza vaccine.

Fig. 5. Change in serum parainfluenza antibody titer with increasing age in the first year of life (solid line connects median values for each age interval)—data obtained from all control vaccinees and from pre-immunization sera in parainfluenza vaccinees—see text.

immunization HIA titers affords an opportunity to assess the quantitative effect of maternal antibody upon successful immunization (fig. 6). It is apparent that four-fold or greater increase in antibody titer occurred in infants whose initial titers were 1:32 or less. Thus of 15 parainfluenza vaccinees with pre-vaccine titers of 1:32 or less, 12 (80 %) experienced a 4× or greater antibody increase, whereas of 16 influenza vaccinees (controls) only 3 (12 %) had 4× or greater increases and these were probably the result of natural infection. At a pre-immunization level of 1:64 more parainfluenza vaccinees had less of a decrease in titer than did controls; 7 of 8 parainfluenza vaccinees had a 4× or less decrease in titer compared to 3 of 11 controls. Unfortunately, this is not clear cut evidence of successful immunization but this trend toward less decrease in titer may reflect the influence of parainfluenza virus antigenic experience.

Discussion

Parainfluenza viruses cause serious respiratory disease during infancy and early childhood. Type 3 virus infects most infants in the first months and years of life. Control measures must be applied sufficiently early to result in significant protection. This study was designed to evaluate the antigenicity of a known potent inactivated alum-adsorbed parainfluenza type 3 vaccine in ear-



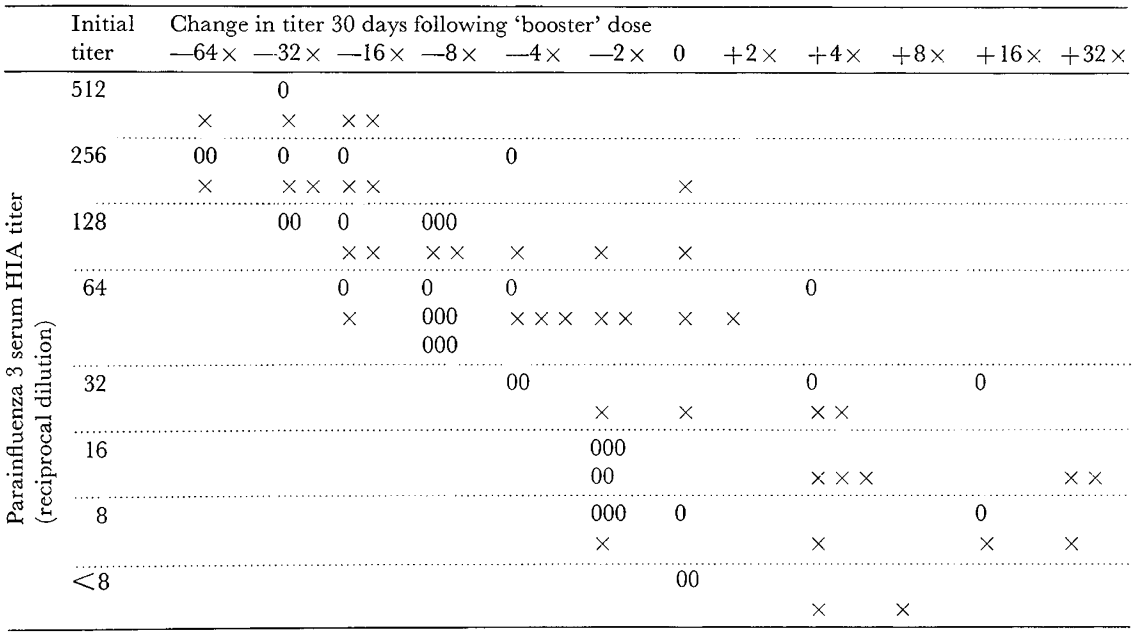


Fig. 6. Comparison of final serum antibody titers with pre-vaccine levels for parainfluenza and control vaccinees (all ages included as determining factor was maternal antibody and not age of child—see text). × = Parainfluenza vaccinees, 0 = Influenza vaccinees.

ly infancy. Two potential problems were investigated: 1. the 'immunologic capability' of infants in the first few months of life; and 2. the influence of passively-derived maternal antibody upon attempted immunization. Our data indicate unequivocal antigenicity of this vaccine preparation when immunization is begun at six months of life. The evidence for successful immunization in some infants at an earlier age is less clear-cut but suggestive. Several infants were unquestionably immunized; one newborn infant with a cord serum antibody titer of 1:8 experienced an eight-fold rise in titer (1:64) following primary immunization. Similarly, two six-week-old and two 12-week-old vaccinees had four-fold or greater antibody increase after immunization. In some of the other infants suggestive evidence for successful immunization is afforded by a smaller decrease in antibody titer than that observed in control infants, by the persistence of a detectable titer following a 'booster' dose compared to undetectable levels in the majority of control infants given the same 'booster' dose; and by the somewhat greater overall antibody response following a 'booster' dose in parainfluenza vaccinees compared to control infants. Unfortunately, the data is not clear-cut largely because maternal antibody was still present at the time of 'booster' immunization. Thus, we were probably observing the combined effect of maternal-modification (? suppression) of the immune response as well as the infant's basic capacity to respond. A further difficulty in interpretation was presented by sporadic infection evidenced by marked antibody rises among control infants who did not receive parainfluenza vaccines initially. Examination of the data for 12-week-old vaccinees illustrates this point. Whereas all of the parainfluenza vaccinees experienced no change or slight increases in titer following a booster dose of parainfluenza vaccine, the control group divided sharply into two sub-groups; those maintaining or decreasing their titers slightly and those with four-fold or greater increases indicative of infection during the month following immunization.

Our data do not provide clear guide-lines to future immunization programs, but do serve to emphasize the difficulties in evaluation of vaccines in the face of homologous maternal antibody and natural infection and reemphasize the value of a control population studied simultaneously.

With regard to the influence of maternal antibody upon successful immunization, our data do provide quantitative information. Lack of suppression of immunization was evident for serum HIA titers of 1:64 or less in parainfluenza vaccinees. This can be appreciated by examination of pre-vaccinal serum anti-body level (maternally-derived) in comparison to post-

immunization titers (fig. 6). Parainfluenza vaccinees clearly and consistently achieved four-fold or greater antibody rises (80 %) below this level whereas controls experienced a lower rate of antibody increase (12 %). It is tempting to assume all vaccinees were immunized and all controls infected. The absolute titers tend to support this contention (table V and fig. 3), but in the absence of viral isolation and specific illness data no valid conclusion can be reached. In general increases in antibody following immunization in parainfluenza vaccinees tended to be moderate in comparison to antibody rises observed in controls.

Serum antibody levels of 1:128 or greater prior to vaccination appeared to prevent immunization. Thus, the majority of newborn parainfluenza vaccinees failed to differ from their controls in either response to primary or booster immunization. Fourteen of 17 newborn vaccinees in both groups had serum antibody levels of 1:128 or greater prior to immunization.

Suppression of serum antibody response to vaccines in young infants by transplacental antibody is not unique to parainfluenza virus type 3 and has previously been noted with live virus vaccines (measles [10], poliovirus [9], vaccinia [7]), inactivated virus vaccines (measles [8], poliovirus [11]) and with a bacterial vaccine (diphtheria toxoid [2]). This response is by no means all or none; rather, it appears graded so that with lower levels of antibody or increased amounts of antigen immunization may be quite successful [5, 3]. Additionally, reliance upon antibody titers in young infants sera following immunization may be insufficient as shown by Krugman for killed measles virus vaccine; infants who failed to develop hemagglutination-inhibition antibody titers following killed vaccine administration at 2, 3 and 4 months of life, responded to subsequent natural challenge and live virus immunization in an attenuated fashion [8].

It is apparent from our experience with this study that further investigation is warranted employing more concentrated antigens in an effort to surmount the probable suppression produced by maternally-derived homologous antibody. Furthermore, 'booster' immunization should be delayed until all maternal antibody has disappeared.

Summary

A controlled clinical trial employing a parainfluenza type 3 vaccine (inactivated, alum-adsorbed) is reported. An attempt was made to evaluate the influence of age and homologous maternal antibody upon immunization by initiating immunization at four age intervals; 2 days, 6 weeks, 12 weeks and 24 weeks of life.

Successful immunization was achieved when immunization was begun at 24 weeks of age. Some infants younger than 6 months were successfully immunized as evidenced by four-fold or greater rise in serum antibody titers. However, the presence of significant levels of maternal antibody and sporadic infection in control infants obscured the effect of immunization. Evidence is presented which suggests, but does not firmly establish, the efficacy of immunization in some infants 12 weeks and younger. Interference with immunization was associated with a maternally-derived hemagglutination-inhibition antibody titer of 1:128 or greater in the preimmunization serum. Below this level successful immunization was achieved in 80 % of infants.

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