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Immunization Against Neonatal Tetanus in New Guinea

Antitoxin Response of Pregnant Women to Adjuvant and Plain Toxoids *

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Immunization of pregnant women in New Guinea with three injections of plain toxoid had previously been shown to prevent neonatal tetanus. In the present study antitoxin levels induced by two oil-adjuvant toxoids (one injection), one $AlPO_4$ toxoid (two injections) and one plain toxoid (three injections) were compared with those induced by the same plain toxoid as used in the earlier study. At term there was no significant difference in the levels for the five toxoids, but those for the plain toxoids later declined rapidly. $AlPO_4$-toxoid titres were significantly higher than the titres for the plain toxoids at the end of a year, but lower than the oil-adjuvant titres, which were the highest and most persistent. However, unacceptable side-effects (induced by subsequent lots of oil-adjuvant toxoids) preclude their routine use at present.

The results indicate that a maternal antitoxin level at delivery of 0.01 unit/ml is protective. Aluminium-compound toxoid rapidly achieved titres that were better than this for at least a year, with minimal side-effects. Hence such toxoids are recommended for maternal immunization to prevent neonatal tetanus.

For more than forty years it has been known that tetanus antibody passes the placenta (Ten Broeck & Bauer, 1922-23), and suggestions were made that newborn infants might be passively protected against neonatal tetanus by active immunization of their mothers (Nattan-Larrier, Ramon & Grasset, 1926, 1927; World Health Organization, 1950). During 1959-61 a field study in the Maprik area of the Sepik District, New Guinea, where the incidence of this disease was 80 per 1000 live-births, indicated the practical value of primary immunization in pregnancy (Schofield, Tucker & Westbrook, 1961). A fluid formol toxoid was used, and three injections (schedule E, described later) were found necessary. Two injections six weeks apart were insufficient to provide complete protection but they reduced the expected incidence by two-thirds.

The three injections of schedule E were practicable at Maprik only because village clinics for pregnant women and for children were set up at the beginning of the field studies. Wide-scale primary immunization during pregnancy by similar multidose schedules cannot yet be attained in many of the parts of the world where the disease is most common —namely, in rural tropical areas. Ante-natal clinics and staff are scarce, communications are severely limited, and illiterate populations have great difficulty in keeping to a schedule of appointments with vaccinators. For these reasons it is important to discover a satisfactory adjuvant toxoid that will produce adequate antitoxin levels after primary immunization in the childbearing period of life with the minimum number of injections—preferably only one.

The collaborative study reported here was arranged in 1961 through the good offices of the late Dr Joseph E. Smadel. The main objectives were:

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(1) To compare maternal antitoxin responses to four preparations of one batch of toxoid, three with different adjuvants in one- or two-dose schedules and one without adjuvant in a three-dose schedule.

(2) To determine in the last trimester of pregnancy the mean and the range of the maternal antitoxin titres produced by schedule E, which provides a basis of known efficacy in practice, and to evaluate the other preparations against this.

(3) To determine persistence of antitoxin levels—information that is useful in establishing when a booster is necessary in subsequent pregnancies.

(4) To compare all five toxoids for side-effects and ease of administration under field conditions.

(5) To relate human antitoxin response to laboratory methods of potency evaluation and to the international standards for tetanus toxoids.[1]

MATERIALS AND METHODS

The toxoids and their administration

Table 1 lists the toxoids and dosage schedules. These schedules were rigidly followed for all women included in the antitoxin study. Toxoids A, B, C and D, kindly supplied by Parke, Davis & Co., were prepared from one batch of toxoid containing 612 Lf/mg non-dialysable protein N. Toxoids A and B were prepared in a similar manner, except that A contained 42.5% mineral oil (Drakeol-6VR) and B contained 42.5% 7-*n*-hexyloctadecane (H-24). Both contained 7.5% Arlacel A and were emulsified with the other ingredients in a 50% aqueous phase (volume), final pH 5.9. The oil-emulsion materials met the criteria described by Wilner et al. in a paper published in 1963. In the same paper these authors describe also the physical characteristics of the emulsions and the factors involved in preparation, as well as the comparative immunological effects of oil adjuvants on influenza virus vaccine. Toxoid C contained 2.5 mg $AlPO_4$ per single dose (0.55 mg Al). Each toxoid contained 0.01% thiomersal for preservative. The potency of each of these toxoids exceeded the minimum requirements of the United States Department of Health Education, and Welfare (*Minimum Requirements: Tetanus Toxoids:* 16 December 1952).

Toxoids A, B, C, and D (plain) were contained in 5-ml rubber-capped glass bottles, and toxoid E (plain), made by the Commonwealth Serum Laboratories, Melbourne, Australia, was contained in 1.0-ml rubber-capped glass bottles. This last-mentioned toxoid was from the same batch, and administered in the same dosage schedule, as in the original field study (Schofield et al., 1961). Throughout this study, 2-ml glass syringes with 22-gauge (0.70-mm) needles were used, and all injections were given into the left deltoid muscle.

TABLE 1
COMPOSITION AND DOSAGE SCHEDULE OF TOXOIDS

Toxoid code[a]	Adjuvant	Lf/ml	Number of doses (0.5 ml)	Total Lf
A	Drakeol	20	1	10
B	H-24	20	1	10
C	$AlPO_4$	10	2[b]	10
D	None	10	3[b]	15
E	None	20[c]	3[b,d]	60

[a] A, B, C and D were prepared from a common parent toxoid.
[b] Six-week interval between injections.
[c] Kf was approximately 24 hours (reported by those of the authors working at the NIH).
[d] 1.0-ml doses.

Selection of women and bleeding schedules

Once a bottle containing toxoid A or B had been broached it became necessary, for reasons described later (see page 694), to finish that bottle the same day. Therefore, the pregnant women, who were met a few at a time in their villages, could not individually be allocated at random to each toxoid. They were allocated in small groups, but all five immunization schedules proceeded concurrently, and the five groups in the antibody study did not differ in mean estimated age. All the women were of the Abelam tribe, described by anthropological and medical workers in a number of papers (Kaberry, 1941; Peters, 1960; Schofield & Parkinson, 1963). Immunization of pregnant women had been practised since early 1960, but tetanus toxoid had never been administered to any other Abelam people. Women who were estimated, by abdominal palpation, as being 4-7 months' pregnant were admitted to the antibody study if they had not previously received any tetanus toxoid. (Sixteen women who, when first seen, were judged to be more advanced in pregnancy were given schedules A, B or C but not bled; and women known to have been previously immunized were given a booster injection of a fluid toxoid but not bled.)

[1] The results of this part of the study are to be given in a subsequent paper.

TABLE 2
NUMBER OF BLEEDINGS IN RELATION TO FIRST TOXOID INJECTION

Toxoid	Scheduled week [a] after first injection											
	0	4	8	12	14	16	18	20	22	24	26	39-64
A	28	27	24	18		10		6		2		24
B	27	27	23	18		5		6		3		19
C	33		25	15		11		8				18
D	20				20		4		7		3	16
E	25				21		6		4		6	9

[a] Bleedings are listed under nearest scheduled week.

A venous blood sample was taken from all women admitted to the antibody study just before the first injection. Further blood specimens during pregnancy were to be collected at specified intervals: monthly, after the first (only) injection of A or B; two weeks after the second (final) injection of C, then monthly; two weeks after the third (final) injection of D or E, then monthly. Specimens were to be collected at six-monthly intervals after delivery, if possible. In practice the second and subsequent bleedings could not always be done at the correct intervals in all cases, and Table 2 indicates how they were distributed in relation to the time of the first toxoid injection. Between 39 and 64 weeks there was only one bleeding per subject, and the total numbers of these individual bleedings are shown in the relevant column of Table 2.

The blood specimens were collected into labelled 30-ml bleeding venules and flown to Melbourne. There the serum was separated, stored frozen and then flown frozen in batches to the National Institutes of Health (NIH), Bethesda, Md., USA. The code A to E was not known outside New Guinea.

Clinical follow-up and reasons for the exclusion of some women during the study

The women injected, even if not in the antitoxin study, were examined twice monthly for side-effects; so were their infants after delivery. Three deliveries were in hospital; the remainder (176) were by traditional methods. Careful inquiry was made about the circumstances and symptoms of 13 stillbirths and of three neonates who had died without being medically examined.

Fifty women who had been bled once and then received their first injection had to be excluded later from the antitoxin study for one of the following reasons:

(1) A live-birth, stillbirth or miscarriage occurred before immunization schedules had been completed, or before the second blood sample had been obtained (41 women). This, of course, was commonest with schedules D and E—the longest and most difficult to follow. (Data from one woman in group C and one in group E, who were delivered before their final injection, have not been included in the pre-delivery antitoxin titres but have been included in over-all data.)

(2) The schedule for the second or third injection was not correctly followed (six women).

(3) Antitoxin was detectable in the first serum specimen and a very rapid rise in titre, of the anamnestic type, followed the first injection (three women). A recheck of the original records revealed that these three women had received toxoid in an earlier pregnancy.

There remained 134 women for the antitoxin studies.

Antitoxin titrations

The details of the test will be given in a subsequent paper. In brief, undiluted and 10-fold dilutions of a serum were mixed with L+/1000 toxin, incubated and then inoculated subcutaneously into three mice per test mixture. Additional mice received a mixture of 0.001 unit of the US Standard Tetanus Antitoxin and the test dose of toxin. The symptoms and deaths were recorded daily for four days. For the analyses given in this paper, only deaths were taken into account. The antitoxin titre of a serum was estimated as the geometric mean of that amount of

serum that provided no protection and the next larger amount that provided protection. For example, if serum diluted 1 : 10 failed to protect while the serum undiluted protected, the antitoxin titre of the serum would be 0.003 unit/ml.

Statistical analyses

The statistical analyses, including geometric mean titres at designated time periods, analyses of variance to test for significant difference between toxoids, and regression curves, were made under the direction of Dr C. J. Maloney of the Division of Biologics Standards, NIH.

RESULTS

Antitoxin responses to the five toxoids

Table 3 gives the results of the titration of each of 518 sera obtained from the 134 women included in the study described in the present report. Pre-injection sera showed no antitoxin at the lowest level tested—i.e., 0.001 unit/ml. After completion of the vaccination schedules the number of bleedings ranged from 1 to 6 per subject, while the latest bleeding per subject ranged from 8 days to 64 weeks.

Only four women failed to show a minimum response. Of these, two were definitely non-responders: subject 103 C was bled at eight weeks (two weeks after final injection) and at 14 weeks; subject 6 E was bled at 14 weeks (two weeks after final injection) and again at 22 and 64 weeks. Subjects 4 E and 8 E were bled only at 52 weeks after vaccination. These latter could have had a rise and decline prior to 52 weeks.

The distribution of the highest titre responses to the five toxoids is given in Table 4. The mean highest titres for the A, B, and C groups are not significantly different, but all three are significantly different from those for both D and E. There is no significant difference between the highest titres for the D and E groups. At the end of one year mean titres for A and B showed no drop; that for C showed a drop from its highest mean, which resulted in a titre significantly below those for A and B but not below the protective titre, which is discussed later. Only titres for D and E showed a significant drop from their highest mean—to a level below 0.01 unit/ml.

The most uniform individual responses occurred with C toxoid; 32 of 34 were at levels between 0.03 unit/ml and 0.3 unit/ml. Responses to B and E showed the widest variation. The time intervals of the bleedings may have influenced the apparent spread, especially in the case of B.

The response to A and B toxoids was gradual and the individual responses were remarkably persistent (Table 3). With A, 14 sera from 24 women (58%) at 39-64 weeks had higher titres than at the previous bleeding; only one (206 A) showed a decline—from 0.3 unit/ml at 12 weeks to 0.03 unit/ml at 64 weeks. With B the rise appeared to be slightly more rapid than with A; at 39-64 weeks 5 of 19 (26%) were higher, while four showed a one-dilution decline. The on page 690 figure gives the mean-titre responses to all toxoids plotted against time and the number of sera per mean and their 95% probabilities.

The oil adjuvants induced the highest and most persistent titres. The $AlPO_4$-adjuvant titre was slightly less in height and in persistence. Both of the plain toxoids induced early titres comparable to that of the $AlPO_4$ adjuvant, but these declined rapidly. E titres had dropped significantly by the end of 14 weeks after the third injection of the toxoid (26 weeks after the first injection).

Regression lines were drawn to fit the mean-titre curves of toxoids A and B. It was found that two straight lines intersecting at a point corresponding to 85 days after the injection (95% confidence limits, 75-96 days) provided the best fit. It appears, therefore, that the optimal antitoxin response to both oil-adjuvant toxoids can be expected to be achieved in a mean of 85 days. The response to toxoid C, in this particular schedule of six weeks between the two doses, was also estimated to reach its optimum by at least 85 days after the first injection, whereas the responses to schedules D and E were, of course, found 98 days after the first injection.

The need for two injections of $AlPO_4$-adjuvant toxoid

One baby born to a women in group C, 34 days after her first injection, developed neonatal tetanus. The mother had received only one of the two injections scheduled for immunization prior to delivery. In order to determine the antitoxin response to one injection nine pregnant women were bled immediately before the second injection. Seven sera showed no detectable titre while two had only 0.003 unit/ml. Two weeks later, sera from three women whose serum titres at six weeks had been less than 0.001 unit/ml—namely, toxoid C subjects No. 111, 112 and 114—had titres of 0.3, 0.3, and 0.03 unit/ml respectively.

Protective antitoxin level against neonatal tetanus

The 132 mothers who were in the pre-delivery antitoxin study bore 120 babies who survived longer

TABLE 3
ANTITOXIN TITRES OF INDIVIDUAL SUBJECTS FROM INITIATION OF IMMUNIZATION THROUGH 64 WEEKS (ANTITOXIN UNITS PER MILLILITRE)

Toxoid A schedule

Subject	Weeks following initial injection									
	0	4	8	12	16	20	24	39	52	64
1	<0.001	<0.001	0.003					0.03		
2	<0.001	0.003	0.3	0.3	0.3	0.3			0.3	
2	<0.001	0.003	0.03	0.3					3.0	
3	<0.001	<0.001	0.003	0.03	0.03			0.03		
6	<0.001	<0.001	0.003	0.003	0.03	0.03		0.03		
10	<0.001	0.003							0.3	
15	<0.001	0.03	0.03							
17	<0.001	<0.001	0.003						0.3	
18	<0.001	<0.001		0.03						0.3
21	<0.001		0.03						0.03	
23	<0.001	<0.001	<0.001	0.003					0.03	
24	<0.001	<0.001	0.003	0.03	0.03					0.3
25	<0.001	<0.001	0.003	0.003	0.03	0.03			0.03	
28	<0.001	0.003	0.03	0.3	0.3				0.3	
36	<0.001	<0.001	0.003	0.003						0.003
37	<0.001	<0.001							0.003	
38	<0.001	0.003								
41	<0.001	<0.001	0.03							
46	<0.001	<0.001	0.03						0.3	
48	<0.001	0.03	0.03	0.3						3.0
60	<0.001	0.03	0.003	0.3	0.3	0.3	3.0			
61	<0.001	<0.001	0.003	0.03	0.03			0.3		
63	<0.001	<0.001	<0.001	0.3	3.0	3.0		3.0		
64	<0.001	<0.001	0.03	0.3	0.3			3.0		
69	<0.001	0.003	0.03							0.3
192	<0.001	0.003	0.3	0.3						0.3
204	<0.001	0.03	0.3	0.3		0.3	0.3		0.3	
206	<0.001	0.003	0.03	0.3						0.03

Toxoid B schedule

Subject	Weeks following initial injection									
	0	4	8	12	16	20	24	39	52	64
1	<0.001	0.03	0.3	0.3		0.3				0.3
2	<0.001	<0.001		0.3						0.03
5	<0.001	<0.001	0.003	0.003						
10	<0.001	0.003	0.03							0.03
13	<0.001	<0.001	0.003	0.03						0.003
14	<0.001	0.003	0.03	0.03						
22	<0.001	<0.001	0.003	0.003		0.03	0.03			0.03
26	<0.001	0.003	0.03							0.03
27	<0.001	0.003								
29	<0.001	0.03	0.3	0.3	0.3	0.3			3.0	
31	<0.001	<0.001	0.03	0.03						0.03
38	<0.001	0.003							0.03	
40	<0.001	0.03								0.3
42	<0.001	0.03	0.03						0.3	
48	<0.001	<0.001	0.03	0.3						
50	<0.001	0.003	0.003	0.03					0.03	
56	<0.001	0.03	0.03							
57	<0.001	0.003	0.03	0.3	0.3			0.3		
58	<0.001	0.003	0.03							
59	<0.001	0.03	0.3	0.3	3.0	3.0	3.0			
65	<0.001	0.03	0.3	0.3				0.3		
66	<0.001	0.003	0.03	0.3	0.3			30.0		
67	<0.001	0.003	0.3	0.3				30.0		
69	<0.001	<0.001	0.03	0.03	0.03	0.3		0.03		
73	<0.001	<0.001	0.003	0.003		0.03				0.003
191	<0.001	<0.001	0.003							
194	<0.001	0.03	0.03	0.3			0.3			0.3

TABLE 3 (continued)

Toxoid C schedule

Subject	Weeks following initial injection										
	0	8	10	12	14	16	18	20	39	52	64
1	<0.001		0.3					0.3		0.03	
3	<0.001									0.03	
6	<0.001	0.03		0.03		0.3		0.3	0.3		
12	<0.001	0.03									0.003
20	<0.001	0.3				0.003				0.003	
22	<0.001	3.0								0.03	
23	<0.001			0.03							0.03
26	<0.001			0.3							0.03
28	<0.001		0.3			0.03		0.03			
30	<0.001									0.3	
31	<0.001		0.3			0.03		0.3		0.3	
38	<0.001		0.3								0.03
39	<0.001			0.3							0.03
39	<0.001		0.03							0.3	
40	<0.001			0.03							
49	<0.001		0.3			0.03					
52	<0.001		0.3		0.03		0.03	0.03		0.03	
53	<0.001		0.3		0.03		0.3	0.03	0.03		
54	Lost		0.3		0.3				0.03		
69	<0.001			0.03							0.03
72	<0.001		0.03								0.03
84	<0.001				0.03		0.03				
88	<0.001							0.03			
93	<0.001		0.03								
96	<0.001	0.003			0.3	0.3		0.3			
97	<0.001	0.3			0.3		0.3				
98	<0.001	0.3			0.03		0.03				
100	<0.001	0.3			0.03						
101	<0.001	0.03									
103	<0.001	<0.001			<0.001						
107	<0.001	0.03									
111	<0.001	0.3									
112	<0.001	0.03									
114	<0.001	0.3									

Toxoid D schedule

Subject	Weeks following initial injection								
	0	14	16	18	22	26	39	52	64
1	<0.001		0.03					<0.001	
2	<0.001	0.03			0.003				0.03
5	<0.001	0.03			0.03				0.03
11	<0.001	0.03							0.03
11	<0.001	0.03						<0.001	
14	<0.001	0.3							0.03
17	<0.001	0.003						<0.001	
28	<0.001	0.3						0.03	
32	<0.001	0.003			0.003				
40	<0.001	0.03						0.03	
43	<0.001	0.3			0.03	0.03		0.03	
47	<0.001	0.03		0.03	0.03			0.3	
51	<0.001	0.003							<0.001
51	<0.001	0.03				0.003			
51	<0.001	0.3		0.3	0.03	0.3		0.03	
52	<0.001	0.003							<0.001
55	<0.001	0.3		0.03	0.03		0.3		
74	<0.001	0.03					0.003		
75	<0.001	0.003		0.03					
201	<0.001	0.003							

TABLE 3 (continued)

Toxid E schedule

Subject	Weeks after initial injection								
	0	14	16	18	22	26	39	52	64
1	<0.001	0.3						0.03	
4	<0.001							<0.001	
6	<0.001	<0.001			<0.001				<0.001
8	<0.001							<0.001	
12	<0.001	0.3						0.03	
34	<0.001	0.03						0.03	
41	<0.001	0.3						0.003	
45	<0.001	0.3							0.003
54	<0.001	0.03			0.03				0.03
70	<0.001	0.03		0.003		0.003			
72	<0.001		0.03			0.003			
76	<0.001	0.03		0.03					
78	<0.001	0.3				0.3			
79	<0.001	0.3				0.003			
80	<0.001	0.03				0.003			
82	<0.001	0.3		0.3	0.3				
83	<0.001	0.03							
85	<0.001	0.03		0.003		<0.001			
87	<0.001			0.03	0.3				
90	<0.001			0.03					
94	<0.001		0.03						
95	<0.001		0.03						
105	<0.001		3.0						
108	<0.001		0.03						
202	<0.001	0.003							

than two days. None developed tetanus. Table 5 gives the time between initiation of immunization and delivery, and the antitoxin level of the last ante-partum serum obtained (one A serum was obtained two days after delivery). The distribution of the antitoxin values induced by each of the toxoids and their geometric mean are summarized in Table 6. There was no significant difference between the means of any of the five schedules. The over-all mean was 0.037 unit/ml.

It should be noted that some bleedings were not made in close proximity to delivery. Although with A and B toxoids significant rises in titres did not occur after 85 days, the maximum mean level was not reached until 24 weeks (see accompanying figure). Hence, several of the values given in Tables 5

TABLE 4

HIGHEST TITRE RESPONSES AND THEIR MEANS, AND THE MEAN TITRES AFTER APPROXIMATELY ONE YEAR

Toxoid	Highest titre (units/ml)								Titre at 39-64 weeks	
	Number of subjects	<0.001	0.003	0.03	0.3	3.0	30	Mean	Number of subjects	Mean (unit/ml)
A	28		4 [a]	7	12	5		0.139	24	0.161
B	27		3 [b]	11	9	2	2	0.124	19	0.135
C	34	1		12	20	1		0.12	18	0.0408
D	20		5	9	6			0.03	16	0.0087
E	25	3	1	12	8	1		0.04	9	0.0041

[a] Last bleedings were at 4, 8, 52 and 64 weeks respectively.

[b] Last bleedings were at 4, 8 and 12 weeks respectively.

MEAN ANTITOXIN TITRES AND 95% CONFIDENCE LIMITS IN WOMEN IMMUNIZED WITH TETANUS TOXOIDS

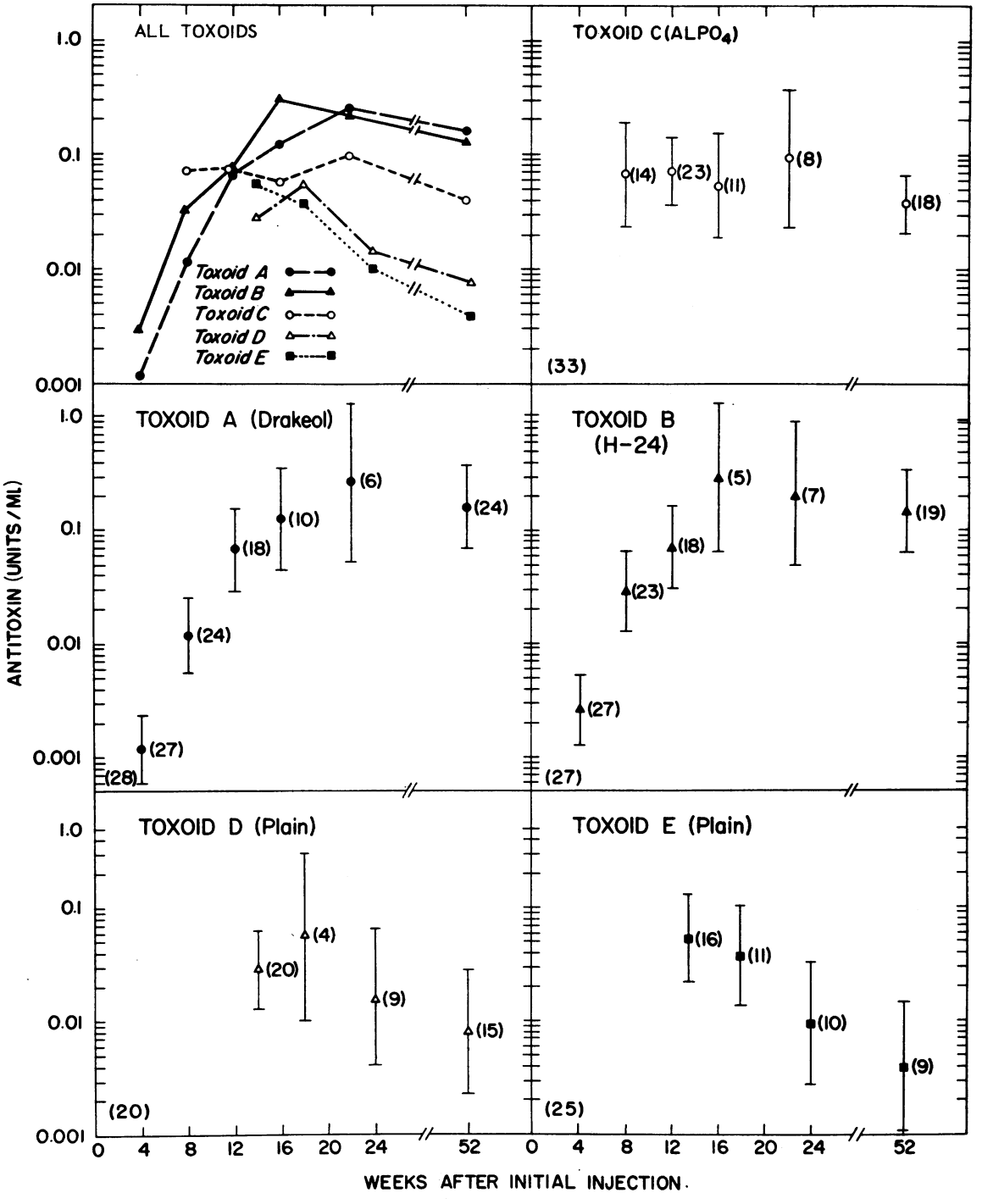

TABLE 5

INTERVAL BETWEEN FIRST INJECTION AND DELIVERY, AND ANTITOXIN TITRE OF LAST ANTE-PARTUM SERUM

Toxoid	Units/ml	Weeks from initiation of immunization to delivery [a]						
		1-4	5-8	9-12	13-16	17-20	21-24	25-28
A	<0.001		16					
	0.003	+2 [b]	12	15, 35	26, 55		75	
	0.03			14, 18, 21, 26		9, 15, 25, 52	21, 25	
	0.3				15, 19	23, 22	59, 63	21, 69
	3.0							5, 30
B	<0.001	8 [c]						
	0.003		13, 23	18	20			
	0.03		7	8, 17, 24	9, 17, 20	40	12	18
	0.3			20	17, 23, 28	1, 18, 20, 24	16, 20	28
	3.0							5
C	<0.001			62 [c], 67 [c]		27		
	0.003					11		
	0.03			4, 6, 7, 10	31, 39	11, 21, 24, 49	12, 20, 27, 58	21, 119, 141
	0.3			5, 7, 28	4, 12, 34	16	15, 20, 21	63
	3.0			28				
D	<0.001							
	0.003				13	40, 44	17, 49	7, 28
	0.03				0, 9	1, 16, 23, 34		2, 14, 18, 19
	0.3					23, 34		7
	3.0							
E	<0.001				91 [c], 92 [c]		14	
	0.003					5, 27	25	6
	0.03				6	21	39, 50	28, 50, 75, 80
	0.3				2, 12	12, 21, 34	10, 42	31
	3.0					24		
	Total	2	5	21	24	33	23	24

[a] Numerals in the columns are the number of days prior to delivery that sera were obtained.
[b] Bleeding two days after delivery.
[c] Pre-immunization serum.

TABLE 6
DISTRIBUTION OF ANTITOXIN TITRES OF SERA OBTAINED NEAREST TO DELIVERY

Toxoid	Antitoxin (units/ml)					Number of sera	Mean titre (unit/ml)
	<0.001 [a]	0.003	0.03	0.3	3.0		
A	1	7	10	8	2	28	0.040
B	1 (1)	4	10	11	1	27	0.055
C	3 (2)	1	17	11	1	33	0.048
D	—	7	10	3		20	0.02
E	3 (2)	4	8	8	1	24	0.031
Total	8 (5)	23	55	41	5	132	0.037

[a] Figures in parentheses indicate number of pre-immunization sera.

and 6 are no doubt lower than at the time of delivery. Only those sera drawn within 30 days of the deliveries at 25-28 weeks post-injection would represent the maximum level. With C toxoid all post-injection values can be considered true delivery values; maximum levels were obtained two weeks after the second injection and these remained relatively constant for 24 weeks or longer (see the figure). On the other hand, with the D and E plain toxoids, titres in sera taken later than 18 weeks after the first injection might have been lower, because the mean values recorded in the figure began to show a decline after 18 weeks. For deliveries at 20-28 weeks the values of all D and E sera not drawn within 15 days of delivery were probably higher than at the time of delivery.

To determine the minimum protective level of antitoxin the values for E are of particular interest because this schedule has been given since 1960 to 900 pregnant Maprik women who bore live infants without any case of neonatal tetanus. The tribal customs that were responsible for the high incidence of 80 cases per 1000 live-births observed previously have not altered in any way and the incidence in villages not served by the ante-natal clinics has not decreased. On indirect evidence the safe human antitoxin level for passive protection against tetanus has been taken to be 0.01 unit/ml (McComb, 1964). Among the 22 pre-delivery E titres (last injection of toxoid in last trimester of pregnancy) there were five below this level. Therefore, among the 900 women, approximately 204 would be expected to have such titres and, if these titres had offered no protection, 16-17 cases of neonatal tetanus would be expected. In fact there were none—a highly significant difference.

It should be noted that: (*a*) the mean titre for E at 24 weeks was 0.01 unit/ml (see figure); (*b*) 7 of 20 delivery titres of D women were 0.003 unit/ml or less; (*c*) one each of A and B women with deliveries within four weeks after vaccination would have had less than 0.01 unit/ml (see figure). In addition, results of work with guinea-pigs now in progress show that the titre that protects 50% of the guinea-pigs against at least 20 MLD of toxin is less than 0.001 unit/ml.[1]

Side-effects of toxoids A to E in the pregnant women

In all, 39 pregnant women were given A toxoid, 34 were given B, 37 were given C, 25 were given D and 44 were given E. The only reactions were at the site of the injections in groups A and B (oil adjuvants), two of which were severe. In one B case a fluctuant swelling appeared, 3 cm × 4 cm, 13 weeks after the injection. It was not hot or tender. Two weeks later it became painful and discharged, then healed leaving a scar. In the second case, in B group, a painful swelling of the deltoid muscle appeared two weeks after the injection; the arm was put in a sling and the reaction subsided in three days.

Minor reactions (nine in A and eight in B) were more common. A diffuse non-tender swelling in the

[1] Findings to be published by those of the authors working at the NIH (M. Pittman, M. C. Hardegree and M. F. Barile).

muscle appeared three to four weeks after the injections and subsided by six to eight weeks. The axillary lymph-nodes were not enlarged or painful and the women's ordinary activities were not affected. Follow-up—for 1½-2 years, so far—has revealed no chronic cysts such as those described by Bell et al. (1961).

Neonatal death and stillbirth rates

There were nine neonatal deaths and 13 stillbirths. Added together, the rates were as follows:

A	5.0%
B	11.1%
C	9.8%
D	8.0%
E	14.9%

These rates are very low for this area. Three pairs of twins were born, all in group B, and one twin died. There was one case of mongolism (E), one infant with an exomphalos (B), one infant born with an opacity of one cornea (E) and one albino stillborn infant (D). The miscarriage rate was approximately 4%.

Side-effects of a second batch of toxoids A, B and C in non-pregnant women

Because of the long-lasting antitoxin levels produced by the adjuvant toxoids among the pregnant women and the low incidence of troublesome side-effects, it was decided to give either A or B (one injection) or C (two injections) to volunteer non-pregnant Maprik women of an estimated age of 12-45 years. The purpose was to provide larger numbers for the study of possible side-effects and to follow their antitoxin levels for some years; also, when the women became pregnant, to compare antitoxin levels before and after one booster injection of toxoids C or D. A census was taken of women who lived in the villages served by the two-weekly clinics and within easy walking distance of Maprik hospital, and all those who had been given tetanus toxoid previously were excluded. After allocation to three groups with a table of random numbers, 999 women were injected in February 1964 with the new A, B or C toxoids, and the second injection of C was given six weeks later.

On this occasion the toxoids were in one-dose, pre-filled, disposable glass syringes with rubber-capped plungers. They were transported and stored in the same manner, and given in the same volume (0.5 ml) by the same route (into the left deltoid muscle) as in the pregnancy study. However, on this second occasion, the preparations—now called A_2, B_2 and C_2—had been made with a different batch of tetanus toxoid, of lower purity than that of the previous A, B, C and D preparations, although with the same Lf/ dose. Otherwise the same or similar constituents were used in the second series as in the first, except that the pH of A_2 and B_2 had been adjusted to 7.0 to improve the stability of the emulsions.

A_2 was given to 327 women, B_2 to 332 and C_2 (two injections) to 340. Many of the women noticed pain 10-12 days after injection. A physician who could not have known which toxoid any of them had received examined 117 complaining of reactions. These reactions were noted in a high proportion of the women who had received A_2 or B_2 and very few of those who had received C_2. In both A_2 and B_2 subjects a hot brawny tender area in the left deltoid muscle was invariably found; in the majority of cases this was 5-7 cm in the direction of the muscle fibres, and slightly less across. The lymph-nodes in the left axilla were palpably larger than those in the right in 16% of the subjects, but the enlargement was never gross and had disappeared by six weeks after the injection. Ten of the 111 A_2 and B_2 women with reactions had temperatures of 100°F-101°F (37.8°C-38.3°C). In 14% of these cases the pain had been severe enough to prevent sleep the previous night. Of the six C_2 women complaining at this stage, four had a local reaction similar to that experienced by the women who had received the oil-adjuvant toxoids, one had enlarged axillary lymph-nodes and one could not sleep for the pain. All the 117 women were given aspirin and examined daily for three days. By that time the pain had ceased, though in most cases the induration remained.

Five weeks after the injection two women reported to the hospital with abscesses. At this stage the regular visiting of all women in their own villages was commenced. By six weeks 35 women had abscesses, of which five had already discharged and healed spontaneoulsy, and many more had painful brawny indurations at the site of the injections that later developed into abscesses. The peak incidence of abscesses was between six and 10 weeks after injection. By the 14th week there had been 103 due to A_2 (31.5%) and 96 due to B_2 (28.9%). Two occurred with C_2 (0.6%). One of these abscesses was similar to those produced by A_2 and B_2, but the other, with marked lymph-node involvement, could have been due to incidental filarial lymph-adenitis or to a bacterial infection.

Twenty-four weeks after the injections arrangements were again made to see the women in their own villages. The results found were as follows:

No abnormality	A_2: 24.3%;	B_2: 28.4%
Swelling present	A_2: 8.5%;	B_2: 7.1%
Abscess (past or present)	A_2: 59.1%;	B_2: 55.3%
Not seen	A_2: 8.1%;	B_2: 9.1%

A comparatively small number of cases of painful indurated swellings seen at six weeks or later had resolved spontaneously by 24 weeks. These are included in the "no abnormality" figures. The figures for "abscess" at 24 weeks include those found by 14 weeks (most of which had healed) and all new abscesses developing after 14 weeks. The latter were less painful and smaller than those that arose earlier. There were no additional abnormalities in the C_2 group.

Many of the early abscesses were aspirated, up to 30 ml of yellow pus being collected in individual cases. All these required subsequent incision. Aerobic and anaerobic cultures of pus aspirated from 59 cases were sterile. No acid-fast bacilli were found. Follow-up of the women is continuing and the toxoids are being investigated to determine the cause of the reactions. It should be mentioned that both the nature of these reactions and the exudates are different from previously reported findings following the injection of vaccines containing oil adjuvants.

Physical stability of the adjuvant preparations A and B

The toxoids A, B, C and D for the pregnancy study were flown, packed in ice in an insulated box, from the USA to Maprik, with an intermediate stop of a few weeks in Port Moresby, where they were held at 4°C. The preparations were unchanged in physical appearance on arrival in Maprik; and they remained so for six months, stored in a refrigerator at approximately 4°C. Then, suddenly, it was found that in all the unused bottles of A and B toxoids the emulsions had broken down completely. (By that time the injections for the antibody studies had been completed.) At the NIH, emulsions of A and B, kept at 4°C under thermostatic control, broke down completely after one year. Also, bottles of emulsions A and B, when sent back to the USA uninsulated, by air freight, were found on arrival to have broken down completely, but they were unaltered when flown back insulated in the manner already described.

Very early in the study it was found that, once two or more doses had been withdrawn from a bottle, the emulsion remaining in it usually broke down within a few days, even though the bottle had been returned to the refrigerator soon after use. After correspondence with the manufacturer it became evident that a little alcohol, from the tincture of iodine or methylated spirits used to sterilize the rubber caps of the bottles, was seeping through the tiny needle-hole made in the caps when the first dose had been withdrawn. No woman was given any A or B emulsion that showed signs of breakdown, but the lack of a good alternative to alcohol for sterilization of the caps led to the modification, already described, in the method of allocating women to the toxoids; and later it influenced us towards pre-filled disposable syringes to immunize the non-pregnant women.

DISCUSSION

The pre-delivery titres of schedule E, considered together with the field success of this schedule in preventing neonatal tetanus, provide good evidence that maternal antitoxin levels in the last trimester of pregnancy of 0.01 unit/ml or less are protective to the infant, at least in the epidemiological circumstances prevalent at Maprik. (We can, of course, provide no evidence on the absolute minimum level that is protective.) The ratio of cord antitoxin to maternal antitoxin in humans is usually 1, according to Nicol et al. (1960) and M. S. Nasution (1962; quoted by S. G. Lo in a personal communication to M. P.), though these investigators recorded a fall slightly below this ratio in 35% and 19% of cases respectively. Infants appear to absorb little antitoxin from mothers' milk (Nattan-Larrier et al., 1927), and colostrum, which is not thought important immunologically in humans (Edsall, 1956), can be disregarded altogether in this study, since Abelam women always express it and discard it before the baby is introduced to the breast (Schofield & Parkinson, 1963). It therefore appears that a level in their blood at birth as low as, or even slightly lower than, 0.01 unit/ml has protected infants at Maprik.

At the time of the deliveries there was no significant difference between the mean antitoxin responses to the five toxoids or in the proportion of titres below 0.01 unit/ml. This is shown by the pre-delivery titres (Table 6) and by the mean titres attained between eight and 20 weeks after the initial toxoid injection (see the figure), at which time the majority of the deliveries occurred. The mean peak titres induced by A, B and C toxoids (Table 4), however, were higher and of longer duration than those induced by D and E toxoids. It can therefore

be concluded that A, B, C and D schedules were no less effective against neonatal tetanus than E had been. D had no apparent advantage over E. A, B and C were better than E from the standpoint of higher levels and longer duration of antitoxin. A and B had the greatest advantage in that only one injection was needed. However, A, B and similar oil-adjuvant toxoids cannot be considered for practical immunization purposes until the cause of the toxic local reactions induced by the second batch is understood. Recently McComb (1964) expressed doubt that alum toxoid is all that it is claimed to be, but there remains no doubt that, under the conditions of this pregnancy study, two doses, each of 5 Lf, of $AlPO_4$ toxoid were superior to three doses, each of 5 Lf, of the same toxoid without adjuvant.

C, which had minimal side-effects in the pregnancy study, remains the best of the five toxoids. It has three great advantages over the plain toxoids D and E:

(1) Only two injections of C are needed to achieve protective levels of maternal antitoxin, whereas three injections of D and E are required.

(2) As a consequence of this, less time is needed before delivery to immunize with C. In practice this is very important, since village women often do not register at ante-natal clinics until far advanced in pregnancy.

(3) Antitoxin levels produced by C, once they have reached their peak, remain at a protective level for a long time—at least a year on our evidence —while E and D titres decline to a statistically significant degree after the 18th week following the first injection. When C toxoid is used, therefore, there can be no danger of completing the immunization too early in pregnancy.

It seems likely that the two injections of C, whether or not given during pregnancy, may produce antitoxin levels high enough and long-lasting enough to provide protective titres during a subsequent pregnancy also. However, until this has been firmly established, safety requires one booster dose of C- or D-type toxoid in each subsequent pregnancy when risk of the disease still exists. E. Massall (in a personal communication to F.D.S., 1961) has stated that, during 1930-40, infants of Indochinese women temporarily living in the New Hebrides had a high rate of neonatal tetanus even though all the women had been fully immunized with three injections of plain toxoid before leaving Indochina. However, the disease no longer appeared after it was arranged that the women should receive one booster dose of plain toxoid in each pregnancy.

Maprik women fully immunized with plain toxoid in one pregnancy have been given one booster injection in subsequent pregnancies. Of about 400 neonates in this group one only developed tetanus; the mother had no detectable antibody in a serum specimen taken two months after the booster injection and 12 days after delivery. (Careful checking of the records revealed that she had received a full schedule E in pregnancy three years previously.) She appears, therefore, to have had no response to the antigen. Such cases are rare but may be commoner in the tropics than elsewhere. McGregor & Barr (1962) found that Gambian children protected all their lives against malarial parasitaemia showed significantly fewer titres below 0.01 unit/ml, in response to two injections of an aluminium-adjuvant toxoid, than comparable children not protected against malaria. Although all the Abelam have lived under malaria control since at least 1959 transmission of malaria continues at Maprik, and in 1963 the parasite rate in adult women was still 7% (Schofield, Parkinson & Kelly, 1964).

Apart from malaria there may be other unknown factors—infective, nutritional or genetic—that modify the responses to tetanus toxoid of people in a tropical environment. Such factors may have influenced the observed variation in the individual responses of the women, which was particularly marked in the A and B groups (Table 3). A few women showed only a minimum rise in titre—0.003 unit/ml——which was constant for over a year, while others showed a gradual rise, two of the titres reaching 30 units/ml. Each woman set her own pattern of response. The measured level of maternal antitoxin that has been demonstrated to be protective in Maprik provides useful guidance for other tropical areas, though it should be remembered that an exposure to *Clostridium tetani* greater than that obtaining in Maprik may require higher levels of maternal antitoxin.

One injection of C was found insufficient to induce as much as 0.001 unit/ml in seven of the nine women tested. However, these findings do not mean that other aluminium-compound adjuvant toxoids might not be protective in a one-injection schedule, particularly if given to women with better antitoxin responses or in circumstances resulting in exposure to smaller numbers of tetanus organisms

than at Maprik. Two injections of E also were insufficient; both in the original study and since, this schedule has been followed in Maprik by a neonatal tetanus incidence of 3%-4%.

Although the oil-adjuvant toxoids at present appear to have certain drawbacks—namely, their local toxic effects and the lack of stability of the emulsions under rural tropical conditions—it is hoped that more research will overcome these problems. Their two advantages—the long persistence of antitoxin and the one-injection dosage schedule—are potentially of great practical importance in many developing areas of the world. The findings described in this paper suggest, however, that at the present time, in those areas where scientific midwifery is absent, immunization with an aluminium-adjuvant toxoid, using two injections for primary immunization, is the best means of preventing neonatal tetanus.

POSTSCRIPT

Since the manuscript was submitted for publication, sera from 48 women obtained two years after the initiation of immunization have been titrated. The mean unitage/ml of the subjects who received toxoids A, B, C, D and E was 0.0631 (10), 0.0549 (12), 0.0478 (11), 0.0098 (7), and 0.0021 (8). The mean titres of the women immunized with toxoids A, B and E had decreased since one year after immunization while those of C and D had remained constant. These results support the recommendation to use aluminium-compound toxoid for maternal immunization to prevent neonatal tetanus.

The need for two injections of the toxoid C has been substantiated: only two of 12 additional subjects had detectable titres, 0.003 unit/ml. Combined, only four of 21 subjects have shown detectable titres prior to the second dose of toxoid.

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RÉSUMÉ

Les auteurs ont étudié l'efficacité respective de cinq préparations d'anatoxine tétanique, pour la prévention du tétanos du nouveau-né par immunisation maternelle, dans une population rurale de Nouvelle-Guinée.

L'immunisation de 134 femmes enceintes fut effectuée d'après l'un des schémas suivants: 1 injection d'anatoxine avec adjuvant huileux (deux préparations différentes); 2 injections d'anatoxine additionnée de phosphate d'aluminium; ou 3 injections d'anatoxine simple (2 préparations différentes). Des échantillons de sang prélevés périodiquement avant l'accouchement, et 6 mois et un an après la première injection servirent au titrage de l'antitoxine sérique.

Lors de la délivrance, les titres d'antitoxine ne différaient guère suivant les préparations utilisées: ils étaient en moyenne de 0,037 u/ml. Chez les femmes immunisées par l'anatoxine simple, les titres moyens diminuèrent de façon significative dès la 12e semaine après la 3e injection; un an après la 1re injection, ils étaient inférieurs à 0,01 u/ml. Les anatoxines avec adjuvant huileux donnèrent les titres les plus élevés (plus de 0,1 u/ml) et les plus persistants. Les titres obtenus par immunisation avec l'anatoxine additionnée de phosphate d'aluminium, un an après la 1re injection, étaient légèrement inférieurs aux titres obtenus avec les anatoxines en émulsion huileuse, mais supérieurs aux titres résultant de l'immunisation par les anatoxines simples, la différence, dans les deux cas, étant significative.

Les anatoxines avec adjuvant huileux ne provoquèrent, chez les femmes enceintes, que des réactions secondaires légères. Une enquête complémentaire effectuée avec d'autres préparations chez des femmes non enceintes montra cependant un nombre élevé de réactions locales intenses qui contre-indiquent, provisoirement, l'emploi de ces anatoxines pour la vaccination antitétanique de routine.

On a pu démontrer qu'un titre d'antitoxine de 0,01 u/ml chez la mère au moment de la délivrance assure une

prévention efficace du tétanos du nouveau-né. L'anatoxine additionnée de phosphate d'aluminium n'a entraîné que peu de réactions secondaires, et permis d'obtenir des titres d'antitoxine élevés, qui un an après la 1re injection étaient supérieurs au taux de protection.

Les auteurs estiment que l'immunisation maternelle par deux injections de cette préparation constitue actuellement la méthode la plus efficace de prévention du tétanos du nouveau-né, étant donné les caractéristiques épidémiologiques locales.

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