Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1970, 43, 469-478

Immunization Against Neonatal Tetanus in New Guinea

5. Laboratory Assayed Potency of Tetanus Toxoids and Relationship to Human Antitoxin Response

MARGARET PITTMAN,[1] ROBERT W. KOLB,[2] MICHAEL F. BARILE,[3] M. CAROLYN HARDEGREE,[3] EDWARD B. SELIGMANN, JR,[4] R. MACLENNAN,[5,6] & F. D. SCHOFIELD[5,7]

Previous papers in this series have shown that plain toxoids induced early primary antitoxin levels in women in New Guinea that were not significantly different from those induced by adsorbed toxoids but that at the end of 1 year the antitoxin levels differed significantly. Protective levels (not less than 0.01 unit/ml) induced by adsorbed toxoids persisted for more than 3 years. Results of laboratory assays of the toxoids reported in this paper show that per total human immunizing dose, the plain toxoids had 72 or less international units (IU) whereas the adsorbed toxoids had approximately 200 IU. The international "unitage" of these toxoids reflected the persistence of the human protective antitoxin level but not the early primary response. The assay results were in agreement with findings of other workers that the mouse as well as the guinea-pig may be satisfactory for potency assay of adsorbed toxoids. The need for determination of the international unitage of tetanus toxoids used in human studies and the confirmation of relationship of this value to persistence of antitoxin levels is emphasized.

One of the five objectives of the study on immunization against neonatal tetanus in New Guinea was to relate human antitoxin response to laboratory methods of potency evaluation and to the international standards for tetanus toxoids (MacLennan et al., 1965). The duration of the antitoxin responses of the women to two series of lots of toxoids up to 40 or 54 months after the first primary injection has been reported (Hardegree et al., 1970). The international "unitages" of the 2 adsorbed and 2 of the plain toxoids reported in this paper were assayed during our participation in the collaborative assay of a lot of dried adsorbed toxoid later designated as the International Standard for Tetanus Toxoid (Adsorbed) (WHO Expert Committee on Biological Standardization, 1967). A preliminary report of the relationship of the international "unitage" of these 4 field study toxoids to the antitoxin levels of the women during 24 months after immunization was made by Pittman et al. (1967). The current report gives the details of the laboratory assays and extends the time of observation on the relationship of the international "unitage" to the persistence of the protective antitoxin levels of the women injected with the $AlPO_4$-adsorbed toxoid to 40 or 54 months after immunization. Refinement in calculations and new data account for slight differences between certain values published in 1967 and those in the present paper.

[1] Chief, Laboratory of Bacterial Products, Division of Biologics Standards, National Institutes of Health, US Department of Health, Education, and Welfare, Bethesda, Md., USA 20014.

[2] Chief, Protocol and Release Section, Office of Director, DBS, NIH.

[3] Laboratory of Bacterial Products, DBS, NIH.

[4] Chief, Laboratory of Control Activities, DBS, NIH.

[5] Division of Medical Research, Department of Public Health, Port Moresby, Territory of Papua and New Guinea.

[6] Present address: Tulane Medical School, New Orleans, La., USA 70112.

[7] Present address: Faculty of Medicine, University College, Nairobi, Kenya.

MATERIALS AND METHODS

Toxoids

Table 1 lists the field study toxoids, designated by letters A to E, and Table 2 indicates the 4 toxoids

TABLE 1

QUALITATIVE ASSAY OF THE TETANUS TOXOIDS FOR MINIMUM POTENCY VALUE

Toxoid code	Type of toxoid	Guinea-pig immunizing dose		Results	
		Lf	ml	So/n [a]	units/ml [b]
D_1	Plain	5	0.5	13/13	
D_2	Plain	5	0.5	12/12	
E	Plain	20	1.0	15/15	
DBS 2A [c]	Plain	5	0.55	13/13	
C_1	$AlPO_4$	5	0.5		>16
C_2	$AlPO_4$	5	0.5		>10–<20
A_1	Drakeol	5	0.25		>16
A_2	Drakeol	5	0.25		>10–<20
B_1	H-24	5	0.25		>16
B_2	H-24	5	0.25		>10–<20
DBS 1J [c]	Alum	5	0.55		8

[a] Number of guinea-pigs that survived without symptoms/number challenged with 10 MLD toxin.

[b] Pooled sera from guinea-pigs immunized with adjuvant toxoids.

[c] DBS house-reference toxoids.

TABLE 2

COMPOSITION AND HUMAN DOSAGE OF TETANUS TOXOIDS

Toxoid code	Adjuvant (mg Al/ml)	Purity (Lf/mg PN)	Lf/ml	Human doses	
				No. of doses (0.5-ml)	Total Lf
C_1	1.1	612	10	2	10
D_1	None	612	10	3	15
C_2	1.1	278	10	2	10
E	None		20 [a]	3 [a]	60
SSI6S [b]	1.0		12.5		
IS A [c]	0.31/10 Lf				
IS B [d]	None	1420			
DBS 2A [e]	None	690	9		

[a] Kf was approximately 24 hours, single human dose was 1.0 ml.

[b] Statens Seruminstitut toxoid, $Al(OH)_3$-adsorbed.

[c] International Standard for Tetanus Toxoid (Adsorbed) dried; 34 Lf, 120 IU and 80 mg/vial. Before drying each vial contained 0.2 ml of 1% $Al(OH)_3$. Calculated as Al_2O_3 there would be 0.31 mg Al per 10 Lf toxoid.

[d] International Standard for Tetanus Toxoid, Plain, dried; 420 Lf and 833 IU/vial.

[e] DBS house-reference, Massachusetts Lot 208.

that were assayed for potency and the reference toxoids. Table 2 also gives certain properties of the toxoids and the human doses of the field study toxoids. Additional information about the latter was given in the reports by MacLennan et al. (1965) and Hardegree et al. (1970). Information on purity of 3 toxoids was not available to us.

Animals

Mice of the NIH strain, approximately 15 g, were used for the active quantitative assays and General Purpose as well as NIH mice, 15 g, were used for the passive antitoxin titration tests. The Hartley strain of guinea-pigs was used in the two types of potency test employed.

Laboratory assays

(1) Qualitative tests were performed as specified in the US Department of Health, Education, and Welfare (*Minimum Requirements: Tetanus Toxoids, December 15, 1952*). The procedures in brief were as follows: (*a*) For plain toxoids, each of 12 or more guinea-pigs (350 g±50 g) was given subcutaneously one-third of the volume of the total human immunizing dose and 6 weeks later was challenged subcutaneously with 10 MLD of toxin; (*b*) For adsorbed toxoids each of 6 guinea-pigs (500 g±50 g) was given one-half of the volume of the total human immunizing dose, subcutaneously, and was bled 6 weeks later. The antitoxin titre of the pooled serum was measured by use of guinea-pigs in relation to the US Standard Tetanus Antitoxin.

(2) The international "unitage" of the toxoids was titrated by the methods used in the collaborative assay of the proposed International Standard for Tetanus Toxoid (Adsorbed).[1] Guinea-pigs (400 g–430 g) were used to assay both the plain and the adsorbed toxoids, and mice were used to assay only the adsorbed toxoids. The potency of each toxoid under test was estimated in relation to the appropriate international standard toxoid (plain or adsorbed).

(*a*) Guinea-pigs in groups of 12 were given a single subcutaneous injection (0.5 ml) of 1 of 4 or 5 two-fold dilutions of a toxoid in saline and challenged 4 weeks later with a subcutaneous injection of 200 μg (20 MLD–40 MLD) of the DBS control toxin P in a volume of 2.0 ml. The animals

[1] J. D. van Ramshorst & T. K. Sundaresan (1965) Collaborative assay of a proposed standard for tetanus toxoid, adsorbed (unpublished document, WHO/BS/788.65).

were observed for 5 days. Three days prior to challenge, 2 ml–3 ml of blood were withdrawn per guinea-pig by cardiac puncture for antitoxin titration. The antitoxin level of the guinea-pigs' sera per toxoid dilution was determined by the mouse-neutralization test that was used for titrating the antitoxin level of the sera of the New Guinea women (Barile et al., 1970). For tests no. 1, 2 and 3, the sera from the 12 guinea-pigs per toxoid dilution were divided into 2 groups of 6 each and an equal portion of serum from each guinea-pig per group was pooled. The geometric mean antitoxin titre of the two serum pools was recorded as the antitoxin response to the particular dilution of toxoid. For tests no. 4, 5 and 6, the sera from the 12 guinea-pigs per toxoid dilution were pooled. For the purposes of calculation, an antitoxin titre of <0.001 unit/ml was assigned a value of 0.0003 unit/ml.

(*b*) Mice in groups of 16 were injected subcutaneously with the dilutions of the adsorbed toxoids prepared for immunization of the guinea-pigs. Dilutions were used on the day of preparation. After an interval of 3 weeks the mice were challenged subcutaneously with 80 μg (20 MLD–40 MLD) of toxin P in a volume of 0.5 ml. The mice were observed for 5 days.

RESULTS

Qualitative potency assay

Table 1 shows that one-third of a human dose of the plain toxoids completely protected guinea-pigs challenged with 10 MLD and that one-half of the total immunizing dose of the adjuvant toxoids induced more than 10 units/ml of antitoxin. Exact antitoxin levels were not determined. The responses to toxoids A_1, B_1 and C_1 were >16 units/ml and responses to toxoids A_2, B_2 and C_2 were >10 units/ml and <20 units/ml. At 6 weeks, when the animals were bled, there was no difference between the responses to the oil-emulsified and the adsorbed toxoids. Barile et al. (1970) showed that, as in the human study, the maximum response of guinea-pigs to the oil-adjuvant toxoids was later than 6 weeks. The antitoxin response of the guinea-pigs to the adsorbed toxoids C_1 and C_2 was significantly higher than the US specified minimum level of not less than 2 units/ml,

" Unitage " assay of the International Standard for Tetanus Toxoid (Adsorbed)

In the WHO collaborative study, we performed simultaneously 3 guinea-pig tests of the proposed International Standard for Tetanus Toxoid (Adsorbed) (IS A) and of the International Standard for Tetanus Toxoid (Plain) (IS B). Three additional tests of each toxoid were performed to determine the potency of some of the field-trial toxoids. The results of each test of each standard are given in Table 3. From the combined test results the calculated value for IS A was 1.57 IU/mg. A value of 1.5 IU/mg was estimated from the results of the participants in the collaborative study and was

TABLE 3
GUINEA-PIG POTENCY ASSAY OF PROPOSED INTERNATIONAL TETANUS TOXOID, ADSORBED

Standard	Dose	Test no.[a]						Total[a] (S/n)	ED_{50}
		1	2	3	4	5	6		
IS B	2.0 IU	11/12	9/11	8/8	9/10	12/12	10/10	59/63	0.666 IU
Plain	1.0 IU	10/12	6/9	8/10	12/12	6/11	10/12	52/66	0.026 [b]
	0.5 IU	1/12	2/12	6/11	3/11	8/12	2/12	22/70	3.7466 [c]
	0.25 IU	0/12	0/11	0/12	1/11	0/12	2/12	3/70	
IS A	2.0 mg	12/12	11/11	12/12	11/11	12/12	12/12	70/70	0.423 mg
Adsorbed	1.0 mg	11/12	11/12	10/11	11/12	10/11	12/12	65/70	0.025 [b]
	0.5 mg	9/12	8/12	9/12	0/12	7/12	7/12	40/72	3.6586 [c]
	0.25 mg	1/12	1/12	1/11	0/10	5/12	7/12	15/69	1.57 IU/mg [d]
	0.125 mg	0/12	0/12	0/11	2/12			2/47	

[a] Results given as no. of survivors/no. challenged.
[b] Standard error.
[c] Slope.
[d] The World Health Organization defined the International Unit (IU) as the activity contained in 0.6667 mg (1.5 IU/mg).

TABLE 4
GUINEA-PIG ASSAY OF PLAIN TETANUS TOXOIDS

Toxoid	Dose (ml)	Test no. [a]			Total [a] (S/n)	ED_{50}	
		4	5	6		IS B [b]	Toxoid
IS B		See Table 3					
D_1	0.1	9/11	11/11	9/12	29/34	0.5831 IU	0.02996 ml
	0.05	8/11	11/12	7/12	26/35		0.046 [c]
	0.025	7/9	6/12	4/11	17/32		2.4288 [d]
	0.0125	3/12	0/12	2/11	5/35		(19.5 IU/ml)
	0.0062	0/12	0/12	0/12	0/36		
E	0.1	12/12	11/11		23/23	0.5831 IU	0.02422 ml
	0.05	11/11	12/12	11/11	34/34		0.026 [c]
	0.025	7/11	7/11	3/12	17/34		7.3406 [d]
	0.0125	0/12	1/12	0/12	1/36		(24.1 IU/ml)
		Test no.					
		1	2	3			
DBS 2A	0.08		12/12	12/12	24/24	0.7635 IU	0.02665 ml
	0.04	10/12	8/12	11/11	29/35		0.031 [c]
	0.02	4/12	2/11	2/12	8/35		5.1526 [d]
	0.01	1/12	0/9	1/12	2/33		(28.6 IU/ml)

[a] Results given as no. of survivors/no. challenged.
[b] ED_{50} of IS B calculated from combined results of concurrent tests.
[c] Standard error.
[d] Slope.

adopted by the WHO Expert Committee on Biological Standardization (1967). We have used the value assigned by WHO to estimate the international " unitage " of the adsorbed toxoids assayed in this study.

" Unitage " of the plain toxoids

Table 4 gives the results of the titration of the protective response of guinea-pigs to 3 plain toxoids and the estimated IU/ml of the toxoids. The potencies of the 3 toxoids D_1, E and DBS 2A, estimated from the ratio of the ED_{50} of the standard to the ED_{50} of the toxoid, were 19.5 IU/ml, 24.1 IU/ml, and 28.6 IU/ml, respectively. Since the field-trial toxoids D_1 and E were administered in total immunizing doses of 1.5 ml and 3.0 ml, respectively, the number of IU administered would have been 29 and 72. (The latter was given as 36 IU by Pittman et al. (1967). The error was due to calculation of the total dose as 1.5 ml instead of 3.0 ml.)

The ratios of the Lf to the IU content of toxoids IS B, D_1, E and DBS 2A (see Table 2 for Lf content) were 0.50, 0.51, 0.83 and 0.32, respectively. One Lf in each toxoid represented 1.98 IU, 1.95 IU, 1.2 IU and 3.18 IU. The relation between the antitoxin titre of the guinea-pig sera and the international " unitage " of the toxoid will be presented below.

" Unitage " of the adsorbed toxoids

Guinea-pig assay. Table 5 gives the dose response of guinea-pigs to the adsorbed standard IS A and 3 adsorbed toxoids. The estimated potencies of the toxoids C_1, C_2 and SSI6S, calculated as for the plain toxoids, were 198 IU/ml, 230 UI/ml and 296 IU/ml, respectively. Hence the total human immunizing doses of 1.0 ml of C_1 and of C_2 would have been

TABLE 5
GUINEA-PIG ASSAY OF ADSORBED TETANUS TOXOIDS

Toxoid	Dose (ml)	Test no. [a]				Total [a] (S/n)	ED_{50}	
		2	3	4	6		IS A [b]	Toxoid
IS A		See Table 3						
C_1	0.02	12/12	12/12	12/12	12/12	48/48	0.4336 mg	0.003281 ml
	0.01	11/11	12/12	10/12	10/11	43/46	0.6504 IU	0.033 [c]
	0.005	7/11	11/11	5/12	12/12	35/46		3.5032 [d]
	0.0025	1/12	6/12	4/12	6/12	17/48		(198 IU/ml)
	0.0012	0/12	0/12		2/12	2/36		
		Test no.						
			3	4	5			
C_2	0.02		12/12	11/11	11/11	34/34	0.4504 mg	0.002938 ml
	0.01		12/12	11/12	10/11	33/35	0.6756 IU	0.038 [c]
	0.005		11/12	9/11	11/11	31/34		3.5365 [d]
	0.0025		0/12	4/12	5/12	9/36		(230 IU/ml)
	0.0012		0/12	4/12	1/12	5/36		
		Test no.						
		1	2	3				
SSI6S	0.01	10/10	12/12	12/12		34/34	0.4357 mg	0.00221 ml
	0.005	11/12	10/12	12/12		33/36	0.6536 IU	0.034 [c]
	0.0025	6/12	6/11	10/11		22/34		4.4171 [d]
	0.0012	1/11	1/11	2/12		4/34		(296 IU/ml)

[a] Results given as no. survivors/no. challenged.
[b] ED_{50} of IS A caculated from combined results of concurrent tests.
[c] Standard error.
[b] Slope.

198 IU and 230 IU, respectively. The values are not significantly different at the 95% confidence level. Our value for SSI6S was significantly higher than that obtained by others in the collaborative study. The reason has not been determined.

The ratios of the Lf to IU content of adsorbed toxoids IS A, C_1, C_2 and SSI6S (see Table 2 for Lf content) were 0.283, 0.051, 0.043 and 0.042. One Lf in each toxoid represented 3.53 IU, 19.8 IU, 23.0 IU and 23.7 IU, respectively. The antigenicities of the toxoids C_1, C_2 and SSI6S were similar and they were approximately 10 times more potent than plain toxoids IS B and D_1. On the other hand, the antigenicity of the dried standard toxoid IS A was only about one-sixth of the antigenicity of the other 3 adsorbed toxoids and it was only 1.8 times more antigenic per Lf than the plain toxoid standard IS B. Standard IS A contains less aluminium than the other adsorbed toxoids and it was subjected to freeze-drying, which might have had an effect on the physical state of the precipitated particles. From the standpoint of potency assaying, it is pertinent to note that the slopes of the dose–response curves of the 4 adsorbed toxoids were not significantly different.

TABLE 6
MOUSE ASSAY OF ADSORBED TETANUS TOXOIDS

Toxoid	Dose	Test no. [a]			Total [a] (S/n)	ED_{50}	Ratio [b] (International units)
		1	2	3			
IS A	2.0 mg	12/16	16/16	15/16	43/48	0.8208 mg	1.0
	1.0 mg	8/16	15/16	8/16	31/48	0.0413 [c]	(1.5 IU/mg)
	0.5 mg	3/16	9/16	2/16	14/48	3.6523 [d]	
	0.25 mg	0/16	0/16	0/16	0/48	0.3488 Lf	
C_1	0.02 ml	15/16	16/16	16/16	47/48	0.0072 ml	114
	0.01 ml	14/16	11/16	10/16	35/48	0.0291 [c]	(171 IU/ml)
	0.005 ml	8/16	3/16	1/16	12/48	4.446 [d]	
	0.0025 ml	1/16	0/16	0/16	1/48	0.072 Lf	
C_2	0.02 ml	15/16	16/16	15/16	46/48	0.0049 ml	167.5
	0.01 ml	15/16	16/16	15/16	46/48	0.0217 [c]	(251 IU/ml)
	0.005 ml	13/16	11/16	2/16	26/48	4.1520 [d]	
	0.0025 ml	0/16	3/16	1/16	4/48	0.049 Lf	
SSI6S	0.02 ml	16/16	16/16	16/16	48/48	0.0068 ml	120.7
	0.01 ml	11/16	14/16	8/16	33/48	0.0348 [c]	(181.0 IU/ml)
	0.005 ml	7/16	9/16	2/16	18/48	4.5065 [d]	
	0.0025 ml	0/16	0/16	0/16	0/48	0.056 Lf	

[a] Results given as no. of survivors/no. challenged.
[b] ISA = 1.0
[c] Standard error.
[d] Slope; standard error of each slope was close to 0.50.

TABLE 7
COMPARISON OF POTENCY ASSAYED BY USE OF GUINEA-PIGS AND MICE

Toxoid	Potency (IU/ml)		Ratio (guinea-pig = 1.0)
	Guinea-pig	Mouse	
C_1	198	171	0.86
C_2	230	251	1.09
SSI6S	296	181	0.61

Mouse assay. Table 6 gives the relative potency (mg equivalent of IS A) and the unitage of the 3 adsorbed toxoids calculated from the combined results of the 3 tests. Table 7 shows that the mouse-potency values of toxoids C_1 and SSI6S were lower than the guinea-pig values while the mouse value of toxoid C_2 was equal to the guinea-pig value. The greatest discrepancy was obtained with toxoid SSI6S with a ratio of only 0.61. This value also disagrees with the ratio obtained in the collaborative study.

Relation of antitoxin response to the international "unitage" of the toxoid

Guinea-pigs. By inspection of the data given in Table 8, it appears that 1.0 IU$\pm$0.5 induced 0.001 unit/ml of antitoxin and that 3.0 IU$\pm$1.0 induced 0.01 unit/ml of antitoxin irrespective of whether the toxoid was plain or adsorbed.

Women. The primary antitoxin responses of the New Guinea women were titrated 2 weeks after the last dose of toxoid. Relative to the first injection, this time was 14 weeks (3 months) for the plain toxoids (3 doses) and 8 weeks for the adsorbed toxoid C_1 (2 doses). For the latter toxoid, Table 9

TABLE 8

ANTITOXIN RESPONSE OF GUINEA-PIGS RELATIVE TO THE INTERNATIONAL "UNITAGE" OF THE TOXOID IMMUNIZING DOSE

Toxoid [a]	Dose	Challenge assay			Antitoxin response (units/ml)
		No. of survivors/ no. challenged	%	IU/dose	
IS B	4.0 IU	22/22	100		0.01
	2.0 IU	59/63	94		0.0045
	1.0 IU	52/66	79		0.0008
	0.5 IU	22/70	31		<0.001
D_1	0.2 ml	20/21	95	3.9	0.003
(19.5)	0.1 ml	29/34	85	1.95	0.003
	0.05 ml	26/35	74	0.98	0.00146
	0.025 ml	17/32	53	0.49	0.00068
E	0.2 ml	12/12	100	4.82	0.03
(24.1)	0.1 ml	23/23	100	2.41	0.01
	0.05 ml	34/34	100	1.20	0.00146
	0.025 ml	17/34	50	0.60	<0.001
DBS 2A	0.08 ml	24/24	100	2.29	0.003
(28.6)	0.04 ml	29/35	83	1.14	0.001
	0.02 ml	8/35	23	0.57	0.00045
IS A	3.0 IU	70/70	100		0.0146
	1.5 IU	65/70	93		0.0056
	0.75 IU	40/72	56		0.00046
C_1	0.02 ml	48/48	100	3.96	0.0056
(198)	0.01 ml	43/46	94	1.98	0.0056
	0.005 ml	35/46	76	0.99	0.001
	0.0025 ml	17/48	35	0.50	<0.001
C_2	0.02 ml	34/34	100	4.6	0.0215
(230)	0.01 ml	33/35	94	2.3	0.0046
	0.005 ml	31/34	91	1.15	0.00145
	0.0025 ml	9/36	25	0.58	<0.001
SSI6S	0.02 ml	24/24	100	5.92	0.0464
(296)	0.01 ml	34/34	100	2.96	0.0215
	0.005 ml	33/36	92	1.48	0.0046
	0.0025 ml	22/34	65	0.74	<0.001

[a] Figures in parentheses give the assayed potency in IU/ml.

TABLE 9

COMPARISON OF THE INTERNATIONAL "UNITAGE" OF THE TOXOID WITH THE DURATION OF HUMAN ANTITOXIN RESPONSE (units/ml) [a]

Toxoid		Months after first injection					
Code	IU [b]	3	6-8	12	24	40	54
C_1	198	0.08 (23) 0.07 [c] (14)	0.100 (8)	0.041 (18)	0.048 (11)	0.032 (9)	0.0075 (6)
C_2	230		0.021 (38)	0.059 [d] (15)	0.018 [e] (12)	0.027 (13)	
D_1	29	0.03 (20)	0.015 (9)	0.008 (15)	0.006 (7)		
E	72	0.05 (16)	0.010 (10)	0.004 (9)	0.002 (8)		

[a] Figures in parentheses indicate the no. of women's sera titrated.
[b] No. of IU per total immunization (guinea-pig assay).
[c] Primary response 2 weeks after second injection (8 weeks after first injection).
[d] 16 months.
[e] 28 months.

shows that the 8-week and the 3-month titres were similar (0.07 unit/ml and 0.08 unit/ml). Although the primary responses to the two plain toxoids and the one adsorbed toxoid were not significantly different, the ranking of the antitoxin levels from low to high were in the same order as the ranking of the international " unitages " of the toxoids. A significant relationship between international " unitage " of the toxoid and human response was shown by the duration of the antitoxin levels. Within less than a year the mean antitoxin titres of the women vaccinated with the plain toxoids had dropped below the protective level (0.01 unit/ml) whereas the mean titres of those vaccinated with the 2 adsorbed toxoids remained above the protective level for more than 3 years. In general, persistence of the mean protective antitoxin level response to toxoids having 72 IU or less per total dose was less than 1 year, whereas persistence of a protective level to toxoids having 200 IU per total dose was longer than 3 years.

DISCUSSION

Several studies have shown that there is a positive relationship between the laboratory-assayed potency of tetanus toxoid and human response. Scheibel et al. (1968) reviewed these studies, presented additional information on the relationship and listed the conditions which they considered essential before the human dose requirements could be expressed in terms of animal potency.

Our study introduces a new parameter for consideration. It is the persistence of the protective antitoxin level relative to the " unitage " of the immunizing toxoids. Plain toxoid D_1 contained 29 IU in the total primary immunization of 1.5 ml and adsorbed toxoid C_1 contained 198 IU in the total dose of 1.0 ml. Three months after the initial injection the mean antitoxin titres of the pregnant women were 0.03 unit/ml and 0.07 unit/ml respectively. The difference was not statistically significant. Within 6 months the mean titre of the D_1 women was significantly lower than that of the C_1 women and by the end of 1 year the former was below the protective titre of 0.01 unit/ml, whereas the mean titre of the C_1 women remained above the protective level for more than 40 months but not for 54 months. With the other plain toxoid E, the total number of IU was 2.5 times that of toxoid D_1, and the early response was 0.05 unit/ml and not different from responses to D_1 or C_1. However, at the end of 6 months the mean titre of toxoid E women had dropped to 0.01 unit/ml. The more rapid decline of the antitoxin level in the toxoid E women than in the toxoid D_1 women might be related to the unusual 24-hour Kf value of toxoid E. Schofield, Tucker & Westbrook (1961) found that the third dose of toxoid E was needed in the last trimester of pregnancy to prevent neonatal tetanus. Hardegree et al. (1970) suggested that the higher response to toxoid C_1 than to toxoid C_2 might have been due to administration during pregnancy. Nevertheless, the mean titre of toxoid C_2 women remained above the protective level for 40 months.

With the New Guinea women the potency of the toxoid reflected the persistence of the protective antitoxin level better than did the antitoxin level 2 weeks after the last primary injection. The latter time has been used in many studies to study the human response to toxoids. The comparison of the antitoxin response relative to IU content in our study was made between plain and adsorbed toxoids and the presence of an adjuvant was no doubt a significant factor in prolonging the protective antitoxin level. From a practical standpoint the results emphasize the inferiority of plain toxoids having 72 IU or less per total dose relative to adsorbed toxoids having about 200 IU.

It should be noted that 200 IU of adsorbed toxoid induced lower titres in the New Guinea women after 2 primary injections than has been reported in the literature. For example, Ikić (1960) reported titres of 0.70 unit/ml, 0.49 unit/ml and 0.03 unit/ml 2 weeks after the second injection of total doses of 168 IU, 36 IU and 7 IU of an adsorbed toxoid; and Scheibel & Larsen (1962) reported average mean titres of two groups each of 0.24 unit/ml and 0.44 unit/ml *(our calculation)* 1 year after 2 injections of a total dose of 250 IU of a toxoid.

There is a need for additional field studies to confirm the relationship of the international " unitage " to persistence of antibody levels. On the other hand, it appears definite that adsorbed toxoid containing around 200 IU, administered in 2 doses, does provide a protective antibody level that persists for 3 years. Although the primary antibody responses of the New Guinea women were lower than the responses reported in the literature, a 10- to 100-fold booster response was obtained when a third injection was given (Hardegree et al., 1970).

Our guinea-pig potency assay results, like those of other investigators, showed the suitability of this animal for determinations of international " unitage ". The ratio of the mouse-assayed potency to the guinea-pig-assayed potency was not different from 1.0 for toxoids C_1 and C_2. However, the ratio was different from 1.0 with toxoid SSI6S due to the high guinea-pig potency. The latter was inconsistent with the value observed in the collaborative study. We believe that investigations are needed to determine the factors which influence mouse-potency assay results. If the cheaper mouse assay could be satisfactorily standardized, it would encourage the specification that potency be expressed in IU.

Koerber & Mook (1943) showed marked differences in response of mice obtained from different sources. Ipsen (1953) showed a 15-fold difference in immunizability of strains of mice. Csizmas (quoted by Pittman, 1967) also found marked differences between strains of mice. The mouse is not satisfactory for assay of an adsorbed toxoid against a plain toxoid standard (Greenberg, 1953; Cohen et al., 1959). Greenberg (1953), however, considered that the mouse was the test-animal of choice for fluid preparations. The suitability of the mouse for adsorbed toxoids tested against an adsorbed standard has been demonstrated (Cohen et al., 1959). With current information about different strains of mice, another look at the feasibility of the use of the mouse for assay of plain as well as adsorbed toxoids may be warranted.

Our comparison of the guinea-pig assayed potency of a dose of toxoid with the antitoxin response of the guinea-pig showed, in general, but not precisely, a direct relationship as also shown by Scheibel (1957).

The observation of the relationship between the potency of the toxoids and the duration of the human protective antitoxin level may have particular significance in those situations where the number of injections that can be made is limited. It is necessary to determine whether this observation is applicable only to plain toxoids *versus* adsorbed toxoids and whether it is applicable to adsorbed toxoids differing in their international " unitage ". This study emphasizes, as have others (Scheibel et al., 1969), the need for the potency of tetanus toxoids to be expressed in IU. Only with this information can quantitative comparisons be made between the human responses obtained with different toxoid preparations.

ACKNOWLEDGEMENTS

We are grateful to Mrs Norma Duffin, Mrs Marion Grabowski and Mr Donald Riggs, Laboratory of Bacterial Products, and Mr H. T. Aylor and Mr O. L. Green, Laboratory of Control Activities, for technical assistance.

RÉSUMÉ

IMMUNISATION CONTRE LE TÉTANOS DU NOUVEAU-NÉ EN NOUVELLE-GUINÉE: 5. ACTIVITÉ DES ANATOXINES TÉTANIQUES MESURÉE AU LABORATOIRE ET SES RAPPORTS AVEC LA PRODUCTION D'ANTITOXINE CHEZ L'HOMME

On a évalué chez la souris et le cobaye l'activité, exprimée en unités internationales (UI), de deux anatoxines simples et de deux anatoxines adsorbées sur phosphate d'aluminium utilisées en Nouvelle-Guinée pour la prévention du tétanos du nouveau-né.

Chez le cobaye, les titres d'antitoxine suscités par les anatoxines simples et adsorbées sont en général fonction de l'activité, en UI, des différentes préparations. Cette activité est responsable de la persistance plus ou moins grande des titres d'antitoxine assurant une protection chez les femmes immunisées, mais est sans rapport avec la réponse immunitaire primaire qui ne varie guère selon les cas. Les titres moyens d'antitoxine, chez les femmes immunisées à l'aide d'anatoxines simples, dont l'activité est de 72 UI ou moins, s'abaissent au-dessous du seuil de protection en moins d'un an, tandis qu'ils persistent à un niveau assurant l'immunité pendant plus de trois ans chez les femmes immunisées par des anatoxines adsorbées dont l'activité est de 200 UI.

L'importance de cette relation entre l'activité UI et la persistance de titres protecteurs d'antitoxine est particulièrement évidente lorsque le nombre des injections d'anatoxine doit être limité pour l'une ou l'autre raison. Il est indispensable que l'activité des anatoxines utilisées chez l'homme soit exprimée en unités internationales.

REFERENCES

Barile, M. F., Hardegree, M. C. & Pittman, M. (1970) *Bull. Wld Hlth Org.*, **43**, 453

Cohen, H., Ramshorst, J. D. van & Tasman, A. (1959) *Bull. Wld Hlth Org.*, **20**, 1133

Greenberg, L. (1953) *Bull. Wld Hlth Org.*, **9**, 837

Greenberg, L. & Benoit R. (1956) *J. Amer. med. Ass.*, **160**, 108

Greenberg, L., Gibbard, J. & Morrell, C. A. (1945/46) *Bull. Hlth Org. L. o. N.* **12**, Extract No. 19, 365

Greenberg, L., Morrell, C. A. & Gibbard, J. (1943) *J. Immunol.* **46**, 333

Hardegree, M. C., Barile, M. F., Pittman, M., Scofield, F. D., MacLennan, R. & Kelly, A. (1970), *Bull. Wld Hlth Org.*, **43**, 439

Ikić, D. (1960) *Immunity in humans and guinea pigs vaccinated with different doses of tetanus vaccine.* In: *Proceedings, International Symposium on Microbiological Standardization*, Opatija, 1960, p. 415

Ipsen, J., Jr. (1953) *J. Immunol.*, **70**, 171

Koerber, W. L. & Mook, G. E. (1943) *J. Immunol.*, **46**, 411

MacLennan, R., Schofield, F. D., Pittman, M., Hardegree, M. C. & Barile, M. F. (1965) *Bull Wld Hlth Org.*, **32**, 683

Pittman, M. (1967) *Mouse strain variation in response to pertussis vaccine and tetanus toxoid.* In: *International Symposium on Laboratory Animals, London, 1966*, Karger, Basel, p. 161 *(Symposia Series in Immunobiological Standardization*, vol. 5*)*

Pittman, M., Barile, M. F. & Hardegree, M. C. (1967) *Comparison of human response with laboratory assays of tetanus toxoid with and without adjuvants.* In: *International Symposium on Adjuvants of Immunity, Utrecht, 1966*, Karger, Basel, p. 295 (*Symposia Series in Immunobiological Standardization*, vol. 6)

Scheibel, I. (1957) *Control of the Danish combined diphtheria-tetanus vaccine with special reference to the assaying of its antigenic potency in international units.* In: *Proceedings of the Third International Meeting of Biological Standardization, Opajita, 1957*, p. 153

Scheibel, I., Chen, B.-L., Bentzon, M. W. & Zia, K. (1968) *Acta pathol. microbiol. scand.*, **73**, 115

Scheibel, I. & Larsen, S. O. (1962) *Immunization of adults with two tetanus vaccines of different antigenic potency when tested in guinea pigs.* In: *Proceedings of the 7th International Congress of Microbiological Standardization, London, 1961*, E. & S. Livingstone, Ltd, London, p. 416

Schofield, F. D., Tucker, V. M. & Westbrook, G. R. (1961) *Brit. med. J.*, **2**, 785

WHO Expert Committee on Biological Standardization (1967) *Wld Hlth Org. techn. Rep. Ser.*, No. **361**, p. 18