ACTIVE IMMUNIZATION AGAINST TETANUS III. RESULTS OF A FIVE YEAR STUDY

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INTRODUCTION

The first batches of purified fluid and purified adsorbed tetanus toxoids (vaccines) ever to be produced in Burma were tested in human volunteers for relative efficacies (antigenic or immunizing potencies) and safety. The adsorbed toxoid was found to be far superior to the fluid as an immunizing agent; both vaccines were safe for human use and a tentative immunization schedule was proposed after nearly three years study (Maung Maung Mya *et al.*, 1973 a, b). The study was continued for more than two years with the addition of two more groups of B.P.I. volunteers to the first three groups. This work was started in December 1969 and carried through till October 1974.

MATERIALS AND METHODS

Fluid tetanus toxoid batch No. E 69001, purified by ammonium sulphate contained 10 Lf antigen per ml and 2700 Lf per mg of protein nitrogen. Adsorbed tetanus toxoid batch No. F 69002, prepared from the same stock concentrate as the fluid toxoid, contained 20 Lf antigen/ml and 2 mg/ml of aluminium phosphate gel as adjuvant. The single dose of fluid toxoid was 1.0 ml I.M while that of the adsorbed toxoid was 0.5 ml I.M. These were the first batches of purified and adsorbed toxoids to be produced in the country by B.P.I. and were used throughout most of this study. Fluid tetanus toxoid batch No. E 72022 was also used to give a booster dose to Group I and II fluid toxoid immunized subjects as the original batch had all been used up.

All the volunteers were healthy, adult, B.P.I. workers of both sexes, ranging in age from 18 to 60 years; 75% of whom were 22 to 44 years old. They had no history of active immunization against tetanus and except for Group V subjects, all volunteers blood samples were taken from before the first dose to confirm their non-immune status. Blood sampling was done at different time intervals after immunization and antibody titrations were performed using the toxin neutralization test in mice (Glenny and Stevens, 1938) at the L+/1000 level. The minimal protective level of serum antitoxin has been taken as 0.01 unit per ml (Looney *et al.*, 1956; McComb, 1964).

RESULTS

Data are presented as mean antitoxin titres, the number and percentage of protected subjects at various time of blood sampling according to study group and the type of vaccine received. The data have been statistically analysed and the probability in terms of significance of difference in means and significance of difference in proportions between fluid and adsorbed vaccine recipients are also shown. Data reported previously are included to provide continuity and to give a comprehensive picture of the nature and course of immunological response.

Table 1 shows that 27 out of 29 (93%) adsorbed vaccine subjects were protected 4 years and 2 months (day 1764) after basic immunization. This indicates the need for a booster dose as the protective index had been 100% previously. Most fluid vaccine sub-

Table 1

Mean antitoxin titres in Group 1 subjects after active immunization against tetanus.

	Day	Absorbed vaccine recipients			Fluid vaccine recipients			Significance in	
		Mean u/ml	No. Protect/tested	% protect	Mean u/ml	Protect/ tested	% protect	means (P)	proportions
B + V 1st dose	1	<0.0012	0/65	0	<0.0012	0/35	0	—	—
V 2nd dose	60	—	—	—	—	—	—	—	—
B	140	0.312	57/57	100	0.054	25/25	100	0.001	—
V 3rd dose	234	—	—	—	—	—	—	—	—
B	337	0.435	31/31	100	0.001	17/21	81	0.001	0.194
B	406	0.270	10/10	100	0.068	9/12	75	>0.01<0.02	0.06
B	594	0.123	32/32	100	0.028	11/20	55	0.001	0.001
B	714	0.088	12/12	100	0.011	3/10	30	0.001	0.001
B	950	0.061	25/25	100	0.011	5/15	33	0.001	0.001
V 4th dose	1264	—	—	—	—	—	—	—	—
B	1764	0.074	27/29	93	0.122	9/10	90	>0.1>0.2	0.764

jects given a booster dose 2 years and 10 months after basic immunization (day 1264) responded well. However, the protection rate 500 days later (day 1764) is only 90% which implies either that the interval of 2 years and 10 months after basic immunization is too long for adequate response to a booster or that the period of protection afforded by the booster is less than 1 year and 4 months. Since there is no significant difference between the mean antitoxin levels or the protective indices of the two types of vaccines, the superiority of the adsorbed vaccine which can match the fluid without benefit of a booster, is reaffirmed.

Table 2 shows that 9 out of 9 (100 %) adsorbed vaccine recipients were protected 3 years and 4 months (day 1500) after basic immunization supporting our previous assumption of a 3 to 4 year protective period. 83% of fluid vaccine subjects were protected 1 year and 4 months after a booster dose (day 1500) given 2 years after basic immunization (day 1000). This probably indicates the necessity of giving a booster to fluid vaccine subjects before an interval of 2 years after basic immunization. As in Table 1, adsorbed vaccine has elicited similar mean antitoxin titres and protective index on day 1500 without the benefit of a booster dose thus

Table 2

Mean antitoxin titres in Group II after immunization against tetanus.

	Day	Adsorbed vaccine recipients			Fluid vaccine recipients			Significance in	
		Mean u/ml	No. Protect/tested	% protect	Mean u/ml	Protect/ tested	% protect	means (P)	proportions
B + V 1st dose	1	<0.0012	0/30	0	<0.0012	0/20	0	—	—
B + V 2nd dose	28	0.0027	2/26	8	0.0012	0/15	0	—	0.194
B + V 3rd dose	283	0.036	19/21	90	0.005	4/15	27	0.001	0.0002
B	723	0.251	22/22	100	0.06	9/13	69	0.001	0.06
V 4th dose*	1000	—	—	—	—	—	—	—	—
B	1500	0.10	9/9	100	0.15	5/6	83	>0.2<0.3	0.37

* Fluid vaccine subjects only; B = Blood sampling; V = Vaccination; (P) = probability.

Table 3

Mean antitoxin titres in Group III subjects after immunization with adsorbed tetanus toxoid.

	Day	Mean u/ml	S.E.	No. protected/ No. tested	% protected
B + V 1 st dose	1	<0.0012	—	0/35	0
B + V 2nd dose	42	0.006	0.001	7/24	29
B	67	0.74	0.124	18/18	100
B + V 3rd dose	200	0.034	0.003	25/29	86
B	730	0.089	0.023	20/20	100
B	1527	0.061	0.160	13/15	87

Dose of adsorbed vaccine = 0.5 ml (10 Lf) I.M.; Minimal protective level = 0.01 u/ml.
B + V = Blood sampling and vaccination. B = Blood sampling only; V = Vaccination only.

giving additional evidence of its superior efficacy.

Table 3 shows that 13 out of 15 (87%) adsorbed vaccine recipients were protected, 3 years and 3 months after basic immunization (day 1527). A booster dose is indicated and the result supports our contention that a booster be given 3 to 4 years after the basic course.

Table 4 shows that 13 out of 17 (76%) of Group IV adsorbed vaccine subjects were protected 266 days (day 308) after the second dose of vaccine, indicating that a shorter interval is required to obtain full protection. The data for Groups II and III are also very similar giving the indication that the third dose of adsorbed vaccine should be given before the fifth month after the second dose, if we intend to protect 100% of normal vaccine responders. However, the response to the third dose in all three groups was very satisfactory. Table 4 also shows the superiority of adsorbed vaccine over fluid vaccine by the immunological responses of Group IV adsorbed vaccine and Group V fluid vaccine subjects.

DISCUSSION

Except for a very limited use in paediatrics, the fluid tetanus vaccine is no longer used in Hungary (L. Erdos, pers comm., 1967). The fluid vaccine is no longer used clinically

Table 4

Antitoxin titres in Group IV and Group V Subjects after immunization.

	Day	Group IV(absorbed vaccine)			Group V (fluid vaccine)			Significance in means (P)	proportions
		Mean u/ml	No. protect/tested	% protect	Mean u/ml	No. protect/tested	% protect		
B + V 1st dose	1	<0.0012	0/32	0	—	0/54	0	—	—
B + V2nd dose(IV)	42	0.0032	2/23	9	—	—	—	—	—
V 2nd dose (V)	46	—	—	—	—	—	—	—	—
V 3rd dose (V)	200	—	—	—	—	—	—	—	—
B + V 3rd dose(IV)	308	0.032	13/17	76	—	—	—	—	—
B(V)	500	0.182	28/26	100	0.046	38/45	84	0.001	0.07
B(IV)	840	—	—	—	—	—	—	—	—
B (V)	1195	—	—	—	—	—	—	—	—
B(IV)	1505	0.193	9/9	100	0.026	11/23	48	0.001	0.007

(IV) = Group IV; (V) = Group V

in the U.S.A. (L. Levine, pers. comm., 1970), and has been discontinued in Australia (CSL Medical Handbook, 1970). When primary immunization is required, Alum Precipitated Vaccine, Purified Toxoid Aluminium Phosphate (PTAP), or Purified Toxoid Aluminium Hydroxide (PTAH) is used (B.P., 1968). All this shows that the adsorbed tetanus vaccine is now the vaccine of choice for active immunization.

The B.P.I. cannot as yet produce sufficient adsorbed tetanus vaccine for the whole country, hence fluid vaccine will have to be used for some time to come. In developing countries tetanus is one of the main causes of morbidity and mortality. The aim here should be to actively immunize persons at risk in the shortest time possible based on a regimen which will give all immunologically normal people high protective antitoxin titres and to maintain them with appropriate booster doses. Only then will these immunized people be protected even without benefit of a booster dose, which is very uncertain in suburban and rural areas, in the event of exposure to tetanus.

The results shown here confirm all of our previous findings and assumptions. The superiority of adsorbed over fluid tetanus vaccine as an immunizing agent is confirmed. Based upon this study following immunization schedules is recommended as being the best. For both vaccines the second dose should be given 4 to 6 weeks after the first; the third dose for fluid vaccine should be 3 to 4 months and for adsorbed vaccine should be 4 to 5 months after the second dose; a booster dose every 1 to 2 years for the fluid and every 3 to 4 years for the adsorbed vaccine after completing the basic course, is necessary. A booster should be given when a fully immunized person is at risk, but he would still be protected without one if the recommended immunization schedules given here have been strictly followed.

SUMMARY

This is the first time in Burma that tetanus toxoids (purified and adsorbed) have been tested in over 250 non-immune adult volunteers and studied for a period of nearly five years. The safety and efficacy of these toxoids have been assessed by immunological, statistical and clinical methods. Both toxoids were found to be safe. The adsorbed toxoid was far superior to the fluid toxoid as an immunizing agent and optimum immunization regimens are proposed and presented.

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