Rev. Inst. Med. trop. São Paulo
12(1):46-54, janeiro-fevereiro, 1970

UDC 615.371
616.981.551

SINGLE DOSE IMMUNIZATION AGAINST TETANUS. PROMISING RESULTS IN HUMAN TRIALS

R. VERONESI [1], A. CORRÊA [2] and D. ALTERIO [3]

SUMMARY

The potential value of a tetanus vaccine capable of immunizing in a single dose is stressed, in view of the high mortality of the disease in developing countries, as well as the lower cost, greater acceptability and considerable simplification of the administration of mass immunization campaigns which would result.

The immune responses to single doses of different toxoids in different concentrations, with and without additional adjuvants, have been assessed and, with high concentrated aluminium hydroxide adsorbed toxoids, 100% protective levels have been achieved in a small group when tested 4 weeks and 8 months after immunization.

The concentrated toxoids, with or without adjuvants were well tolerated.

INTRODUCTION

The importance of developing an effective single dose antitetanus vaccine is obvious in view of the estimated 200,000 deaths caused by tetanus which annualy occur throughout the world. Most of the world's population is not immune against tetanus and, probably, several years will elapse before this picture changes, particularly in developing countries.

The considerable human and material resources required by developing countries to eradicate preventable diseases can be substantially reduced if we can provide safe and effective vaccines and devise methods of administration which render mas immunization programmes simple and economically feasible. If longterm immunity to tetanus can be achieved with one, instead of the currently required three doses, it could permit release of Public Health Personnel for other essential programmes.

Results obtained with laboratory animals have encouraged the belief that protection against experimental tetanus can be conferred by a single dose of potent toxoid.

During the II Internacional Conference on Tetanus (Bern, 1966) the need for a single dose method of inducing long term immunity to tetanus was stressed by a number of participants. ECKMANN [2] in the official inauguration of the meeting said: — "Within the same very few years there have been almost universal efforts to simplify active immunization, as it became clear that whole population could only be successfully vaccinated by the means of a single dose method". SCHOFIELD [17] who had already confirmed the effectiveness of a single dose, for protection of the umbilical tetanus amongst New Guinea tribes, said: — "Obviously, for mass immunization against neonatal tetanus, any adjuvant toxoid which

(1) Professor at the Medical Sciences School, Santos, São Paulo (Faculdade de Ciências Médicas de Santos. São Paulo) and the São Paulo University Medical School, Brazil

(2) Chief, Department of Biological Controls, "Instituto Pinheiros", São Paulo, Brazil

(3) Assistant Physician, Department of Tropical and Infectious Diseases, "Hospital das Clínicas", São Paulo University Medical School, Brazil

enabled primary immunization to be achieved with only one injection would be very advantageous, especially if it produced long-lasting, well-sustained antitoxin levels which would make booster injections unnecessary for many years to come". PATEL [12] agreed with Schofield that the single dose would be desirable in India. In the "Panel of Immunology" held in the IInd International Conference on Tetanus we [23] expressed the following opinion: — "It must be emphasized the difficulties facing a mass inoculation campaign in under-developed countries and feel that one-shot injection method could help overcome them. A single dose of an adequate vaccine strengthened with a special adjuvant, is able to confer a high degree of protection against tetanus and millions of individuals should benefit from it in countries where it is very difficult to inject a large population with 2 or 3 doses of toxoid in a short period of time. People immunized with a single shot should be advised to have a booster dose of toxoid whenever necessary. With this precaution full protection could be afforded and mortality would decrease significantly".

Finally, MÉRIEUX [10] stated: — "The fundamental reason for the relative failure of tetanus prevention by classical methods lies, in our opinion, in their complexity. The three first inoculations of antigen, at intervals of several weeks, have to be followed by booster injections. If such a program is practicable, and often enough practised in countries which enjoy a high standard of sanitation, it is, at present, impracticable in the very numerous developing countries, where there is a lack of medical personnel, an embryonic social organization and an inadequate communications system. Also, we have been led to consider antitetanus vaccination with the aim of simplifying it and putting it within the reach of all, even of those who live in the most backward countries".

SMITH [18] had already shown that a single dose of potent toxoid was able to protect mice and guinea-pigs when inoculated with tetanus spores 10 to 12 days later. In man, protective levels of antibody were not observed until a month after a single dose. It was shown also that during the first 24 months following the injection of the single dose of commercial toxoid (adsorbed with aluminium phosphate) the incidence of the umbilical tetanus dropped [11]. Thus, there was evidence that immunity could be achieved with a single dose, although not in 100% of cases, with toxoids of normal strength (± 20 Lf). Using toxoids of higher potency (100-200 Lf) it should be possible to obtain more reliable results.

In 1965, MACLENNAN et al. [9], in studying the potency of several adjuvants, found that the incorporation of the tetanus toxoid in mineral oil emmulsion afforded better results *as a single dose* than were obtained with two doses of aluminium phosphate adsorbed toxoid or three doses of fluid toxoid. However, when tested by SCHOFIELD [17] in New Guinea, this adjuvant caused a high incidence of sterile abscesses at the inoculation site. In previous work [21] we showed promising possibilities in immunization with single dose of potent toxoids whose antigenicity was enhanced by the addition of special adjuvants. In these experiments we compared, in laboratory animals, the antigenic effectiveness of several preparations of tetanus toxoid with adjuvants. It was concluded that best results were achieved by adding Adjuvant 65, a water-in-oil emulsion of peanut oil, aluminium monosterate and Arlacel A [13, 24]. We investigated the efficacy of this preparation in human beings and achieved 100% responses [21].

In 1968, CODJIA [1], in Dakar, duplicated Mérieux's experiments and confirmed the practicability of immunization with concentrated toxoids, although he observed some undesirable reactions to toxoid concentrated four hundred fold.

In the last two years there have been several efforts to improve one shot tetanus immunization. This paper reports our recent results.

Three possible approaches to a satisfactory single dose method of tetanus immunization are: 1) The use of concentrated toxoids; 2) The addition of further adjuvants to commercial toxoids; 3) A combination of concentrated toxoids with adjuvants.

The concentrated toxoid method was successfully employed by MÉRIEUX [10] and, later on, by CODJIA [1], using products with concentrations 400 to 500 times greater than the commercial product available in France. According to EDSALL [3] the possibility of

immune-paralysis secondary to concentrated toxoids would only occur with doses much higher than those employed by the French workers. It is known also, that toxoid in massive single dose is satisfactorily tolerated by man and laboratory animals [14].

The use of commercial toxoid with addition of adjuvants was successfully employed by Schofield in New Guinea [17], as well as by ourselves [21] in Brazil. It is known that a good adjuvant may enhance the potency of a commercial toxoid up to as much as 1,000 times [19].

The third method combines concentration of the toxoid with the use of adjuvants. At least on theoretical grounds, the combination of a concentrated antigen with a good adjuvant should be the safest way of achieving the goal of the one shot tetanus immunization, once a proper balance of the components is attained. This was our aim in the present trial.

MATERIAL AND METHODS

The toxoids employed were provided by the Wellcome Research Laboratories, Beckenham, England and by the "Instituto Pinheiros, São Paulo, Brazil".

The toxoids from the Wellcome Research Laboratories were all adsorbed on aluminium hydroxide, at 1.3 mg Al^{+++}/ml, and provided at three concentrations. A fourth preparation, at the middle concentration, contained and additional adjuvant, "Arquad", a dialkyl quaternary ammonium salt [4, 7, 16]. The four toxoids were as follows:

Aluminium hydroxide adsorbed tetanus toxoid, batch PX 225, 20 Lf/0.5 ml; Aluminium hydroxide adsorbed tetanus, toxoid, batch PX 213, 50 Lf/0.5 ml; Aluminium hydroxide adsorbed tetanus toxoid plus Arquad, batch PX 214, 50 Lf/0.5 ml; Aluminium hydroxide adsorbed tetanus toxoid, batch PX 226, 200 Lf/0.5 ml.

The toxoid from the "Instituto Pinheiros, São Paulo" contained 4-6 units/ml (Minimum Requeriments Tetanus toxoid unit), was alum precipitated, and incorporated in Adjuvant 65 in the ratio of 3 parts toxoid to 1 part adjuvant. We did not follow the proportions (1:1) originally described by WOODHOUR et al. [24] in order to have a less thick product. Strong agitation of the vial just before injecting made the product an emulsion.

The total number vaccinated was 350. The injections were given intramuscularly, except for those receiving the vaccine by pressure jet (Dermojet) as indicated under results. The subjects were followed up for a period of 4 weeks to observe the development of any clinical reactions which could be ascribed to the vaccination.

A random sample was bled before, 4 weeks and 8 months after vaccination for the estimation of circulating antitoxin. Antitoxin titrations were performed at the Wellcome Research Laboratories, Beckenham and at the "Instituto Pinheiros, São Paulo".

RESULTS

In presenting the results, individuals showing a titre of 0.01 u/ml or greater, before vaccination, were regarded as immune and have been excluded from the tables.

Four weeks after a single dose of 20 Lf adsorbed toxoid, 57% (4 out of 7) had reached a titre of 0.01 u/ml or higher (Table I (a)). In a previous trial, when we gave 4-5 units alum precipitated toxoid (Pinheiros), we found 50% rose to protective levels 3 weeks after as single dose, although 100% reached protective levels after a second dose [19].

Therefore, we can conclude that commercial toxoids, whose strength varies widely [5], are not potent enough to be used safely in a single dose and are only suitable for 2- or 3-dose schedules.

Table I, (b) to (e), shows the distribution of responses to increased amounts of adsorbed tetanus toxoid. As the dose of toxoid is increased there is a steady increase in the proportion of people with protective antitoxin levels until, with doses of 200 and 400 Lf, 100% protection is achieved. Later blood samples taken 8 months later showed that 100% of those individuals injected with 100 Lf absorbed tetanus toxoid also had titers $\geq$ 0.01 u/ml. The results shown in Tables I and IV served as screening for the choice of the best toxoid to be used as a single-shot Tetanus vaccine. So, we bled, 8 months later, only those individuals pertaining to groups *c*, *d* and *e*

from Table I and groups *a* and *b* from Table IV, because these were the groups showing the more convincing results. With the relatively small numbers involved these results cannot be relied upon, but the progressive improvement is encouraging.

TABLE I

Distribution of tetanus antitoxin titres 4 weeks after a single dose of tetanus toxoid adsorbed on Aluminium hydroxide

	u/ml < ⩾	0.01	0.01 — 0.02	0.02 — 0.05	0.05 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	2 — 5	no. of subjects	Geom. mean titre	% ⩾ 0.01 u/ml
Tetanus toxoid, Adsorbed													
Lf dose	vol.												
(a) * 20 Lf	0.5 ml	3	2	1			1				7	⩽ 0.017	57
(b) * 50 Lf	0.5 ml	3	1	2				1			7	⩽ 0.021	57
(c) 100 Lf	1.0 ml	2		1			2	1		1	7	⩽ 0.11	64
(d) 200 Lf	0.5 ml		3	1		1	2			1	8	⩽ 0.069	100
(e) 400 Lf	1.0 ml		1	2		1	3	1			8	⩽ 0.096	100

* These two groups (*a* and *b*) were not followed up 8 months later

TABLE II

Distribution of tetanus antitoxin titres 8 months after a single dose of tetanus toxoid adsorbed on Aluminium hydroxide

	u/ml < ⩾	0.01	0.01 — 0.02	0.02 — 0.05	0.05 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	2 — 5	5 — 10	no. of subjects	Geom. mean titre	% ⩾ 0.01 u/ml
Tetanus toxoid, Adsorbed														
Lf dose	vol.													
(c) 100 Lf	1.0 ml		1	1	2	1			1		1	7	⩽ 0.174	100
(d) 200 Lf	0.5 ml			1	1	2	1					5	⩽ 0.11	100
(e) 400 Lf	1.0 ml				1	2	1	1	1			6	⩽ 0.14	100

Table III show the results achieved with a single dose of one M.R.T. unit (Pinheiros toxoid) in Adjuvant 65. Twenty-three out of 26, 89%, showed antitoxin titres $\geq$ 0.005 u/ml 5 weeks later, and 15 out of 26, 58%, $\geq$ 0.01 u/ml. Sera with titres $\geq$ 0.005 u/ml prior to vaccination have been excluded. Unfortunately it was not possible to follow up serologically this group at the end of the 8th month.

TABLE III

Distribution of tetanus antitoxin titres 5 weeks after a single dose of tetanus toxoid incorporated in Adjuvant 65

u/ml < ⩾	0.005	0.005 — 0.01	0.01 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	no. of subjects	Geom. mean titre	% ⩾0.005 u/ml
Tetanus Toxoid, fluid in Adjuvant 65	3	8	7	4	1	2	1	26	⩽ 0.040	89

When a further adjuvant, the quaternary ammonium compound Arquad, was incorporated with the toxoid adsorbed on aluminium hydroxide we found that the antigenicity was further enhanced (Table IV). The proportion of subjects showing protective titres at the 30th day was of 64% with the 50 Lf toxoid and 56% with the 100 Lf. However, at the end of the 8th month, titrations of serums of the same group demonstrated that 100% of them were showing titers $\geq$ 0.01 u/ml (Table V). Also it was observed that among the individuals of Table IV the titers were substantially higher than those of Table I.

We concluded that the potent Aluminium hydroxide adsorbed toxoids, with and without Arquad, posses a long-acting antigenic power which answer for the later appearance of protective levels of circulating antibodies. Analysis of Table I gave us the impression that 100 Lf toxoids (without Arquad) is the minimum *safe-potency-limit* to be considered for the purpose of single-shot immunization against Tetanus. When further adjuvant was added (Arquad) the *safe-potency-limit*, as shown by our experiments, was the 50 Lf toxoid. Further experiments are needed to establish the *real* minimum *safe-potency-limit* for such products when used for the purpose of one-shot immunization against Tetanus.

In all the groups receiving Aluminium hydroxide adsorbed vaccines, but especially those containing Arquad, there are individuals with very high titres, side by side with others with low titres. The addition of Arquad has served to exaggerate this difference, two individuals showing titres of 5-10 u/ml and two of 20-50 u/ml. At present we have no explanation to offer for the wide gulf between the good and poor responders (previous experience with toxoids?).

Three groups of people received tetanus toxoid by pressure jet ("Dermojet") injection which delivers the toxoid part intradermally and part subcutaneously. The results are set out in Table VI. The proportion of responders was low, and the method is probably unsuitable for single dose immunization. This group was not followed-up 8 months later.

TABLE IV

Distribution of tetanus antitoxin titres 4 weeks after a single dose of tetanus toxoid adsorbed on Aluminium hydroxide with added Arquad

		u/ml < ⩾	0.01	0.01 — 0.02	0.02 — 0.05	0.05 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	2 — 5	5 — 10	10 — 20	20 — 50	50	no. of subjects	Geom. mean titre	% ⩾ 0.01 u/ml
Tetanus toxoid, Adsorbed with added Arquad																		
Lf dose	vol.																	
(a) 50 Lf	0.5 ml		5	2	1			1		2	1	1		1		14	⩽ 0.10	64
(b) 100 Lf	1.0 ml		4		1				2			1		1		9	⩽ 0.13	56

TABLE V

Distribution of tetanus antitoxin titres 8 months after a single dose of tetanus toxoid adsorbed on Aluminium hydroxide with added Arquad

		u/ml < ⩾	0.01	0.01 — 0.02	0.02 — 0.05	0.05 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	2 — 5	5 — 10	no. of subjects	Geom. mean titre	% ⩾ 0.01 u/ml
Tetanus toxoid, Adsorbed with added Arquad															
Lf dose	vol.														
(a) 50 Lf	0.5 ml				3	2	2	3		2			12	⩽ 0.168	100
(b) 100 Lf	1.0 ml			1		2		1		1		1	6	⩽ 0.263	100

TABLE VI

Distribution of tetanus antitoxin titres 4 weeks after a single dose of tetanus vaccine given intradermally by pressure jet

			u/ml < ⩾	0.01	0.01 — 0.02	0.02 — 0.05	0.05 — 0.1	0.1 — 0.2	0.2 — 0.5	0.5 — 1	1 — 2	2 — 5	no. of subjects	Geom. mean titre	% ⩾ 0.01 u/ml
Tetanus Vaccine, Adsorbed by "Dermojet"															
	Lf dose	vol.													
(a)	10 Lf	0.1 ml		4									4	⩽ 0.0071	0
(b)	40 Lf	0.1 ml		11	1		1					1	14	⩽ 0.014	21
containing Arquad															
(c)	10 Lf	0.1 ml		5	1			1					7	⩽ 0.012	30

Reactions to the toxoids

Neither with the adsorbed toxoids in high concentration (200 and 400 Lf/ml) nor with the additional adjuvants used (Arquad and Adjuvant 65) did we see reactions other than those commonly occurring with the usual commercially available toxoids.

The following side effects were observed:

1. Toxoid adsorbed on aluminium hydroxide:
   a) 20 Lf: 1 subject with local pruritus, 2 with headache on the day of inoculation.
   b) 50 Lf: 1 case of local pain at the site of inoculation lasting 48 hours.
   c) 100 Lf: no reactions.
   d) 200 Lf: no reactions.
   e) 400 Lf: 1 case of local pain at the site of inoculation and in the homolateral axillary area lasting 4 days, subsiding afterwards.
   f) 10 Lf by Dermojet: 1 case of local pain lasting 1 day, and 1 case of local pruritus and inflammation lasting 5 days.
2. Toxoid incorporated in Adjuvant 65:
   No noteworthy side effects.
3. Adsorbed toxoids containing Arquad:
   a) 50 Lf: 2 cases of local pain 24 and 48 hours after inoculation respectively, and 1 case of pain and local inflammation (20 mm diam.) starting 3 days after inoculation and lasting a further 3 days.
   b) 100 Lf: no noteworthy side effects.
   c) 10 Lf by Dermojet: 2 cases of local pruritus and erythema (30 mm diam.) at the site of inoculation lasting 3 and 5 days respectively.

VERONESI, R.; CORRÊA, A. & ALTERIO, D. — Single dose immunization against tetanus. Promising results in human trials. *Rev. Inst. Med. trop. São Paulo* 12:46-54, 1970.

It is well known that tetanus toxoids are reasonably well tolerated [5], even when high concentrations are injected [1,2]. With our 400, 200 and 100 Lf/ml toxoids we did not meet the reactions frequently observed by CODJIA [1] when highly concentrated toxoids were injected. The reactions found by SCHOFIED [14] when using a mineral oil emulsion adjuvant were not observed, neither with Adjuvant 65 nor with Arquad.

In conclusion, we regard the present results as indicating that single dose immunization is possible with potent Aluminium hydroxide adsorbed toxoids and the addition of other adjuvants can raise responses still further.

RESUMO

Imunização antitetânica com vacina de dose única. Resultados promissores em experimentações humanas.

Foi realçada a importância em se produzir uma vacina antitetânica de dose única, em face dos elevados índices de mortalidade por tétano nos países em desenvolvimento. Tal vacina seria altamente desejável por permitir sensível barateamento do custo dos programas de imunização em massa, maior rapidez na execução dos programas além de melhor receptividade popular. As respostas imunológicas às diferentes doses dos diferentes toxóides testados demonstraram que com toxóides altamente concentrados, adicionados ou não de outros adjuvantes, são obtidos níveis protetores de antitoxinas circulantes em 100% dos casos. As titulagens de anticorpos foram realizadas um e oito meses após a vacinação com dose única dêsses toxóides.

A experimentação em sêres humanos demonstrou perfeita tolerância aos toxóides altamente concentrados, adicionados ou não dos novos adjuvantes testados.

ACKNOWLEDGMENTS

We hereby acknowledge the assistance of the following people to prepare this paper: Dr. David Gall and Dr. A. H. Griffith from the Wellcome Research Laboratory for providing us with the toxoids, the dosage of antitoxins and also for the judicious comments and corrections made during the preparation of this paper; Prof. Ciro Camargo and the "Instituto Pinheiros" in São Paulo for the preparation of special toxoids and Mr. José Tavares, technician of the same Institute for the dosage of antitoxins; Dr. Manuel C. Fonseca, from the Medical Department of the Police Guard in São Paulo for the permission to carry out the immunization in his institution; Dr. Thomaz Camanho Netto, Chief Med. Department of Souza Cruz Co. for the permission to immunize the employées in the Company.

REFERENCES

1. CODJIA, D. N. — *L'immunisation antitetanique par injection unique d'anatoxine concentrée.* Thesis. Dakar, 1968.

2. ECKMANN, L. — Chairman's opening address. *In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 17-18.

3. EDSALL, G. — *Panel and discussions on Immunology. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 225-236.

4. GALL, D. — The adjuvant activity of aliphatic nitrogenous bases. *Immunology* 11: 369-386, 1966.

5. GREENBERG, L. — The relative immunizing efficiency of Tetanus toxoid preparations. *Bull. W. H. O.* 12:761-768, 1955.

6. GRIFFITH, A. H. — *Clinical reactions to Tetanus toxoid. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 299-306.

7. HEGEDUS, L.; RETHY, L.; JUHASZ, V. P. & BACSKAI, L. — Comparative study of the adjuvant activity of endotoxin of biological origin with Arquad 2HT. *Kiserl. Osvostud.* 20:139-142, 1967.

8. HEINIG, A. — Experimentelle untersuchungen über den entritt der immunität nach einmaliger Tetanus-Schutzipflung. *Arch. Vet. Med.* 8:394-403, 1954.

9. MacLENNAN, R.; SCHOFIELD, F. D.; PITTMAN, M.; HARDEGREE, M. C. & BARILE, M. F. — Immunization against tetanus in New Guinea. Antitoxin response of pregnant women to adjuvant and plain toxoids. *Bull. W.H.O.* 32:683-697, 1965.

10. MERIEUX, Ch. — *Single shot primovaccination against Tetanus by needleless injectors. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 423-436.

11. NEWELL, K. W.; DUEÑAS LEHMAN, A.; LE BLANC, D. R. & GARCEZ, O. N. — *Tetanus neonatorum: epidemiology and prevention. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 261-270.

12. PATEL, J. C. — *Panel and discussions on Immunology. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 323-329.

13. PECK, H. M.; WOODHOUR, A. F.; METZGAR, D. P.; McKINNEY, S. E. & HILLEMAN, M. R. — New metabolizable immunologic adjuvant for human use. II — Short term animal toxicity tests. *Proc. Soc. Exp. Biol. Med.* **116**:523-527, 1964.

14. PRÉVOT, A. R. — *Proposition of a worldwide experiment to erradicate Tetanus. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, 337-340.

15. RAYNAUD, M.; LEMETAYER, E.; NICOL, L. & TURPIN, A. — Action preventive specifique précoce de L'anatoxine tétanique employée a doses massives. *Congrés Internat. Microbiol.* (Rome) **2**:784, 1953.

16. RETHY, L.; HEGEDUS, L.; JUHASZ, V. P. & BACSKAI, L. — A study of the immunological adjuvant activity of certain quaternary ammonium bases. *Kiserl. Orvostud.* **20**:135-138, 1967.

17. SCHOFIELD, F. D. — Maternal immunization against Tetanus of the newborn. *In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 271-274.

18. SMITH, J. W. G. — Tetanus prophylaxis. *Brit. Med. J.* **1**:373, 1964.

19. UNGAR, J. — *Panel and discussions on Immunology. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 323-329.

20. VAN HEYNINGEN, W. E. & MILLER, P. A. — The fixation of tetanus toxin by ganglioside. *J. Gen. Microbiol.* **24**:107-119, 1961.

21. VERONESI, R.; CORRÊA, A.; ZUCCAS, W. A. & LOPES, O. S. — Tetanus immunization with a single dose of vaccine. Importance and possibilities for mass immunization programs. Preliminary report on experimental studies. *Rev. Inst. Med. trop. São Paulo* **8**:83-88, 1966.

22. VERONESI, R.; GUIDOLIN, R.; CORRÊA, A.; MARTINEZ, A. A. & MEIRA, D. A. — Toxóide tetânico e gamaglobulina antitetânica humana. Como, porque e para que? *J. Brasil. Med.* **10**:549-552, 1966.

23. VERONESI, R. — *Panel and discussions on Immunology. In Principles on Tetanus.* Hans Huber Publ., Bern & Stutgart, 1966, pp. 323-329.

24. WOODHOUR, A. F.; METZGAR, D. P.; STIM, T. B.; TYTELL, A. A. & HILLEMAN, M. R. — New Metabolizable Immunologic Adjuvant for human use. I — Development and animal immune response. *Proc. Soc. Exp. Biol.* **116**:516-523, 1964.

Recebido para publicação em 30/6/1969.