Factors affecting the immunogenicity and potency of tetanus toxoid: implications for the elimination of neonatal and non-neonatal tetanus as public health problems

V. Dietz,[1] A. Galazka,[2] F. van Loon,[1] & S. Cochi[3]

An estimated 400 000 deaths occur annually from neonatal tetanus (NT). In 1989 WHO adopted the goal of eliminating NT as a public health problem worldwide. To achieve this, and to control non-neonatal tetanus (non-NT), WHO recommends that newborns be passively protected at birth by the antepartum administration of at least two doses of tetanus toxoid (TT) to their mothers and that all children subsequently receive at least three doses of diphtheria–tetanus–pertussis (DTP) vaccine. For this strategy to be effective, the TT used must be immunogenic. Potential factors that may affect TT immunogenicity need to be evaluated if NT is to be eliminated and if non-NT is to be controlled. Although data are conflicting, concurrent malarial infection may decrease the immune response to TT; however, malarial chemoprophylaxis may enhance the immune response. Malnutrition does not appear to affect immunogenicity; nevertheless, one study suggests that vitamin A deficiency is associated with an impaired immune response. Although it has been postulated that placental transfer of tetanus antibody is impaired in African women, a survey of the published literature suggests that this is not the case. Freezing TT has been shown to decrease its potency, but its impact on immunogenicity needs more evaluation.

Introduction

In 1989 the World Health Assembly adopted a resolution calling for the global elimination of neonatal tetanus (NT) as a public health problem.[a] WHO identified two strategies to reach this goal:[b] achieve and maintain high coverage levels with tetanus toxoid (TT) by giving at least two doses to women; and ensure access to clean birth practices. The recommendation to administer TT during pregnancy, or more comprehensively to all women of childbearing age, is based on studies demonstrating that the offspring of women given two doses of properly spaced TT are protected against the development of NT (1–3). To maximize the effect, the first two doses of TT should be given at intervals of at least 4 weeks, with the second dose being given at least 4 weeks prior to delivery (4, 5).[c] WHO currently recommends that women complete a series of five doses of TT. Furthermore, to control non-neonatal tetanus (non-NT) WHO recommends that all children complete a primary vaccination series that includes three doses of diphtheria–tetanus–pertussis (DTP) vaccine. In 1994 WHO estimated that 46% of pregnant women had received two doses of TT and that 79% of 1-year-

[1] Medical Epidemiologist, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, GA, USA. Currently at: Division of Parastic Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Burfurd Highway NE, Atlanta, GA, USA. Requests for reprints should be sent to Dr Dietz at the latter address.

[2] Medical Officer, Expanded Programme on Immunization, Global Programme for Vaccines and Immunization, World Health Organization, Geneva, Switzerland.

[3] Chief, Polio Eradication Activity, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, GA, USA.

[a] Resolution 42.32. *Handbook of resolutions and decisions of the World Health Assembly and the Executive Board*, Vol. III, third edit. (1985–92). Geneva, World Health Organization, 1993.

[b] *Prevention of neonatal tetanus through immunization.* Unpublished document WHO/EPI/GEN/86.9 Rev 1, 1986 (available upon request from Global Programme for Vaccines and Immunization, World Health Organization, 1211 Geneva 27, Switzerland).

Reprint No. **5756**

[c] **Galazka A.** *Immunization of pregnant women against tetanus.* Unpublished WHO document EPI/GEN/83/5, 1983 (available upon request from Global Programme for Vaccines and Immunization, World Health Organization, 1211 Geneva 27, Switzerland).

olds had been protected with three doses of DTP (6).[d] Thus, elimination of NT may be unrealistic unless activities are greatly accelerated to increase coverage.

Although the administration of TT prevents NT, the disease has reportedly developed in infants whose mothers have received two or more doses of TT (7, 8). Such observations, although anecdotal, raise questions about the potency of TT (7–9) and about what factors can affect TT immunogenicity and what is their operational significance for both the elimination of NT and for the eventual control of non-NT. There is also concern about whether women in the tropics have a lower rate of transplacental antibody transfer to the developing fetus than women in nontropical areas. Other factors that could affect TT immunogenicity include concurrent malarial infection, the impact on vaccine potency of both high and low (i.e., freezing) temperatures (which could occur during transportation and storage), malnutrition and vitamin A deficiency, pregnancy status, the age of the recipient at the time of TT vaccination, and the tetanus toxin load. The present article evaluates the impact of selected factors on TT immunogenicity and potency. Excluded is the impact of different immunization schedules and intervals between doses (1)[e] as well as the impact on immunogenicity of human immunodeficiency virus/acquired immunodeficiency syndrome infection (HIV/AIDS).

Sources of data

An extensive review of the published literature, predominantly in English, was carried out to identify studies that evaluated the impact on the immune response to TT of concurrent malarial infection and chemoprophylaxis, malnutrition, vitamin A deficiency, pregnancy status, placental transfer, age of vaccinee, and the effects of temperature on the vaccine. Reviewed also were published and unpublished WHO reports. Although studies of the immunogenicity of TT in pregnant women are the most desirable for assessing the impact on NT elimination, most studies involved children and DTP. We included such studies in the review since they are

relevant for the global control of non-NT and also have implications for the immunogenicity of TT for NT control.

Standard definitions were used to interpret potency testing and immunogenicity study data. Standard potency testing, as defined by WHO, involves a lethal challenge test in mice, with the degree of protection of the TT concerned being compared with a standard reference TT; the results are expressed in International Units (IU) (10), and TT vaccines with ≥40 IU per human dose are considered potent. Tetanus antitoxin levels are measured using in-vivo and in-vitro methods.[e] The neutralization test, an in-vivo test using mice, is sensitive for determining even low levels of tetanus IgG antitoxin. In-vitro tests, including passive haemagglutination, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA), are less sensitive than the mouse neutralization test and have poor correlation at antibody levels <0.16 IU. Also, in-vitro tests may record higher levels of antibody than neutralization tests since they detect also IgM or other nonspecific antibodies. However, a recently developed antigen-competition ELISA (11), as well as the toxin-binding inhibition test (TOBI), have good correlation at low levels. A TT vaccine with an in-vivo level of 0.01 IU/ml of serum is considered protective (5),[e] whereas a level of 0.1 IU/ml by in-vitro testing is generally considered protective.

Results

Malaria infection

For complete evaluation of the impact of concurrent malarial infections on the immune response to TT, immunogenicity studies should compare individuals infected with *Plasmodium* parasites and those who are free of such infection. Also, the role of parasitaemia load and of antimalarial therapy on the immune response to TT should be evaluated. However, withholding chemotherapy from persons, especially pregnant women, who are infected with malarial parasites for at least 2 months (1 month between TT doses and 1 month for the second blood specimen) is unethical in prospective randomized trials. Observational studies have been used to compare the responses of persons receiving or not receiving chemoprophylaxis; the results have been conflicting (Table 1). Only one study evaluated the impact of malaria on the immunogenicity of TT in pregnant women, while there are no studies of nonpregnant women of childbearing age (12). Most of the studies evaluated the immunogenicity of TT (or DTP) in children.

---

[d] *Information system.* Unpublished document WHO/EPI/CEIS/95 1995 (available upon request from Global Programme for Vaccines and Immunization, World Health Organization, 1211 Geneva 27, Switzerland).

[e] **Galazka A.** *The immunological basis for immunization. Part 3: tetanus.* Unpublished document WHO/EPI/GEN/93.13, 1993 (available from Global Programme for Vaccines and Immunization, World Health Organization, 1211 Geneva 27, Switzerland).

Table 1: Comparison of studies to assess the impact of malarial infection or chemoprophylaxis on the immune response to tetanus toxoid (TT)

Country	Ref.	TT administration	Treatment groups (GP)	Blood samples:	% with protective levels	P
Kenya	12, 13	2 doses	GP 1: n = 73 pregnant women, with no parasitaemia GP 2: n = 107 pregnant women, with parasitaemia Both groups received chloroquine weekly	days 0, 30	—[a]	NS[b]
Gambia	14	2 doses at 6-week intervals	GP 1: n = 16, 150mg of chloroquine weekly from birth GP 2: n = 14, pyrimethamine weekly from birth GP 3: n = 36, no prophylaxis but treated as needed	days 10, and 14 after TT2	GP 1: 86% GP 2: 87% GP 3: 61%	<0.02[c] <0.02
Nigeria	15	1 dose	GP 1: n = 26, >5% parasitaemia GP 2: n = 25, 0.5–5% GP 3: n = 31, sick controls GP 4: n = 34, healthy controls All received pyrimethamine on day 2 and day 9 after TT, GP 1 and GP 2 were given chloroquine	day 16	GP 1: 19% GP 2: 28% GP 3: 42% GP 4: 50%	0.02[c] <0.05 NS
Gambia	16	2 doses	GP 1: 19 children with malaria GP 2: 106 children without malaria	?	GP 1: 89% GP 2: 100%	0.02
Papua New Guinea	17	1 booster dose	n = 161, 69% with malaria	days 0, 28	Difference in mean antibody levels	>0.1
Nigeria	19	3 doses	GP 1: n = 199 children, weekly chloroquine from birth GP 2: n = 186 children, without weekly chloroquine	days 0, 30	Post-vaccination titres: GP 1: 1.31 ± 0.25 GP 2: 1.25 ± 0.31	NS

[a] Rise in geometric mean titre.
[b] NS = not significant.
[c] P-value between group without prophylaxis or healthy control versus those treated.

The immunological response to two doses of TT (containing 10 Lf units and 3 mg AlPO$_4$) was evaluated in two groups of pregnant Kenyan women (12, 13) who were also given chloroquine on days 0, 28, and 56 of the study. Group 1 ($n = 73$) had no documented parasitaemia during the study, while group 2 ($n = 107$) had parasitaemia at least once during the study period. There were no differences in the geometric mean titres (GMTs) between the two groups of women in terms of their primary and secondary immune responses, nor was any difference observed between primigravidae and multigravidae. The study results suggest that the presence of malaria parasitaemia does not interfere with immune response; however, all the women received chemo-prophylaxis, which suppresses current infections.

McGregor et al., in the Gambia, studied the immune response to two doses of TT in three groups of 2-year-olds (14) who received 0.5-ml doses of TT (unspecified Lf and AlPO$_4$ contents) at 6-week intervals. Group 1 ($n = 16$) consisted of children who received 150 mg of chloroquine weekly from birth, group 2 ($n = 14$) of children who received pyrimethamine weekly from birth, and group 3 ($n = 36$) of children who did not receive malarial prophylaxis but who were treated for malaria as needed. Blood samples were drawn 10–14 days after TT2. Protective titres ($\geqslant 0.01$ IU/ml) were more likely to develop in children who received chemoprophylaxis (groups 1 and 2) than in children who did not (86% and 87% versus 61%, resp., $P < 0.02$). However, the distribution of antitoxin levels among those who developed a protective antitoxin level was the same in all three groups. Although chemoprophylaxis resulted in better immune response to TT, the level of parasite infection was not assessed.

Greenwood et al., in Nigeria, evaluated the impact of malarial infection when one dose of TT was administered to young children (15). Sera were obtained 16 days after injecting one dose of TT (0.5 ml, unknown Lf and AlPO$_4$ contents) to four groups of children (aged 6 months to 6 years) at an outpatient clinic. Group 1 ($n = 26$) included children with >5% *Plasmodium falciparum* parasitaemia, group 2 ($n = 25$) had parasite levels of 0.5–5%, group 3 ($n = 31$) were sick nonmalarial controls, and group 4 ($n = 34$) were healthy controls. All were given pyrimethamine twice, on day 2 and day 9 after TT administration. Groups 1 and 2 were treated with chloroquine. Although 50% of healthy controls (group 4) responded (defined as a $\geqslant 2$-fold increase in antibody level by double gel diffusion), only 19% in group 1 ($P < 0.02$) and 28% in group 2 ($P < 0.05$) responded. There were no significant differences in immune response between either the two parasitaemia groups or the healthy group and the sick controls. Although no information was provided on the children's vaccination status, and double gel diffusion is not sensitive, the results suggested that malarial infection at the time of TT administration reduced the immune response to TT. Both higher parasitaemia load and chronic infections may influence the immune response, and it was recommended that TT should not be given during the rainy season, when malaria transmission may increase. Some of these findings were confirmed by Edsall et al. (16), who reported a decreased immune response to two doses of TT given to 19 children with malaria, compared with 106 noninfected children (89% versus 100%, resp., $P = 0.02$).

The impact of malarial infections on TT booster doses administered to 161 children (mean age = 9.8 years) in Papua New Guinea was studied by Corrigall (17); one dose of TT of unspecified composition was administered. Serum specimens were obtained on days 0 and 28, and no treatment was provided. Only those children with pre-existing antibody levels on day 0 were considered previously immunized and were included in the study of secondary immune responses. There was no difference in the mean rise in antibody levels between children with or without parasitaemia ($P > 0.1$). No apparent correlation was found between parasitic density and the rise in antibody titre.

In Burkina Faso, Monjour et al. compared the immune response of two doses of TT in 126 children with untreated malarial parasitaemia and in 159 children who were given amodiaquine once a week for 6 weeks (18). The first group had parasitaemia on days 0 and 35. The treated group had no parasitaemia on day 35, although an unreported number were infected on day 0. No differences in antibody levels were reported after either one or two doses of TT.

The immune response to three doses of DTP was evaluated in Nigeria among 199 children protected since birth with chloroquine and 186 age-matched controls who had not received weekly protection (19). There were no significant differences in the mean increase in antibody titre between the two groups (1.31 ± 0.25 versus 1.25 ± 0.31, resp.); 99% of children in both groups seroconverted. However, the mean increase in antibody titre was significantly lower among children who were parasitaemic when they received the first dose of DTP (1.12 ± 0.33 versus 1.32 ± 0.26, $P < 0.01$).

An adequate antibody response to three doses of DTP during malarial chemoprophylaxis has been reported in Nigeria (20). In addition, animal studies suggest that the immunosuppressive effect of *P. yoelii* on the immune response to tetanus toxoid (21) could be overcome by using an adjuvant and pertussis vaccine with TT. Thus, caution is required

in assuming the equivalence of immune responses to DTP and TT in the presence of malarial infections.

Malnutrition

We found no studies that assessed the impact of malnutrition on the response of pregnant women to TT; instead, most studies involved the administration of DTP to malnourished children. However, available data suggest that the efficacy of TT is not decreased under conditions of malnutrition (22) but that vitamin A deficiency may decrease the immune response to TT (23). Although cellular and humoral immune responses are impaired in children with severe protein–calorie malnutrition (PCM), malnutrition in general appears not to depress humoral immunity. The primary immune deficiency is related to cell-mediated immunity (22); for example, a case–control study of 76 malnourished children aged 6 months to 6 years and 41 age-matched controls failed to detect suppression of humoral immunity (24). Depression of cellular immunity has been reported in malnourished children with infections and among those with PCM and kwashiorkor (25, 26); however, the immune responses to tetanus and diphtheria toxoids were satisfactory among three groups of children (27) who received DTP (children with normal nutritional status, children with mild-to-moderate malnutrition, and children with severe PCM). There were no significant differences in tetanus antibody titre levels among the three groups. Adequate antibody responses to TT were also exhibited by 90 malnourished children aged 12–36 months; however, the antibody levels were lower for these children than for healthy controls ($P > 0.05$) (28). In addition, the antibody response to TT among 26 infants with fetal growth retardation did not differ from that of healthy neonates (29). Similarly, the immune responses of healthy children ($n = 357$), mild-to-moderately malnourished ($n = 116$), and severely malnourished children ($n = 33$), all of whom received three TT doses given 1 month apart, were examined 1 month after TT3; protective titres developed in 98.3% of the healthy children (30), in 97.4% of those who were mild-to-moderately malnourished, and in 93.9% of those who were severely malnourished.

In contrast, a reduced response to tetanus toxoid after primary immunization was observed in 21 children aged 7–20 months who had protein–energy malnutrition (31). In addition, animal studies suggest that when protein-deficient mice were given high protein diets before TT administration their antibody response improved (32).

Studies of the impact of vitamin A deficiency on the immune response to TT of children (but not of pregnant women) have also resulted in conflicting findings. A total of 25 children who showed ocular signs of vitamin A deficiency and who were attending a nutrition clinic were compared with 24 children who lacked signs of vitamin A deficiency. Each child received one dose of TT and antibody levels were measured 2 weeks after vaccination; protective levels of IgG antibodies were produced in all the children (33). A further study compared the antitoxin levels after two doses of TT among 46 vitamin-A-treated children and 49 controls (34); the difference in levels between the two groups was not significant and there was no correlation between initial serum levels of vitamin A and subsequent antitoxin levels.

A randomized, double-masked, placebo-controlled trial of clinically normal children and xerophthalmic children aged 3–6 years showed that among both groups the IgG response to tetanus toxoid was significantly greater following vitamin A ingestion than after a placebo (23). Of the clinically normal children, 44% had subclinical vitamin A deficiency; nevertheless, children who were supplemented with vitamin A until they reached normal vitamin A levels had higher IgG responses to TT than those who received placebo ($P < 0.05$).

In rats the response to diphtheria and tetanus toxoid injection was poorer among animals that were vitamin-A-deficient than among controls (35). Also, vitamin-A-depleted rats produced very low concentrations of tetanus-toxoid-specific IgM and IgG antibodies in the primary and secondary responses (36).

Impact of pregnancy status on the response to TT

No controlled trials designed specifically to compare the response to TT of pregnant and nonpregnant women were identified. However, several studies of TT immunogenicity reported the impact on TT immunogenicity of the pregnancy status of the recipient (37, 38). For example, one study of the duration of primary antitoxin responses noted differences in the proportion of pregnant and nonpregnant women who developed protective levels after receipt of TT: 9 of 39 (23%) nonpregnant women and 2 (4%) of 50 pregnant women failed to develop protective levels after two doses of absorbed TT (10 Lf), corresponding to a relative risk (RR) of 5.8 for not responding to TT (95% confidence internal (CI) = 1.3, 25.2; $P < 0.007$).

Rate of placental transfer of tetanus antibody

Transplacental transfer of immunoglobulins is limited to IgG, while IgM, IgA and IgD are excluded

from fetal circulation (*39*). In addition, there are specific Fc receptors in the placenta only for IgG; placental transfer is initiated by the binding of IgG to the Fc receptors, followed by receptor-mediated endocytosis and active transport. Different maternal antibodies must compete for the limited numbers of receptors, thus influencing the transport of a particular antibody to the fetus. Studies indicate that the concentrations of IgG in maternal and in cord sera are essentially the same (*40, 41*) and that IgG is actively transported from mother to fetus by the placenta. In addition, data suggest that there is a linear relationship between placental transfer of immunoglobulins and gestational age, such that active transport increases with gestational age. In one study fetal concentrations of IgG immunoglobulin approximated the maternal levels at 38 weeks' gestation and continued to increase until birth, reaching more than twice the maternal levels by delivery (*42*). These observations provided, in part, the basis for giving TT during pregnancy and for recommending that the second dose be given in the third trimester, ideally at least 4 weeks before birth and 4 weeks after the first dose.

Immunoglobulin levels are, in general, higher among the populations of tropical countries than among those of nontropical countries. Thus, mean IgG levels were higher among Gambian, Gabonese or Nigerian adults than British, French or Swiss adults (*43–45, 47*). This difference may be related to the wide range of antigenic stimuli provided by the variety of infections that occur in tropical countries.

Several workers have suggested, however, that a reduction in placental transfer of tetanus antibody occurs in the tropics, particularly in Africa, and therefore have questioned the effectiveness of pro-tection provided by TT administered to mothers (*46–48*). This conclusion is based on the observation that the cord/maternal (C/M) ratios of tetanus antibody are higher among European than African women, with the lower C/M ratios in African women being taken to indicate a defect in the transplacental transfer of tetanus antibodies. If correct, this could have implications for vaccination practices in Africa, since pregnant women who have received two or more doses of TT might not transfer sufficient quantities of TT antibodies to the fetus to protect against NT.

Mean maternal levels of tetanus antibody (IU/ml) are higher among women in Gabon than among those in France and higher in Nigeria than in the United Kingdom (Table 2). Although lower, the differences persisted when the levels were evaluated for cord sera. In addition, the C/M ratios were different among women from tropical and European countries. In Gabon and Nigeria (as well as in Thailand (*49*) and India (*50*)), where maternal IgG levels are high, the levels in cord blood are lower than the corresponding maternal levels; in contrast, in France and Switzerland cord levels are higher than maternal levels (i.e., the C/M ratios are >1.0 in France and Switzerland and <1.0 in tropical countries). These C/M ratios were, however, obtained using *in-vitro* techniques to determine tetanus antibody levels. In maternal sera both IgG and IgM were determined, resulting in misleadingly high antibody levels (regardless of the country of origin) compared with those obtained using *in-vivo* neutralization techniques. In contrast, since only IgG levels could be determined in cord sera, the C/M ratios would be expected to be lower but in tropical countries the reduction in C/M ratios greater, since maternal levels

Table 2: **Tetanus antibody levels in maternal and cord sera in selected countries**

Country	Mean tetanus antibody level (IU/ml):		C/M ratio	Ref.
	Mother (M)	Cord (C)		
Gabon[a]	1.11	0.91	0.82	*46*
France	0.44	0.80	1.81	
Nigeria[b]	2.37	1.93	0.81	*48*
United Kingdom	1.11	1.53	1.38	
Thailand[c]				*49*
After first dose	0.183	0.043	0.23	
After second dose	0.508	0.341	0.67	
India[b,d]	0.87	0.85	0.98	*50*

[a] Radioimmunoassay.
[b] Enzyme-linked immunosorbent assay.
[c] Hemagglutination inhibition.
[d] Only IgG levels were measured in the maternal sera, while in the other studies, total tetanus antibody was measured.

would be higher because of the greater levels of IgM from other sources.

The increase in the C/M ratio with the number of TT doses in the above-mentioned study in Thailand (49) suggests that as the amount of antibody produced in response to TT administration increases, there is a corresponding increase in the level in cord sera. Also, in the Indian study (50), where only IgG was determined in maternal sera, the C/M ratio is close to unity (0.98). Furthermore, in any comparison of European and African mothers, differences in the timing of immunization must be taken into account. In Europe women are routinely immunized on several occasions in childhood and may have received booster doses as adults. In contrast, women in Africa are usually only immunized during pregnancy, and, on average, have received fewer doses of TT; also, if they are given TT early in pregnancy or with a shorter than ideal interval between the first and second doses or between the second dose and delivery (51), placental antibody transfer will be lower than expected.

Common placental infections in the tropics, e.g. malaria (52), can produce pathological changes in the placenta that reduce antibody transfer to the fetus. For example, in a malarious area of Papua New Guinea, the C/M ratio of tetanus antibody among pregnant women with heavy placental malarial infections was considerably lower (0.18) than that among women without such infections (0.83). About 10% of babies born to mothers whose placenta was heavily infected with *P. falciparum* failed to acquire protective levels of tetanus antibody, despite adequate maternal antibody concentrations (53).

Impact of the recipient's age on immune response to TT

Tetanus toxoid has been administered primarily to two groups: women of childbearing age to prevent NT; and children (with DTP) to prevent non-NT. Most studies (1–5, 54) on the immunogenicity of TT have been carried out on these groups. The numerous studies on the TT immunogenicity in adults for the prevention of NT have been carried out on pregnant women, often with little regard to their age. The studies of the impact of age on immune response have focused on the response to TT among the elderly and not among different age groups of young adult pregnant women. Although the results of such studies have no direct impact on NT, they are relevant to the control of non-NT and have therefore been included here. Several studies have reported that the majority of cases of tetanus in developed countries involve over-60-year-olds (55–58). Thus,

93% of cases in France, 61% in Poland, 67% in Switzerland, and 58% in the USA have involved persons aged >60 years. In contrast, studies in developing countries suggest that a much smaller proportion of tetanus cases occur in adults, and less than 10% of reported cases in Nigeria, Burkina Faso and India have occurred in persons >60 years of age (59–61).

Nevertheless, the results of studies on the influence of age on tetanus antibody response are not consistent: some have documented a satisfactory response among the elderly (62–64), while others have shown that the immune response of the elderly is slower and less vigorous than that of younger persons (65–69).

In one study, two doses of TT vaccine administered to persons aged 61–94 years with a 7-month interval between doses increased levels of tetanus antibody to >0.1 IU/ml among all recipients (62). Also, 81–95% of individuals aged 40–93 years who were immunized with two primary doses of adsorbed TT separated by an interval of 40 days followed by a booster dose 1 year later exhibited tetanus antibodies (Table 3) (63). The mean antibody levels 1 year after the first two doses showed no evidence of decline, and 2 months after the third dose all vaccinees had protective levels of antibodies (mean, >1 IU/ml). There was no clear difference in the immune response of persons aged 40–60 years and those aged 81–96 years (Table 3). The tetanus antibody levels 8 years after the third dose of TT were considerably lower (64), however, with only 50–83% of all recipients exhibiting levels of at least 0.01 IU/ml.

Other studies of the impact of age on the response to TT have reported less encouraging results. Two groups of adults (65–84-year-olds and 25–34-year-olds) were vaccinated twice with adsorbed TT (5 Lf) (66). Both the mean titres of total and IgG tetanus antibody were significantly lower for the elderly group, who also exhibited a greater decrease in IgG antibody levels than the younger adults. Similar

Table 3: **Tetanus antibody levels among adults after primary immunization with two doses of tetanus toxoid and a booster dose** (ref. 63).

| Age (years) | % with mean antitoxin levels >0.01 IU/ml: | | |
	30 days after 2nd dose	12 months after 2nd dose	2 months after 3rd dose
40–60	91 (0.07)[a]	81 (0.027)	100 (1.23)
61–70	87 (0.05)	78 (0.057)	100 (1.11)
71–80	81 (0.05)	79 (0.053)	100 (1.19)
81–96	95 (0.07)	89 (0.023)	100 (1.25)

[a] Figures in parentheses are the mean levels (IU/ml).

Table 4: **Impact of freezing on the potency of absorbed tetanus toxoid (TT)** (see ref. *73*)

	n	<0.01 IU[a]	≥0.01–1.9 IU	≥2.0 IU	*P*
			% with titres:		
10 days after TT1:					
Unfrozen	121	50	50	NR[b]	
Frozen, ×1	116	53	47	NR	0.64
Frozen, ×4	119	54	46	NR	0.51
10 days after TT2:					—[c]
Unfrozen	113	0	35	65	
Frozen, ×1	109	0	38	62	0.7
Frozen, ×4	114	0	54	46	0.006
10 days after TT3:					—[c]
Unfrozen	70	0	0	100	
Frozen, ×1	64	0	0	100	
Frozen, ×4	90	0	9	91	0.008

[a] A level ≥0.01 IU is considered protective.
[b] NR = not reported.
[c] Test of significance does not include group for <0.01 IU.

findings have been reported by Rethy & Rethy (*68*). Compared with other age groups, a lower proportion of older adults developed protective tetanus antibody titres after a primary series of two doses of TT. In addition, the fall in tetanus antibody levels during the year following the primary series was more accentuated in the older adults than in the younger persons, and the response to the booster dose was also weaker among the older adults.

Finger et al. found that only one-third of recipients aged 60–98 years developed protective titres after the second dose of TT (*65*). However, the third dose exerted a significant booster effect, causing an 80% increase in the proportion of individuals who were protected. Masar et al. have also suggested that age has an impact on the response to TT (*67*). Booster doses of TT were administered to two groups of adults who had been vaccinated at least 10 years previously: those aged 40–57 years and those aged ≥60 years. Among recipients who were seronegative, the immune responses were slower among the elderly than among those who were middle-aged: 7 days after vaccination, only 11% of the elderly had seroconverted compared with 86% of the middle-aged. However, it is difficult to interpret these data since the two groups were not characterized in terms of their previous vaccination history.

Age therefore appears to be an important factor that can impair the immune response to TT. Tetanus antibody response among the elderly may be more delayed than among younger individuals and is less vigorous than that of younger adults. Nevertheless, there is every indication that elderly persons can respond to TT vaccination and will be protected after the receipt of a primary series.

Effect of storage temperature on TT

Low storage temperatures adversely affect TT potency, while high temperatures have a smaller impact.[f] TT stored at 22 °C for 45 days did not exhibit any significant deterioration in potency (*70*). When stored at 35 °C for 45 days, the potency of two samples of TT decreased from 134.6 IU to 72 IU and 82 IU, respectively, but both were still above the minimum level of 40 IU. However, significant loss of potency was observed after 15 days of storage at 45 °C. Other studies have also reported on the stability of TT over long storage periods (*71, 72*).

Only one study was found on the impact on TT immunogenicity of freezing, although other studies evaluated the impact of freezing on the tetanus component of DTP or DT (*73*). The one study concerned, conducted on army recruits in India, used four different lots of TT, half of which were adsorbed (Table 4). In each of the four lots there were three study groups: TT that was frozen once; TT frozen four times; and TT not frozen. Blood samples were drawn on day 0 and day 10 after TT1, TT2 and TT3; there was an interval of 6 weeks between TT1 and TT2, and 6 months between TT2 and TT3. All titres before vaccination were <0.005 IU and protective titres developed in all vaccinees. Thus, 10 days after the receipt of TT3, which had been frozen four times, 100% of the 89 subjects had protective titres of >0.01 IU (Table 4); however, only 91% of those who

Table 5: **Impact of freezing on the potency of the tetanus component of DTP and DT vaccines at different temperatures** (adapted from ref. 74, 75)[a]

Vaccine	Storage temperature (12 hours)			
	−5°C	−10°C	−20°C	−30°C
DTP (sample):				
1	Not tested	—	—	—
2	—	—	NSD[b]	—
3	NSD	—	—	NSD
4	—	NSD	—	29% decrease
5	—	NSD	—	25% decrease
6	—	NSD	—	NSD
7	Not tested	—	—	—
DT (sample):				
1	NSD	—	—	—
2	61% decrease[c]	—	—	55% decrease

[a] DTP = diphtheria–tetanus–pertussis; DT = diphtheria–tetanus.
[b] NSD = no significant difference between potency of vaccine stored at +4°C.
[c] Even with the decrease, the potency was reported to be >40 IU (minimum WHO requirements).

received TT frozen four times had titres >2.0 IU, compared with 100% of subjects who received unfrozen TT ($P = 0.008$) and 100% of subjects who received TT that had been frozen once.

In two WHO-sponsored studies conducted by six laboratories, the impact of freezing DTP and DT on the potency of the tetanus component (when stored at +4°C, −5°C to −15°C for 12 hours, and −20°C to −35°C for 12 hours) was examined (74, 75). No other studies of the impact on tetanus potency of freezing DTP were found. As shown in Table 5, freezing DTP at −5°C to −15°C for 12 hours (which simulates freezing in a refrigerator) or at −20°C did not reduce its potency. Two samples of DT were tested after storage at −5°C: one had no significant change in potency, while the potency of the other sample, although it had decreased by 61% was still adequate. However, the potency of three of five samples tested when stored at −35°C for 12 hours (which simulates vaccine frozen in a freezer) decreased by 25% to 55%, while two samples showed no significant decrease in potency. In addition, the potency of the tetanus component in one unadsorbed sample of DTP vaccine was unchanged after freezing at −5°C and −30°C.

Discussion

In this review we have summarized what is known about various factors that may affect the performance of TT. Although numerous studies were found that addressed several potential factors, the data available on most of them do not permit definitive conclusions to be made about their precise impact on

TT potency or immunogenicity in the field.

Malaria infection, but not malnutrition, may suppress immune response to TT; however, further investigations are needed to determine the operational significance of this, especially the impact of malaria infection on the immune response to TT of pregnant women, since most studies were conducted on children. In addition, research is needed to determine whether in malarious areas, TT should be given with chemoprophylaxis during the rainy season (15). Using case–control studies, investigators evaluating risk factors for NT and non-NT should assess the malarial and nutritional status of mothers. If reports of TT failures are verified, both the role of concurrent malarial infection and nutritional status, especially vitamin A deficiency, should be evaluated.

There are few data that compare the immune response to TT of pregnant and nonpregnant women. Those data that are available (often on small sample sizes) were obtained in field trials and not in controlled studies designed to detect differences in TT immunogenicity between such women. This is not, however, significant for programmes since many countries recommend that all women of childbearing age, regardless of their pregnancy status, be vaccinated with TT to prevent NT. These observations need to be further investigated to assist in identifying the most appropriate group of women to receive TT to prevent NT.

Available data suggest that placental transfer of TT antibodies is not impaired as previously had been held to be the case. However, the mechanisms involved in the transfer of immunity have not been investigated rigorously in the tropics. Studies should be undertaken to confirm whether the different cord/

maternal ratios observed in Africa arise only be-
cause of variations in the sensitivities of different
techniques used to determine the activity of antibod-
ies present in maternal and cord sera or because of
impairment of the passage of tetanus antibodies
across the placenta. The influence of the characteris-
tics of the antibodies, i.e., the class, subclass, avidity,
etc., produced using various immunization schedules
should be further investigated. Epidemiological in-
vestigations of tetanus immunization failures in
pregnant women (i.e., development of NT in infants
born to women who received at least two TT doses)
should be supported by serological studies on the
quantity and quality of antibodies.

Those studies that have evaluated the effects of
age on TT responses suggest that the elderly can
respond adequately. This has greater importance for
control of non-NT than for control of NT, especially
in developed countries. However, in many countries,
large cohorts of women who are reaching child-
bearing age were immunized in early childhood with
only the primary series of DTP vaccine or with addi-
tional booster doses of TT. It is important to deter-
mine both the circulating antibody levels long
periods after primary vaccination with DTP as well
as the extent of immunological memory and capacity
to respond to a booster dose of TT.

Storing TT frozen may contribute importantly
to poor TT performance (J. Lloyd, personal commu-
nication, 1993). However, we found only one study
that evaluated the effect of freezing TT on im-
munogenicity. TT that had been frozen even four
times was immunogenic when given as a second or
third dose in the primary series. Freezing DTP af-
fects its potency, but not consistently. More studies
are needed to determine the impact of repeated
freeze–thaw cycles on TT. Changes in its potency
and immunogenicity should be evaluated under cir-
cumstances that replicate field experiences, e.g. the
impact of freezing in the cold boxes used during
vaccination sessions. Freezing changes the composi-
tion and the appearance of TT, causing flocculation.
More research is needed to determine whether fail-
ure to shake the vaccine vial thoroughly results in
lower immunogenicity in field use.

In summary, TT coverage is still unacceptably
low, and immediate activities need to be accelerated
to meet the goal of <1 non-NT case per health dis-
trict. While there is an urgent need to accelerate
activities to increase coverage as the top priority,
studies are also needed to identify risk factors for NT
in individual countries, and to more fully understand
those factors that affect immune response to TT and
whether the results obtained with DTP or DT in
children apply to TT in pregnant women. Failure to
address these issues adequately runs the risk of com-

promising the efforts of many countries actively
involved in realizing both the goal of global elimina-
tion of NT as a public health problem and of control-
ling non-neonatal tetanus.

Résumé

Facteurs influant sur l'immunogénicité et l'activité de l'anatoxine tétanique: répercussions sur l'élimination du tétanos néonatal et non néonatal en tant que problème de santé publique

Le tétanos est une cause majeure de morbidité
et de mortalité à l'échelle mondiale, et entraîne
chaque année environ 400 000 décès par tétanos
néonatal. En 1989, l'OMS s'est fixé comme objec-
tif l'élimination à l'échelle mondiale du tétanos
néonatal en tant que problème de santé publique.
Afin d'atteindre cet objectif et également pour lutter
contre le tétanos non néonatal, l'OMS recommande
de protéger les nouveau-nés à la naissance par
l'administration antépartum d'au moins deux doses
d'anatoxine tétanique à la mère, et d'administrer
ensuite à tous les enfants au moins trois doses
de vaccin antidiphtérique–antitétanique–anticoque-
lucheux (DTC). Pour que cette stratégie soit ef-
ficace, l'anatoxine tétanique utilisée doit être
immunogène. Les facteurs susceptibles d'influer
sur son immunogénicité doivent être évalués si
l'on veut atteindre les objectifs fixés. Bien que les
données divergent selon les études, il semble que
le paludisme puisse interférer avec l'immunité
néonatale en supprimant la réponse immunitaire
de la mère à l'anatoxine et en réduisant le trans-
fert transplacentaire d'anticorps. La malnutrition,
y compris l'avitaminose A, ne semble pas affec-
ter l'immunogénicité de l'anatoxine. Cependant,
d'après les résultats d'une étude, l'avitaminose A
pourrait tout de même être associée à une altération
de la réponse immunitaire. Il a parfois été postulé
que, chez les Africaines, le transfert transplacen-
taire de l'anticorps antitétanique pouvait être
diminué mais, d'après des rapports publiés, il
semble qu'il ne soit pas modifié en l'absence
d'infection palustre. D'après un examen des
articles publiés, aucun essai contrôlé n'a cherché
spécifiquement à comparer la réponse à l'anatoxine
tétanique chez la femme enceinte et non enceinte.
L'âge semble jouer un rôle important, en retardant
la réponse immunitaire à l'anatoxine chez les sujets
âgés. Certaines études semblent cependant
indiquer que ces derniers répondent à la vaccina-
tion antitétanique et sont protégés par l'admini-
stration d'une première série d'injections.

La congélation de l'anatoxine tétanique au cours du stockage entraîne une perte d'activité, mais son impact sur l'immunogénicité doit être mieux évalué. On n'a trouvé dans la littérature qu'une seule étude consacrée à l'impact de l'emploi d'une anatoxine ayant gelé sur la réponse immunitaire. Même après quatre cycles de congélation-décongélation, l'anatoxine donnait des titres protecteurs chez toutes les personnes vaccinées. D'autres études montrent que la congélation a un effet sur l'activité de l'anatoxine tétanique, mais de façon non reproductible.

Toutes ces études sur l'impact de différents facteurs sur l'immunogénicité de l'anatoxine tétanique ont des répercussions directes sur les programmes. Par exemple, s'il était démontré que le paludisme nuit à la protection contre le tétanos néonatal, cette conclusion pourrait avoir des conséquences importantes sur le moment à choisir pour les vaccinations ou sur la nécessité d'évaluer la situation des vaccinés en ce qui concerne le paludisme. De plus, d'autres études doivent être effectuées pour déterminer l'impact de cycles répétés de congélation-décongélation sur l'anatoxine. Les recommandations actuelles préconisent la destruction des flacons d'anatoxine tétanique ayant gelé, mais pourraient devoir être revues si de nouvelles études laissent à penser que, dans certaines circonstances, une anatoxine tétanique ayant gelé est encore immunogène et conserve son activité.

References

1. **Rashman M et al.** Use of tetanus toxoid for the prevention of neonatal tetanus. 1. Reduction of neonatal mortality by immunization of non-pregnant and pregnant women in rural Bangladesh. *Bulletin of the World Health Organization*, 1982, **60**: 261–267.
2. **Schofield FD, Tucker VM, Westbrook GR.** Neonatal tetanus in New Guinea, effect of active immunization in pregnancy. *Lancet*, 1961: 785–789.
3. **Newell KW et al.** The use of toxoid for the prevention of tetanus neonatorum. *Bulletin of the World Health Organization*, 1966, **35**: 863–871.
4. **Chen ST et al.** Timing of antenatal tetanus immunization for effective protection of the neonate. *Bulletin of the World Health Organization*, 1983, **61**: 159–165.
5. **Jones TS.** The use of tetanus toxoid for the prevention of neonatal tetanus in developing countries. In: *Recent advances in immunization, a bibliographic review*. Washington, DC, Pan American Health Organization, 1983 (Scientific Publication No. 451).
6. Progress toward the global elimination of neonatal tetanus, 1989–1993. *Morbidity and mortality weekly report*, 1994, **43**: 885–887; **43**: 893–894.
7. **Owa JA, Makinde OO.** Neonatal tetanus in babies of women immunized with tetanus toxoid during pregnancy. *Tropical doctor*, 1990, **20**: 156–157.
8. **Ghosh JB.** Prevention of tetanus neonatorum. *Indian pediatrics*, 1990, **27**: 210.
9. **Owa JA, Makinde OO.** Neonatal tetanus in babies of immunized mothers. *Journal of tropical pediatrics*, 1990, **36**: 143–144.
10. **Dobbelaer R.** A comparison between the World Health Organization and European Pharmacopoeia methods for the potency testing of adsorbed tetanus toxoid. In: Manclark CR, ed. *Proceedings of an informal consultation on the World Health Organization requirements for diphtheria, tetanus, pertussis and combined vaccines, Geneva 30 May–1 June 1988*. Bethesda, MD, USA, U.S. Public Health Service, Food and Drug Administration, 1991.
11. **Simonsen O, Bentzon MW, Heron I.** Modification of the ELISA for the estimation of tetanus antitoxin in human sera. *Journal of biological standardization*, 1987, **15**: 143–157.
12. **Expanded Programme on Immunization.** Influence of malaria on the immune response to tetanus toxoid in pregnancy. *Weekly epidemiological record*, 1982, **59**: 45–46.
13. **Brabin BJ et al.** The influence of malaria and gestation on the immune response to one and two doses of adsorbed tetanus toxoid in pregnancy. *Bulletin of the World Health Organization*, 1984, **62**: 919–930.
14. **McGregor I, Barr M.** Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1962, **56**: 364–367.
15. **Greenwood BM et al.** Immunosuppression in children with malaria. *Lancet*, 1972, **22**: 169–172.
16. **Edsall G et al.** Malnutrition and the immune response to tetanus toxoid. In: *Proceedings of the Fourth International Conference on Tetanus, 6–12 April 1975, Dakar, Senegal*. Lyon, Fondation Mérieux, 1975: 683–685.
17. **Corrigall RJ.** Asymptomatic malaria parasitaemia and the antibody response to tetanus toxoid vaccination. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, **82**: 540–541.
18. **Monjour L et al.** Etude de l'immunité humorale et cellulaire après vaccination antitétanique chez l'enfant african malnutri et paludéen. *Bulletin of the World Health Organization*, 1982, **60**: 589–596.
19. **Gilles HM et al.** The Malumfashi Project — an epidemiological, clinical and laboratory study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1983, **77**: 24–31.
20. **Bradley-Moore AM et al.** Malaria chemoprophylaxis with chloroquine in young Nigerian children. II. Effect on the immune response to vaccination. *Annals of medical parasitology*, 1985, **79**: 563–573.
21. **Tarzaali A, Viens P, Quevillon M.** Inhibition of the immune response to whooping cough and tetanus vaccines by malaria infection, and the effect of pertussis adjuvant. *American journal of tropical medicine and hygiene*, 1977, **26**: 520–524.

22. **Katz SP.** Effects of malnutrition and parasitic infections on the immune response to vaccines. Evaluation of the risks associated with administering vaccines to malnourished children. In: *Recent advances in immunization.* Washington, DC, Pan American Health Organization, 1983: 81–89 (Scientific Publication No. 451).

23. **Semba RD et al.** Depressed immune response to tetanus in children with vitamin A deficiency. *Journal of nutrition*, 1992, **122**: 101–107.

24. **Neumann CG et al.** Immunologic responses in malnourished children. *American journal of clinical nutrition*, 1975, **28**: 89–104.

25. **Chandra RK.** Immunocompetence in undernutrition. *Journal of pediatrics*, 1972, **81**: 1194–2000.

26. **Geefhuysen J et al.** Impaired cellular immunity in kwashiorkor with improvement after therapy. *British medical journal*, 1971, **2**: 527.

27. **Reddy V, Bhaskaram C, Raghuramulu N.** Immunological responses in malnourished children. *Indian pediatrics*, 1977, **14**: 255–258.

28. **Chandra RK.** Immunocompetence in undernutrition. *Journal of pediatrics*, 1972, **81**: 1194–2000.

29. **Chandra RK.** Fetal malnutrition and postnatal immunocompetence. *American journal of diseases of children*, 1975, **129**: 450–454.

30. **Ghosh S et al.** DPT immunization and malnutrition. *Indian pediatrics*, 1980, **2**: 123–125.

31. **Chandra RK, Chandra S, Gupta S.** Antibody affinity and complexes after immunization with tetanus toxoid in protein–energy malnutrition. *American journal of clinical nutrition*, 1984, **40**: 131–134.

32. **Price P, Bell RG.** The effects of nutritional rehabilitation on antibody production in protein-deficient mice. *Immunology*, 1976, **31**: 953.

33. **Kutty PM, Mohanram M, Vinodini R.** Humoral immune response in vitamin A deficient children. *Acta vitaminol Enzymol*, 1981, **4**: 231–235.

34. **Brown KH et al.** Failure of a large dose of vitamin A to enhance the antibody response to tetanus toxoid in children. *American journal of clinical nutrition*, 1989, **33**: 212–217.

35. **Krishnan S et al.** Effect of vitamin A and protein–calorie undernutrition on immune responses. *Immunology*, 1974, **27**: 383–392.

36. **Kinoshita M et al.** Immunological memory to tetanus toxoid is established and maintained in the vitamin-A-depleted rat. *FASEB journal*, 1991, **5**: 2473–2781.

37. **Hardegree MC et al.** Immunization against neonatal tetanus in New Guinea. 2. Duration of primary antitoxin responses to adjuvant tetanus toxoids and comparison of booster responses to adjuvant and plain toxoids. *Bulletin of the World Health Organization*, 1970, **43**: 439–451.

38. **Katitch RV.** Valeur de l'immunité antitétanique, après injection de rappel, chez les vaccinées en cours de gravidité. *Revue d'immunologie, et de thérapie antimicrobienne*, 1960, **24**: 521–524.

39. **Brambell FWR.** *The transmission of passive immunity from mother to young.* Amsterdam, North-Holland, 1970.

40. **Kohler FP, Farr RS.** Elevation of cord over maternal IgG immunoglobulin: evidence for an active placental IgG transport. *Nature*, 1966, **210**: 1070–1071.

41. **Gitlin D et al.** The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. *Journal of clinical investigation*, 1964, **43**: 1938–1951.

42. **Pitcher-Willmot RW, Hindocha P, Wood CBS.** The placental transfer of IgG subclass in human pregnancy. *Clinical and experimental immunology*, 1980, **41**: 303–359.

43. **Michaux JL, Heremans JF, Hitzig WH.** Immunoglobulin levels in cord-blood serum of Negroes and Caucasians. *Tropical and geographical medicine*, 1966, **18**: 10–14.

44. **Logie DE et al.** Plasma immunoglobulin concentrations in mothers and newborn children with special reference to placental malaria. Studies in the Gambia, Nigeria, and Switzerland. *Bulletin of the World Health Organization*, 1973, **49**: 547–554.

45. **Rowe DS et al.** Plasma immunoglobulin concentrations in a West African (Gambian) community and in a group of healthy British adults. *Clinical and experimental immunology*, 1968, **3**: 63–79.

46. **Gendrel D et al.** Transfert placentarie des anticorps antitétaniques et protection du nouveau-né. *Archives françaises de pediatrie*, 1990, **47**: 725–729.

47. **Gendrel D et al.** Placental transfer of tetanus antibodies and protection of the newborn. *Journal of tropical pediatrics*, 1990, **36**: 279–282.

48. **Hood N et al.** Placental transfer of tetanus toxoid antibodies in Nigerian mothers. *Annals of tropical paediatrics*, 1994, **14**: 179–182.

49. **Sangpetchsong V et al.** Transfer rate of transplacental immunity to tetanus from non-immunized and immunized mothers. *South-east Asian journal of tropical medicine and hygiene*, 1984, **15**: 275–280.

50. **Sundar S et al.** Study of anti-tetanus IgG and IgM levels in maternal and cord sera by enzyme-linked immunosorbent assay (ELISA). *Indian journal of pediatrics*, 1990, **57**: 763–770.

51. **Maselle SY et al.** Maternal and foetal tetanus toxoid antibody levels following immunization in pregnancy. *Journal of obstetrics and gynaecology of Eastern and Central Africa*, 1989, **8**: 11–14.

52. **Galbraight RM et al.** The human materno-foetal relationship in malaria: histological, ultrastructural and immunopathological studies of the placenta. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1980, **74**: 61–72.

53. **Brair ME et al.** Reduced transfer of tetanus antibodies with placental malaria. *Lancet*, 1994, **343**: 208–209.

54. **Orenstein WA, Weisfield JS, Halsey NA.** Diphtheria and tetanus toxoids and pertussis vaccine, combined. In: *Recent advances in immunization: a bibliographic review.* Washington, DC, Pan American Health Organization, 1983 (Scientific Publication No. 451).

55. **Zuber PLP et al.** Tetanus in Switzerland 1980–1989. *European journal of epidemiology*, 1993, **9**: 617–624.

56. **Lonbard L, Lepoutre A.** Le tétanos en France en 1991 et 1992. *Bulletin épidémiologique hebdomadaire*, 1993, **28**: 125–126.
57. **Blaszczyk J.** [Tetanus in 1993]. *Przegl epidemiologiczny*, in press (in Polish).
58. **Prevots R et al.** Tetanus surveillance — United States, 1989–1990. *Morbidity and mortality weekly report*, 1992, **41**(suppl. 8): 1–9.
59. **Afonja AOG, Jaiyeola BO, Tunwashe OL.** Tetanus in Lagos: a review of 228 adult Nigerian patients. *Journal of tropical medicine and hygiene*, 1973, **76**: 171–174.
60. **Cosnard G, Joullie M, Davin R.** Le tétanos à l'hôpital de Ouahigouya (Haute Volta). *Médecine tropicale*, 1977, **37**: 45–49.
61. **Patel J, Mehta BC.** Tetanus: study of 8697 cases. *Proceedings of the Fourth International Conference on Tetanus, Dakar, Senegal 6–12 April 1975*. Lyon, Foundation Mérieux, 1975: 233–241.
62. **Ruben FL, Nagel J, Fireman P.** Antitoxin responses in the elderly to tetanus-diphtheria (TD) immunization. *American journal of epidemiology*, 1978, **108**: 145–149.
63. **Salomonova K, Vizev S.** Immunological reactivity of senescent and old people actively immunized with tetanus toxoid. *Zeitschrift für Immunitätsforschung*, 1973, **146**: 81–90.
64. **Salomonova K, Vizev S.** Secondary response to boostering by purified aluminum-hydroxide-adsorbed tetanus antitoxin in aging and in aged adults. *Immunobiology*, 1981, **158**: 312–319.
65. **Finger H et al.** [Tetanus immunity in senium]. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Originale Reihe B*, 1975, **161**: 188–193 (in German).
66. **Kishimoto S et al.** Age-related decline in the in vitro and in vivo synthesis of anti-tetanus toxoid antibody in humans. *Journal of immunology* 1980, **125**: 2347–2352.
67. **Masar I, Kamienicka L, Novakova I.** Immune response of elderly to boosters of tetanus. In: Nistico G, et al. *Proceedings of the Eighth International Conference on Tetanus, Leningrad, 25–28 August 1987*. Rome, Pythagora Press, 1989: 251–253.
68. **Rethy LA, Rethy L.** The age and sex dependent features of the development of human immune memory. *Annales immunologiae Hungaricae*, 1986, **26**: 551–562.
69. **Rotivel Y et al.** Simultaneous vaccination of the elderly with fluid polymerized tetanus toxoid and influenza vaccines. In: Nistico G et al., eds. *Proceedings of the Eighth International Conference of Tetanus Leningrad, 25–28 August 1987*. Rome, Pythagora Press, 1989: 420–427.
70. **Kumar V, Sahai G, Kumar A.** Studies on the stability of tetanus and pertussis components of DTP vaccine on exposure to different temperatures. *Indian journal of pathology and microbiology*, 1982, **25**: 50–54.
71. **Kindt H et al.** Stability of DTP vaccine. *Journal of biological standardization*, 1974, **2**: 183–187.
72. **Stainer DW, Hart FE.** The stability of bacterial vaccines at elevated temperatures. *Developments in biological standardization*, 1978, **41**: 249–253.
73. **Menon PS et al.** Field trial on frozen and thawed tetanus toxoid. *Indian journal of medical research*, 1976, **64**: 25–32.
74. **Expanded Programme on Immunization.** The effects of freezing on the appearance, potency and toxicity of absorbed and unabsorbed DTP vaccine. *Weekly epidemiological record*, 1980, **55**(50): 385–392.
75. **Expanded Programmed on Immunization.** The effects of freezing on the appearance, potency and toxicity of absorbed and unabsorbed DTP vaccine. *Weekly epidemiological record*, 1980, **55**(51): 396–398.