

Rational approaches to reduce adverse reactions in man to vaccines containing tetanus and diphtheria toxoids§

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Adverse reactions to routine vaccines are obstacles to the mass vaccination campaigns. Though the absolute safety of any injectable vaccine cannot be guaranteed, the adverse side effects to vaccines can be minimized by practicing existing scientific knowledge. Adverse side effects to tetanus and diphtheria toxoids have been known for many years and there have been ways to minimize these reactions. These procedures did not get wide acceptance, because the current partially purified tetanus and diphtheria vaccines meet the regulatory requirements and the manufacturers are reluctant to change the established procedures of production due to the amount of work involved in the regulatory issues under the current Good Manufacturing Practices (GMP). Due to the recent epidemic of diphtheria in the independent states of the former Soviet Union, and its potential for spread to other European Countries, vaccination campaigns with tetanus and diphtheria vaccines received a new boost with several international agencies. In this report, we review the causes for adverse reactions to tetanus and diphtheria vaccines and offer practical suggestions for minimizing these reactions. The major issues in minimizing adverse reactions to these vaccines include: (1) purifying the toxins before detoxification as the reactogenic accessory antigens get covalently bound to the toxins during detoxification; (2) either using well-tolerated adjuvants which do not elicit the production of antigenic specific IgE antibodies responsible for adverse reactions or by using non-adjuvanted highly immunogenic polymerized antigens; (3) checking the status of immunity by recently developed rapid serological methods or by the Schick skin-test for diphtheria to avoid allergic or Arthus-type reactions. These approaches are applicable to industrial scales and would result in a pure, less reactogenic and better characterized toxoids antigens which would be more suitable for combined vaccines comprising highly purified acellular pertussis components, polysaccharide-protein conjugates and other antigens. © 1998 Elsevier Science Ltd. All rights reserved

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Vaccination has been the most cost effective way of controlling infectious diseases. Success of vaccination programs depends upon a number of factors, the most important being the acceptance of immunization programs by the masses and physicians. One of the critical factors which may affect the acceptance of a routine vaccine is safety with regard to adverse local and systemic side effects. There have been instances, particularly with pertussis vaccines, where rejection by majority of the population to vaccination in several countries lead to an increase in the cases of pertussis¹⁻⁶. Sometimes, to minimize adverse reactions,

the immunization programs are modified, which may also lead to resurgence in the incidence of the disease. The recent example is the epidemic of diphtheria in the New Independent Republics of the USSR which was due to a low immunization coverage and use of an adult type tetanus-diphtheria (Td) vaccine for primary immunization of children to prevent adverse reactions⁷⁻¹⁰. The Td vaccine, due to its low content of diphtheria toxoid, did not elicit long lasting protection. Therefore, for successful mass immunization purposes, adverse side effects should be minimized. Adverse reactions to diphtheria-tetanus-pertussis (DTP) vaccines have usually been attributed to the pertussis component, though tetanus and diphtheria toxoids have been known to cause adverse side effects¹¹. With the development and licensing of acellular pertussis vaccine with tetanus and diphtheria toxoids (DTaP) in several countries^{5,6,12}, which would ultimately replace the DTP vaccine, it is logical to increase the purity of tetanus and diphtheria components with a view to minimizing adverse reactions after DTaP vaccination.

Currently used tetanus and diphtheria toxoids in most of the DTP and DTaP vaccines are partially purified^{13,14} (antigenic purity approximately 60%) in contrast with highly purified pertussis components of acellular pertussis vaccine (> 90%)^{5,6,15}. Purifying tetanus and diphtheria toxins would also lead to the widespread use of the toxoids, particularly diphtheria toxoid for immunogenic booster doses in adults without adverse reactions. Though there is sufficient scientific data to relate adverse reactions after tetanus and diphtheria vaccinations due to the presence of a high proportion of contaminants in the vaccine (accessory antigens), there are also several reports which link adverse reactions to the presence of high levels of circulating antitoxic antibodies¹⁴.

Most of these early studies employed 'so-called highly purified toxoids' that have been purified after formaldehyde treatment, thus containing reactogenic contaminating antigens covalently linked to the toxoid¹⁴, or used partially purified toxin, which was considered a highly purified preparation at the time (1938)⁵¹. Later, more purified toxins were developed by growing the organisms in fermentors (discussed later). In this report, we review the existing scientific knowledge relating to the adverse reactions to tetanus and diphtheria toxoids with a view to obtaining useful information so that more rational approaches may be adopted in minimizing adverse reactions, especially to diphtheria toxoid.

ADVERSE REACTIONS TO TOXOIDS

The occurrence of reactions to vaccines consisting of either toxoids or inactivated and attenuated micro-organisms have been reported on many occasions. The reactions observed were either local or systemic of the immediate, anaphylactic type, often mediated by IgE antibodies or delayed type, mediated by T-cells. Although most of the reactions are usually benign, severe reactions (with necrosis, high fever) as well as anaphylactic reactions occasionally occurred with certain vaccines¹⁶. All types of allergic responses could be elicited in humans after diphtheria vaccination^{17,18}. Various mechanisms have been described to be

involved in these reactions. These include: (1) sensitization to the vaccine antigen or contaminating accessory antigen (impurities in the vaccine)¹⁹ by prior vaccination or prior sub-clinical infection, (2) production of reaginic type (IgE) antibodies to vaccine antigens which are preferentially elicited with certain adjuvants, such as aluminum containing adjuvants²⁰, and (3) Arthus-type reactions due to the presence of high levels of circulating specific antibodies in response to frequent vaccine boosters¹⁹.

ROLE OF ADJUVANTS

The adjuvant used for increasing the immune response of poorly immunogenic toxoids may also contribute to the adverse side effects²¹. Careful selection of an adjuvant by keeping a balance between adjuvanticity and reactogenicity, and risk-benefit ratio could minimize adverse reactions. Although mineral adjuvants have been widely used for many years, they still have limitations, including local reactions and the ability to elicit IgE antibodies (with aluminum-containing adjuvants^{11,13,21-27}). Various adjuvant preparations are currently being investigated, in particular for weak antigens with a view to enhance or to modulate the immune response²⁸⁻³⁰. A long survey of the effects of any new adjuvant is required before it can be used as a safe and non-oncogenic formulation for routine immunization purposes³¹.

The choice of an adjuvant is of major importance, as was recently documented in Swedish studies²⁰. Following the discontinuation of DTP vaccination in Sweden for fear of adverse reactions due to the pertussis component, children were vaccinated with the DT toxoids adsorbed onto aluminum hydroxide. Most of the DT vaccinated children showed sensitization to the DT components when boosted at the age of 10 years. Many vaccinees exhibited vaccine-related adverse reactions²⁰. However, some of them immunized with the non-adjuvanted DTP preparation remained free of reactions. These studies led the authors to initiate a search for alternatives to aluminum adjuvants. The authors further suggested that aluminum hydroxide, when used as adjuvant, might sensitize the vaccinees to components other than the vaccine antigens. Anaphylaxis reactions, including death, have been observed after immunization with DT vaccines³². Anaphylactic reactions are mediated by IgE antibodies, which trigger release of histamine and other pharmacologically active substances, and aluminum containing adjuvants have been shown to increase the production of total and antigen specific IgE antibodies^{11,13,21-27}. Though allergens adsorbed onto aluminum hydroxide have been used to hyposensitize atopic individuals, use of such preparations has decreased, since it became clear that aluminum-containing adjuvants elicit Th2 type response stimulating the production of IgE antibodies^{13,27}.

The use of adjuvants, such as calcium phosphate, that do not induce production of IgE antibodies and the efficacy of which has been demonstrated for many years in France and other countries with adsorbed simple or combined D, T, P, inactivated polio vaccines and even with allergen extracts, appears to be prefer-

able to avoid vaccine-related (IgE-mediated) adverse reactions^{11,13,19,21,27,33-42}.

Non-adjuvanted irreversibly inactivated glutaraldehyde polymerized vaccines and allergens developed at and patented by the Pasteur Institute, also showed high immunogenicity in humans and animals comparable to that of preparations adsorbed onto mineral adjuvants⁴³⁻⁴⁶.

In the search for an alternate adjuvant to aluminum adjuvants (hydroxide or phosphate) to reduce the adverse reactions after a booster immunization of adults with tetanus and diphtheria toxoids, Aggerbeck *et al.*⁴⁷ showed that calcium phosphate adsorbed DT vaccine was significantly more immunogenic in humans than the aluminum hydroxide adsorbed preparation, in spite of the significantly higher potency of the latter preparation in laboratory animals. However, the calcium phosphate adsorbed preparation caused more adverse reactions than the aluminum preparation due to subcutaneous injection of the vaccines (cf. intramuscular routes). In a recently published study⁴⁸, the dose of calcium phosphate preparation was reduced to half of the aluminum preparation. Both preparations showed similar immunogenicity in man, but the calcium phosphate preparation caused fewer adverse reactions than aluminum preparation⁴⁸. A booster with both preparations and plain vaccine raised IgE levels in man. The authors attributed this to sensitization with aluminum adsorbed vaccines during primary immunization 20 years earlier⁴⁸.

ROUTE OF ADMINISTRATION

The route of administration of vaccines, particularly those adsorbed onto mineral adjuvants, plays an important role in avoiding adverse local reactions. Subcutaneous injection of adsorbed vaccines may induce nodules which are not observed when the vaccine is administered intramuscularly, as recommended in many countries, particularly in the United States^{38,49}. Although vaccines adsorbed onto mineral adjuvants are generally injected by the intramuscular route, there have been instances when adsorbed vaccines were inoculated subcutaneously, leading to increase in local side effects^{38,47}.

SENSITIZATION TO VACCINE AND CONTAMINATING ACCESSORY ANTIGENS

Severe adverse reactions, including urticaria, were observed during the late 1950s in response to booster vaccination of the Pasteur Institute staff with combined diphtheria and tetanus toxoids (unpublished data). Skin tests were carried out with dilute diphtheria or tetanus toxoids and it was found that sensitization was directed exclusively to the crude diphtheria toxoid⁵⁰. Subsequent vaccination of the subjects with a toxoided purified toxin adsorbed onto calcium phosphate did not elicit undesirable reactions, indicating that the adverse reactions were not to the toxoid but to contaminating accessory antigens present in partially purified diphtheria and tetanus vaccines.

Systematic studies were performed to confirm these findings and a large number of subjects were intra-

dermally skin tested so as to identify the sensitizing component(s) in diphtheria toxoid preparations. The purpose of the studies was to identify if the reactions were due to the toxin molecule proper or to the contaminating accessory antigens in the vaccine⁵⁰. Skin tests were performed with the following preparations.

- (1) D1, crude diphtheria toxoid preparation (150 Lf mg⁻¹ protein nitrogen)
- (2) D2, toxoided purified diphtheria toxin (pure toxoid) preparation (purity 3000 Lf mg⁻¹ protein nitrogen)
- (3) D3, permeate from crude ultrafiltered toxoid
- (4) D4, crude ultrafiltered diphtheria toxoid

All preparations were diluted to 6 Lf ml⁻¹ for toxoids (D1, D2 or D4) or a level corresponding to this concentration for the permeate from ultrafiltration (D3). Each preparation was injected intradermally at 0.1 ml volume in the upper part of the arm of each of 200 healthy individuals. The diameter of any erythema and papula was measured after 24 h. The overall results of the tested subjects showed 75% positive reactions to crude toxoids (D1 and D4) and 10% to pure toxoid (D2), whereas positive reactions with preparation D3 were infrequent and mild⁵⁰. Table 1 shows the percentage of response according to the severity of reactions to all four preparations in 60 randomly selected subjects. The majority of the subjects did not show reactions to pure toxoid (D2) and permeate from ultrafiltered toxoid (D3). The reactions to these preparations were usually mild. In contrast, the majority of the subjects showed reactions to crude toxoid preparations (D1 and D4) and many individuals showed large local reactions to these preparations. Most tested subjects were over 20 years old, except four children of 2, 7, 7 and 15 years old, respectively, showed to be not sensitized to pure toxoid (D2) or weakly to crude toxoid (D1)⁵⁰.

Therefore, it was concluded that the adverse reactions to the crude or partially purified diphtheria toxoids were directed mostly to accessory antigens or to other proteins present in the culture filtrates, distinct from the toxin molecule. These antigens are coupled by covalent methylenic bridges to toxin during formalinization of crude toxin.

Earlier studies of Lawrence and Pappenheimer^{18,51,52} on 186 medical students and nurses using a purified diphtheria toxoid and a formalinized preparation of crude diphtherial proteins cultivated in excess of iron (P-fractions), found that a significant proportion of Schick-positive individuals showed delayed reactions to the P-fraction or accessory antigens, whereas a small

Table 1 Positive skin reactions of 60 healthy individuals to various preparations of diphtheria toxoids^a

Preparation	Negative	Skin reactions %		
		≤ 1 cm	2-3 cm	≥ 4 cm
Crude toxoid, D1	22	35	37	7
Pure toxoid, D2	85	7	5	3
Permeate from ultrafiltration of crude toxoid, D3	70	18	8	3
Retente from ultrafiltration of crude toxoid, D4	38	15	25	22

^aFrom ref. 50

number of individuals were highly sensitive to purified toxoid. Though these studies indicated the importance of purified toxin in toxoid preparations, the results did not clarify the role of the toxoid molecule in adverse reactions. It is interesting to note that the purified diphtheria toxin (prepared in 1938), as used by Pappenheimer and coworkers^{51,52} with a purity of approximately 2000 Lf mg⁻¹ protein nitrogen, was not a highly purified preparation, unlike the crystalline toxin with > 3000 Lf mg⁻¹ protein nitrogen. But at that time (1938), it was considered a highly purified toxin preparation, since higher purity could not be achieved with the then available purification techniques, and also fermentors for producing diphtheria toxin were not available. In fermentors, much higher concentrations of toxin (200–250 Lf mg⁻¹) are produced in a shorter time (36 h) than in flat bottom bottles (65 Lf ml⁻¹ after 8–10 days). Therefore, the concentration of accessory antigens is lower in fermentor grown culture supernatants of *Corynebacterium diphtheriae*, as compared with those grown in bottles under static conditions, and highly purified toxins are easily prepared⁵⁵.

ROLE OF NON-TOXIGENIC AND OTHER CORYNEBACTERIA

Delayed-type hypersensitivity is most commonly induced by infection with the related organisms. Non-toxicogenic *C. diphtheriae* and other closely related corynebacteria are usually present among the normal flora of the human respiratory tract and skin. Non-toxicogenic strains have been known to cause a mild diphtheria-like disease^{9,10,18,38}. With a view to evaluating the role of various corynebacteria, skin tests were performed with culture filtrates of toxigenic and non-toxicogenic diphtheric or non-diphtheric corynebacteria⁵³. Toxigenic strains were from the collection of the Pasteur Institute (Biotype Gravis, strains Gr 52110 and Gr 52111; and Biotype Mitis, strains Mi 52112 and A98 biotypes) or had been isolated in Paris from clinical diphtheria cases (H1, H2 and H3). Three non-toxicogenic strains of Scandinavian origin (B5, B7 and B12) and one non-toxicogenic strain (C4) which could be converted into a toxigenic form following infection with the phage β were included in the study. Eight non-diphtheric corynebacteria (Table 2), also from the Culture Type Collection of the Pasteur Institute were studied, when grown under optimal growth

Table 2 Skin reactions to non-diphtheric corynebacteria cultures of nine healthy subjects^a

Preparation	Negative	Number of reactions		
		≤ 1 cm	2–3 cm	≥ 4 cm
Crude toxoid	1	0	2	6
Pure toxoid	4	0	4	1
<i>C. pseudodiphthericum</i>	4	4	1	0
<i>C. xerosis</i>	9	0	0	0
<i>C. renale</i>	7	2	0	0
<i>C. fermentans</i>	6	2	1	0
<i>C. belfanti</i> X 30	2	5	1	1
<i>C. belfanti</i> X 31	3	4	1	1
<i>C. acnes</i>	6	3	0	0
<i>C. fermentans flavum</i>	8	1	0	0

^aFrom ref. ⁵³

conditions. *C. diphtheriae* strain (PW8, type Toronto) used for the toxin production in vaccine preparation was cultivated at two different iron concentrations which are known to affect toxin production: 150 µg l⁻¹ of culture medium, optimal for toxin production, or 4000 µg l⁻¹ of culture medium, which inhibits toxin synthesis almost completely⁵³. The culture filtrates were formalinized in the same way as crude toxoids. They were subsequently ultrafiltered, dialyzed and filtered under sterile conditions. Skin tests were performed on 26 healthy subjects as described above. Each individual was injected with 4–8 preparations from different strains. Pure and crude diphtheria toxoids were inoculated as controls to each subject.

The results showed that the subjects strongly sensitized to crude toxoid were unresponsive or mildly responsive to the filtrates of non-diphtheric corynebacteria cultures, indicating that sensitization with these strains was uncommon (Table 2). In contrast, frequency of positive reactions to the culture filtrates of the various diphtheric strains irrespective of their toxigenicity was similar to those with the crude toxoid (Table 3). Excess iron in the culture medium for toxin production did not inhibit the production of the sensitizing antigens. As reported in a previous study, reactions to pure toxoids were much lower than to the crude toxoid. But a few subjects showed sensitization to the toxoid molecule proper and responded positively to low concentrations of diphtheria toxoid. The infrequent reactions to pure toxoid were always less severe than those observed to crude or partially purified toxoids. Delayed-type hypersensitivity reactions were observed in Schick-negative individuals to both toxoid molecule and toxoid-free P-fraction or accessory antigens^{18,52,54}.

Double immunodiffusion tests were carried out with antitoxic preparations purified by peptic digestion of sera from horses hyperimmunized with crude toxoid. In addition to the specific antitoxin, these sera contained several antibodies directed against accessory antigens^{53,55–57}. The results showed that all toxigenic and non-toxicogenic diphtheric strains shared common

Table 3 Skin reactions to toxigenic and non-toxicogenic strains of *Corynebacterium diphtheriae* cultures in 26 healthy subjects. This was performed in three different experiments and results are pooled for crude and pure diphtheria toxoids and details reported in ref. ⁵³

Preparation	Negative	Number of reactions		
		≤ 1 cm	2–3 cm	≥ 4 cm
Crude toxoid	4	3	13	6
Pure toxoid	17	2	6	1
PW8; Fe-150 µg	1	0	3	2
PW8; Fe-4000 µg	1	1	4	0
<i>Clinical isolates</i>				
H1	1	0	5	0
H2	1	0	5	0
H3	1	0	5	0
Gravis 52110	1	0	5	0
Gravis 52111	1	0	4	1
Mitis 52112	1	2	3	0
Mitis A 98	1	0	5	0
<i>Non-toxicogenic strains</i>				
C4	3	7	8	2
B5	5	6	9	0
B7	5	5	9	1
B12	6	4	8	2

antigens secreted by these bacteria, whereas non-diphtheric strains had no antigens detectable with the antitoxic sera⁵³. Consequently, as could be expected, the antigens secreted by the non-diphtheric strains are distinct from those secreted by diphtheric strains⁵³. In other not very sensitive precipitation reaction (ring test) studies, crude diphtheria toxoid gave a strong reaction with rabbit serum raised to the so-called pure toxoid as well as with a rabbit serum directed to accessory antigens or P-fractions, whereas this anti-P serum containing no antitoxin showed across reaction with the purified toxoid due to the low level of contamination⁵¹.

These results confirm that crude toxoid would elicit reactions in individuals who are sensitized to accessory antigens. No history of prior diphtheria was found in many sensitized subjects. Some of them had not been vaccinated against the disease. Therefore, sensitization may be due to prior contact with bacteria secreting sensitizing antigens, in particular with the non-toxicogenic strains which are known to frequently infect the upper respiratory tract and skin^{9,10,18}.

SCHICK TEST

The Schick test has been a useful method to determine immunity to diphtheria^{18,38,46,58}. A positive Schick reaction, characterized by an area of inflammation at the site of injection, is due to lack of circulating antitoxin in the blood. The Schick test, performed by the intracutaneous injection of crude and pure diphtheria toxins containing 1/50 of a lethal dose of toxin for the guinea pig on the same subject in either arm, proved that 65% of positive reactions to crude toxin⁵⁸ were Schick negative when tested with pure toxin showing immunity to diphtheria. This was confirmed by titration of neutralizing antibodies in the sera of individuals showing a positive Schick reaction to crude toxin and a negative Schick reaction to pure toxin. The positive reaction to crude toxin resulted from sensitization of individuals to accessory antigens. Therefore, a reliable Schick test may only be performed with pure toxin. A pseudo-Schick positive reaction detected with crude toxin would lead to injection of a crude or partially purified vaccine to a sensitized but immunized subject with the risk of severe allergic or Arthus-type reactions. Therefore, a simple protocol using a pure Schick toxin was described^{50,55,58} to detect non-immunized subjects.

IMMUNOLOGICAL BASIS OF SENSITIZATION

Both immediate type and delayed-type sensitivity reactions occurred following injection with crude diphtheria toxoid. Passive transfer of sensitization to pure toxoid to negative subjects with the serum of strongly sensitized subjects did not occur, because sensitization is likely to be of cellular type⁵⁰. In contrast, sensitization to crude toxoid could be transferred, but, unlike typical Prausnitz-Küstner reaction occurring after 20–30 min, the reactions were negative or of low intensity and became clearly positive only after 24–48 h⁵⁰. This indicates that the sensitizing antibodies are of reaginic nature. Kuhns and Pappen-

heimer⁵⁹ also reported the atopic reagin nature of the sensitizing antibody responsible for immediate type reactions.

Passive cutaneous anaphylaxis (PCA) reactions in guinea pigs were elicited by the intradermal administration of sera from sensitive and negative subjects. Crude and purified toxoids at concentrations ranging from 100 to 300 Lf ml⁻¹ were administered intravenously followed by 0.25–0.5 ml of 1% Evans blue solution within 4–6 h. Tests were positive only when the sera contained precipitating antitoxic antibodies and showed that human diphtheria antibodies obtained even from non-allergic subjects could bind to guinea pigs dermal cells and give positive reactions with pure and crude toxoids, which was not the case for the sensitizing antibodies against accessory antigens. These findings showed that intradermally administered human antitoxic antibodies were fixed by the skin of guinea pigs to give positive PCA reactions⁵⁹.

Untoward reactions are usually more common in older children and adults and are mostly directed against formalinized corynebacterial proteins, which are present in crude toxoids^{18,58}. A small amount of toxoid (5 Lf or less) can cause severe local and systemic reactions with fever and uneasiness in sensitive individuals^{60,61}.

ARTHUS-TYPE REACTIONS

Occurrence of mild Arthus-type reactions has been reported only in a few subjects with boosted diphtheria toxoid¹⁸. Arthus-type reactions are more common with tetanus toxoid and are related to high circulating antibody levels^{19,62}. These could be avoided by titrating antibodies prior to administering the vaccine or also by checking the immune status of an individual with pure Schick toxin. Antibodies can be easily measured by a simple and rapid passive haemagglutination test⁶³. Arthus-type reactions observed with tetanus vaccines can be avoided by giving boosters injections at least at 10-year intervals⁶².

PURIFIED DIPHTHERIA VACCINE

Purity of tetanus and diphtheria toxoids is expressed in antigenic purity (Lf mg⁻¹ of protein nitrogen)⁶⁴. Antigenicity in terms of Lf is determined against an equine antitoxin raised against a partially purified toxoid. The antitoxin also contains antibodies to accessory antigens. Vaccines with more than 2000 Lf mg⁻¹ protein nitrogen are considered as highly purified preparations, although these contain reactogenic accessory antigens covalently linked to the toxoid molecule. Vaccines made by detoxifying crude toxins with formaldehyde, instead of highly purified toxin, result in cross-linkage of reactogenic accessory antigens and also amino acids, peptides and low-molecular weight proteins with the toxin molecule^{11,18,19,35,62,65,66,76,77}. Such types of preparations, even though they exhibit a purification degree of more than 2000 Lf mg⁻¹ protein nitrogen are still reactogenic^{7,14,61}. An attempt to avoid untoward reactions when immunizing or boosting adults or sensitized subjects by the administration of low doses of crude or partially purified toxoids is expected to be mostly unsuccessful because of the poor

Table 4 Immunity to tetanus after a single and two doses of toxoided purified tetanus toxin vaccine (30 Lf dose ⁻¹) adsorbed onto calcium phosphate in absence of adverse reactions^a

Time of control	Number of infants	Immunization status		Mean titre (IU ml ⁻¹)
		Not protected (<0.02 IU ml ⁻¹)	Protected (≥0.02 IU ml ⁻¹)	
Before vaccination	261	246 (94%)	15 (6%)	≤0.1
Two months after 1st injection	260	3 (1%)	257 (99%)	0.5
One year after 1st injection (before 2nd injection)	117	0	117 (100%)	0.2
Two months after 2nd injection	54	0	54 (100%)	2.4

^aFrom ref. ⁷⁵

immunogenicity of such toxoids. Furthermore, even at low doses these toxoids could still produce allergenic effects^{19,50,67}.

In contrast, vaccines prepared by detoxification of homogeneous toxin preparations are devoid of the accessory antigens and have been safely used in France for about 20 years without side effects for immunizing newborns, children, adults and elderly including allergic subjects or those having contraindications to vaccination^{33-35,68-74}. Protection could also be afforded by two injections at one-year intervals^{35-37,68-75}. A single dose of such a vaccine protected the majority of the individuals (Table 4), since high doses of the antigen could be used for immunization due to lack of reactogenicity of pure toxoids⁷⁵. Such a vaccine should represent a good candidate for a single dose tetanus vaccine, which is being developed by the World Health Organization in collaboration with several laboratories.

These results emphasize the need for 'toxoided pure toxins' which can be injected at higher doses and can be used as single or combined diphtheria vaccines, avoiding the need for preparations of adult type low-dose vaccines (Td). As stressed earlier, preparations with low diphtheria antigen are not only poorly immunogenic, particularly for primary immunization, but can still be reactogenic.

Purification of diphtheria toxin produced in fermentors is easily accomplished^{14,55}. Detoxification under controlled conditions results in formation of non reversible toxoids^{14,76}. In contrast, toxoids prepared by detoxification of crude toxins, followed by subsequent purification of the crude toxoid, while achieving high antigenic purity, are still reactogenic, due to cross-linkage of accessory antigens by covalent methylenic bridges to the toxin as a result of formalin action. The use of purified toxins for the preparation of vaccines has been since recommended by the European Pharmacopoeia⁶⁶ and other scientists^{14,18,77}.

CONCLUSIONS

Vaccination is the ultimate way to control infectious diseases, but adverse reactions occur in a high proportion of adults. Local and systemic reactions following whooping-cough immunization of babies have frequently been reported, but were most times mild. Severe reactions, especially those ascribed to brain damage, have also been observed, but did not exceed normal background and could not be related to vaccination^{5,82}.

The current diphtheria outbreak in the New Independent Republics of the USSR is related to the

giving up of systematic diphtheria vaccination, as well as to the use of an adult type Td vaccine, as a result of the warning of pediatricians against possible severe adverse side effects to the vaccine. As a consequence, vaccine coverage became inadequate^{8,38,83}.

Immunity to diphtheria in many industrialized countries is low, particularly in older subjects, making these populations prone to diphtheria^{9,10,63,78-81}. Disease outbreaks can most often be ascribed to faded immunity, because boosting of individuals was no longer performed due to adverse reactions. These results stress the need for the administration of booster injections to adults with safe purified vaccines, whenever possible after measuring circulating antibody titers so as to avoid adverse reactions.

In order to contain the diphtheria epidemic in the New Independent Republics of the former Soviet Union and to prevent it from further spread to European countries, use of vaccines devoid of undesirable side-effects is recommended. This can be achieved by using

- (1) toxoided purified toxins,
- (2) well-tolerated adjuvants that do not enhance production of total or antigen specific IgE antibodies administered by the intramuscular route,
- (3) glutaraldehyde-polymerized toxoided purified toxins without adjuvants.

It is worth mentioning again that the calcium phosphate adjuvant is a natural constituent of the body and is now even used as a 'Skeletal Repair System' (S.R.S.)^{84,85}. For this reason, it is devoid of reactogenicity and well tolerated as reported before in histopathological studies⁸⁶⁻⁸⁸, while exercising powerful immunostimulating activity without inducing formation of IgE antibodies, which would increase the risk of the occurrence of IgE mediated allergic reactions in the population. Results and benefits of several vaccination campaigns with calcium phosphate adsorbed vaccines in absence of adverse reactions have been reported in this review.

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