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Increased Dosage of Diphtheria Toxoid for Basic Immunization of Adults

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Basic immunization of adults with increased dosages of a diphtheria toxoid vaccine (2100 flocculation units (Lf)/mg) was evaluated. Three injections of 7.5 Lf or 15 Lf diphtheria toxoid were given to 243 adults who had a history of no more than one previous vaccine injection. Systemic reactions were rare in both groups. Following the first two injections, local reactions (> 5 cm) were observed in 6–14% of the adults. After the third injection, 35% of adults in the 15 Lf group reported a local reaction (> 5 cm) compared to 11% in the 7.5 Lf group ($p < 0.001$). The 15 Lf dose elicited a better antitoxin response than the 7.5 Lf dose. In a seronegative subgroup including 65 vaccinees who showed no booster response to the first vaccination, 79% had a postvaccination titer of ≥ 0.1 IU/ml and 28% a titer of ≥ 1.0 IU/ml after the third injection of 7.5 Lf. The corresponding numbers in the 15 Lf group were 94% and 44%, respectively. The study demonstrates that 7.5 Lf and 15 Lf diphtheria toxoid of high purity can safely be given to adults for basic immunization. The higher dose is more immunogenic but local reactions increase after the third injection.

Two small-scale outbreaks of diphtheria in Göteborg (1) and Stockholm, Sweden, in 1984 and 1985 demonstrated the importance of immunity to diphtheria. The outbreaks differed from the classical epidemics in that only a minority of the infected patients were children. At this time it was shown that a large proportion of adults in Sweden had low or no measurable levels of antibody to diphtheria toxin (2).

Basic immunization of infants in Sweden consists of a three-dose schedule with 15 Lf diphtheria toxoid in each injection. Immunization of adults with such doses was found in earlier studies to induce a high rate of adverse reactions (3, 4). Therefore, a much lower dose has been administered routinely to adults in Sweden and in many other countries. In a recent Swedish study, however, it was found that three injections of 2 Lf or 6.25 Lf diphtheria toxoid as basic immunization did not result in a satisfactory immune response (5).

The present study was undertaken to establish whether higher doses of a regular diphtheria toxoid vaccine could be given for basic immunization of adults.

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Materials and Methods

Altogether 243 healthy adults working at the Volvo Aircrafts Engine Factory in Trollhättan, Sweden, volunteered for the study. Criterion for inclusion was a reported history of no more than one previous inoculation with diphtheria toxoid. The study was approved by the Ethical Committee of the University of Göteborg. The participants were randomly divided into two groups and given subcutaneous injections of either 7.5 Lf or 15 Lf diphtheria toxoid in the left arm. The injection volumes were 0.25 ml and 0.5 ml, respectively. Three injections were given, the second injection two months after the first, and the last injection eight months after the second.

The group receiving 7.5 Lf comprised 116 participants of whom 61% were males; the mean age was 50 years (range 26–64). The group which received 15 Lf comprised 127 participants of whom 54% were males; the mean age was 49 years (range 28–64).

The diphtheria vaccine used (lot No. D 125) contained 2000–2400 Lf/mg protein-N, and was a regular vaccine prepared at the National Bacteriological Laboratory, Stockholm, Sweden. The vaccine consisted of 30 Lf/ml of diphtheria toxoid, which was adsorbed to aluminium phosphate (corresponding to 1 mg Al/ml) and suspended in phosphate buffered saline with 0.01% thiomersal of pH 6.0. The vaccine was estimated to have a potency of 2 IU/Lf.

Venous blood was drawn from the vaccinees prior to each inoculation and eight weeks after the third inoculation. All serum samples were stored at −20°C until analyzed. The diphtheria antitoxin response was assessed in paired samples by the microculture neutralization test in Vero cells (6). Results obtained by this method have been shown to correlate well with results obtained by the method of Jensen (7). The plates were examined after four days of incubation. Antitoxin titers were quantified in international units (IU) in relation to the WHO Standard; titrations were performed in triplicate in each test.

Adverse reactions were recorded by the vaccinees on a special form during the first week after each immunization. Systemic reactions were registered as signs of malaise such as general discomfort, headache and inability to work. The vaccinees were instructed to measure the body temperature on the morning after vaccination, and if the temperature was over 37.5 °C, to continue measuring it every morning until fever was no longer present. The highest body temperature noted during this period was entered on the form. Local reactions were registered as local tenderness, redness or swelling. Local reactions of clinical significance were defined as redness or swelling > 5 cm in diameter.

Statistical analysis for the sample size was based on the assumption there would be local reactions in 10% of the recipients of the 7.5 Lf diphtheria vaccine at each injection. A 25% increase in the rate of reactions was considered acceptable in view of the expected improved antitoxin response. In order to show this difference with a type I error of 0.05 and a type II error of 0.2, 70 subjects in each group were required as the final sample size. The analyses of the adverse reactions and antitoxin response were made by a computer programme (8) using the unpaired t-test or the chi-square test with Yates correction for comparisons between the groups. When the expected value was less than five, Fisher's exact test was used for statistical analysis.

Results

Of 243 vaccinees, 211 (87%) completed all three injections according to the schedule. Seventeen individuals (7%) completed two injections while 15 individuals (6%) completed only one injection. In addition, five vaccinees after the second injection and four after the third injection did not complete the adverse reactions form. Two vaccinees did not return for collection of a serum sample after the third injection.

Systemic reactions were few and mild after the first two injections (Table 1). No significant differences were observed between the two vaccine doses. Only five vaccinees after the first injection and six after the second injection noted a body temperature above 37.5 °C or inability to work.

After the third injection a non-significant tendency towards a higher rate of systemic reactions was noted in recipients of 15 Lf diphtheria toxoid. In this group, eight vaccinees reported fever and two had to stay home from work for 2 to 4 days. In the 7.5 Lf group two vaccinees had fever after the third injection but required no medical attention except telephone advice.

Local reactions occurred at a frequency of about 40% after the first two injections for both toxoid doses. After the third injection a significant increase in the frequency of local reactions was registered for the 15 Lf group compared to the 7.5 Lf group (74% versus 44%, $p < 0.001$). Adverse local reactions (> 5 cm in diameter) and systemic reactions to the two different doses of diphtheria vaccine are shown in Table 1. After the third injection, 35% of vaccinees in the 15 Lf group reported more pronounced local reactions compared to 11% in the 7.5 Lf group ($p < 0.001$).

In nine vaccinees, six in the 7.5 Lf group and three in the 15 Lf group, adverse reactions were reported as the reason for not completing the vaccination schedule. One female developed leukemia eight months after the second injection and was not able to continue the study. She received the 7.5 Lf dose. Another female became pregnant and did not continue the study. The remaining 21 individuals who did not complete all three vaccinations reported no adverse reactions on their record forms after the injections but failed to return for follow-up despite several reminders.

The vaccinees were separated into three different groups according to the prevaccination titers and serologic response to the first injection of diphtheria toxoid. Since no serum was obtained from the 15 individuals who completed the first injection only, they were excluded from this evaluation. Seventy-two subjects (32%) had a prevaccination titer below the limit for the analytical method (0.003 IU/ml) and did not have a fourfold or greater increase in their first postvaccination titer, i.e. there was no booster response following the first injection. These individuals were regarded as previously non-vaccinated (group A). Sixty-two individuals (27%) had a prevaccination titre of < 0.003 IU/ml but reacted with a booster response as seen in individuals having received earlier immunization (group B). Ninety-four (41%) had a prevaccination titer of ⩾ 0.003 and

Table 1: Number of vaccinees with adverse reactions to 7.5 Lf and 15 Lf diphtheria toxoid.

Type of reaction	Injection I		Injection II		Injection III	
	7.5 Lf (n = 116)	15 Lf (n = 127)	7.5 Lf (n = 104)	15 Lf (n = 119)	7.5 Lf (n = 91)	15 Lf (n = 116)
Local redness or swelling > 5 cm	12 (10%)	7 (6%)	10 (10%)	17 (14%)	10 (11%)[a]	41 (35%)[a]
General discomfort or headache	19 (16%)	21 (16%)	13 (12%)	12 (10%)	9 (10%)	19 (16%)

[a] $p < 0.001$.

were regarded as previously completely or partly immunized (group C). Postvaccination antitoxin levels in these three groups are shown in Table 2. Group A included 34 subjects vaccinated with 7.5 Lf and 38 vaccinated with 15 Lf. The corresponding numbers of subjects vaccinated in group B were 31 and 31, and in group C 42 and 52 respectively.

The frequency of adverse reactions is shown in Table 3. No significant differences in the frequency of systemic reactions were noted between the three groups. The proportion of local reactions increased after each dose of vaccine. After the second and third dose, the frequency of more pronounced local reactions was slightly higher in group B than in group A ($p < 0.05$). Individuals in group C showed an intermediate rate of local reactions.

Table 2: Diphtheria antitoxin levels (geometric mean titer) in the three different groups defined according to prevaccination titers and serologic response to the first injection of diphtheria toxoid.

Serum	Group A[a] (n = 72)	Group B[b] (n = 62)	Group C[c] (n = 94)
Prevaccination	< 0.003	< 0.003	0.027
Postvaccination Injection I	< 0.003	0.21	2.04
Postvaccination Injection II	0.0056	0.079	0.87
Postvaccination Injection III	0.55	1.75	5.3

[a] Prevaccination titer < 0.003 IU/ml and no fourfold or greater titer increase following injection I (no booster-response). Seven vaccinees did not complete the study.
[b] Prevaccination titer < 0.003 IU/ml with a fourfold or greater increase in titer following injection I (booster-response). One vaccinee did not complete the study.
[c] Prevaccination titer ⩾ 0.003 IU/ml. Eleven vaccinees did not complete the study.

The serological results for all vaccinees are shown in Table 4. After the third injection the geometric mean titer was significantly higher in the recipients of 15 Lf than in the recipients of 7.5 Lf (2.39 IU/ml and 1.42 IU/ml respectively, $p < 0.05$).

Postvaccination titers equal to or above 0.01 IU/ml, 0.1 IU/ml and 1.0 IU/ml after the second and third inoculations in the group without previous diphtheria immunization (group A) are shown in Table 5. After two injections of vaccine, titers in 21% of the 7.5 Lf recipients and 56% of the 15 Lf recipients reached 0.01 IU/ml ($p < 0.01$), i.e. the minimum antitoxin level generally believed to give protection. After three inoculations all vaccinees had titers above this level. The level of 0.1 IU/ml was reached in 79% of the 7.5 Lf recipients after the third inoculation, as compared to 94% in the 15 Lf group. A level of 1 IU/ml, often described as desirable for long-term protection (5), was reached in 8 of 29 (28%) recipients of the 7.5 Lf dose and 16 of 36 (44%) recipients of the 15 Lf dose (not significant).

In the group with prevaccination titers of ⩾ 0.003 IU/ml (group C), titers in all vaccinees reached the level of 0.01 IU/ml after two injections of vaccine and 0.1 IU/ml after three injections. In the group with prevaccination titers of < 0.003 IU/ml but with a booster response after injection I (group B), titers in all vaccinees reached 0.01 IU/ml after two injections and 0.1 IU/ml after three injections in all but 2 of 31 recipients of 7.5 Lf.

Discussion

The present study demonstrates that, in contrast to the general belief, the dosage of 15 Lf diphtheria toxoid given to infants for basic immunization in Sweden can also be safely given to adults. No severe

Table 3: Number of vaccinees with adverse reactions in relation to prevaccination antitoxin titres.

Type of reaction	Injection I			Injection II			Injection III		
	Group A[a] (n = 72)	Group B[b] (n = 62)	Group C[c] (n = 94)	Group A[a] (n = 70)	Group B[b] (n = 61)	Group C[c] (n = 92)	Group A[a] (n = 65)	Group B[b] (n = 59)	Group C[c] (n = 83)
Local redness or swelling > 5 cm	5 (7%)	2 (3%)	6 (6%)	5 (7%)[d]	13 (21%)[d]	9 (10%)	10 (15%)[d]	20 (34%)[d]	21 (25%)
General discomfort or headache	8 (11%)	9 (15%)	9 (10%)	8 (11%)	5 (8%)	12 (13%)	9 (14%)	9 (15%)	10 (12%)

[a] Prevaccination titer < 0.003 IU/ml and no fourfold or greater titer increase following injection I (no booster-response). Seven vaccinees did not complete the study.
[b] Prevaccination titer < 0.003 IU/ml but with a fourfold or greater increase in titer following injection I (booster-response). Three vaccinees did not complete the study.
[c] Prevaccination titer ⩾ 0.003 IU/ml. Eleven vaccinees did not complete the study.
[d] $p < 0.05$.

Table 4: Diphtheria toxin antibody levels (geometric mean titer) in vaccinees following three injections of 7.5 Lf compared to 15 Lf diphtheria toxoid.

Serum	Time after first injection (months)	Mean titer (IU/ml)		
		7.5 Lf	15 Lf	
Prevaccination Injection I	0	0.0049	0.0043	NS
Prevaccination Injection II	2	0.11	0.11	NS
Prevaccination Injection III	10	0.07	0.11	NS
Postvaccination Injection III	12	1.42	2.39	$p < 0.05$

NS = not significant

adverse reactions occurred. After the first two injections, the frequency of local and mild systemic reactions to the 7.5 Lf and 15 Lf doses was similar. After the third injection, the 15 Lf dose induced significantly more and larger local reactions. Therefore, the lower dosage may be preferable for booster injections.

The results of the present study and our previous booster study (9), which showed that 7.5 Lf of diphtheria toxoid can safely be given to adults, differ from findings of earlier studies where such doses in adults were found to elicit numerous and serious adverse reactions (3, 4). The previous studies were however conducted in an adult population with a higher degree of natural immunity to diphtheria. Also, the early studies used crude vaccines of 20–30% purity with regard to diphtheria toxoid. A third factor of possible importance is purification of the toxin prior to toxoiding in the preparation of the regular Swedish diphtheria vaccine. The early studies used vaccines that were purified after toxoiding, a method still practiced in preparation of the majority of diphtheria toxoids in current use.

The role of high preimmunization antitoxin titers does not seem to be great. In order to simulate the routine clinical situation, the inclusion criterion in the present study was a history of immunization of the subject. Therefore, the finding that almost 70% of the vaccinees showed serological evidence of previous immunization was not surprising. Despite the antitoxin titers, the reactions were very moderate. The same lack of severe reactions was noted in our booster study where many subjects had high prevaccination diphtheria antitoxin levels (9).

Impurities in the vaccine were shown to be a cause of side-effects as early as 1950 by Pappenheimer et al. (4) who compared a crude vaccine with a highly purified preparation. The study demonstrated a lower rate of adverse reactions with the highly purified preparation. The regular toxoid vaccine used in the present study had the same degree of purity as the highly purified preparation used by Pappenheimer et al. (4).

The purification of the toxin prior to toxoiding, as compared to the more common practice of purification after toxoiding, seems to emerge as an important factor in the reduction of side-effects. Most manufacturers use a medium containing enzymatic digests of bovine origin to culture the diphtheria bacteria (PW-8 strain) (10).

Formaldehyde treatment before purification results in a large amount of impurities, some of bacterial and some even of bovine origin, which are covalently linked to the toxoid and therefore impossible to remove (11). One way to avoid this is to purify the toxin before formaldehyde treatment. In another study (12) we found that the side-effects of toxoid vaccines were associated with the tetanus toxoid content and not with the diphtheria toxoid content (12). The regular Swedish tetanus toxoid has a similar degree of purity as the diphtheria toxoid, as measured in Lf/mg protein-N, but is purified after toxoiding which probably results in a more crude product, even if the tetanus bacilli are grown in a semisynthetic medium. The same observation was made in

Table 5: Number of previously non-immunized individuals (group A) with antitoxin levels up to 0.01, 0.1 and 1.0 IU/ml after injection II and injection III.

Postvaccination titer	Injection II[a]		Injection III[b]	
	7.5 Lf (n = 29)	15 Lf (n = 36)	7.5 Lf (n = 29)	15 Lf (n = 36)
≥ 0.01 IU/ml	6 (21%)[c]	20 (56%)[c]	29 (100%)	36 (100%)
≥ 0.1 IU/ml	0 (0%)	0 (0%)	23 (79%)	34 (94%)
≥ 1.0 IU/ml	0 (0%)	0 (0%)	8 (28%)	16 (44%)

[a] Samples collected eight months after injection II prior to injection III.
[b] Samples collected eight weeks after injection III.
[c] $p < 0.01$.

a booster study in 6-year-olds (13) where as little as 0.75 Lf tetanus toxoid in combination with 2.5 Lf diphtheria toxoid induced equal or higher rates of adverse reactions than 7.5 Lf diphtheria toxoid given to adults (9).

The purity of the diphtheria and tetanus toxoids and the production method might influence the size of the dose of single or combined vaccine that can be administered for immunization of adults. The worldwide recommended dose is usually not higher than 2 Lf diphtheria toxoid in combination with varying amounts of tetanus toxoid (3, 14, 15). Such a low diphtheria toxoid dose was found in our previous study to be insufficient to induce protection in a population where antitoxin titers had decreased to very low levels (12). One exception to the low dose recommendations is found in Denmark where the recommended dose is 12.5 Lf diphtheria toxoid for basic immunization of adults (5, 16). Military recruits have safely received such booster doses in combination with 12 Lf tetanus toxoid (16). To our knowledge, Denmark is also the only country where both toxoids are made by purification of the toxins prior to toxoiding.

The present study demonstrates the need for adequate doses of diphtheria toxoid for long-term protection. Although titers in all truly non-immune vaccinees reached the $\geqslant 0.01$ IU/ml level after three injections of either doses, titres in over 20% of the 7.5 Lf group failed to reach the 0.1 IU/ml level and in over 70% failed to reach the 1.0 IU/ml level. Diphtheria antitoxin titers drop with time (16), but after basic immunization the protection is supposed to last for at least ten years. The importance of higher doses was shown by the results in the present study after two injections when titers reached the 0.01 IU/ml level in significantly fewer individuals in the 7.5 Lf group than in the 15 Lf group (21% versus 56%). During an epidemic such individuals may have poor protection for many months in spite of an intensified vaccination program.

In conclusion, the present study using a regular vaccine administered in doses of 15 Lf showed that such a dosage is necessary to reach a titer of 0.1 IU/ml after three injections in 90% of previously non-immune vaccinees. These observations confirm the findings of Scheibel et al. (17) and Larsen et al. (5) who showed that basic immunization of adults with 1 Lf, 5 Lf or 6.25 Lf did not induce adequate titer levels in adults. Vaccine purity and the purification method used may be crucial for the safe administration of higher doses in adults. The use of pure products in high dosages seems to be the preferable way to induce and maintain adequate diphtheria antitoxin levels.

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