Contents

Preface

This series of modules on the immunological basis for immunization has grown out of the experience of persons working with the WHO Expanded Programme on Immunization (EPI). The EPI was established in 1974 with the objective of expanding immunization services beyond smallpox, with emphasis on providing these services for children in developing countries.

Six vaccine-preventable diseases have been included within the EPI since its beginning: diphtheria, measles, pertussis, polio, tetanus, and tuberculosis. To protect newborns against neonatal tetanus, tetanus toxoid is administered to the mother either during her pregnancy or prior to pregnancy during the childbearing years.

Two more vaccine preventable-diseases will be addressed by the EPI during the 1990s. The World Health Assembly has set the target of including yellow fever vaccine in the EPI by 1993 in countries where this disease poses a risk. Hepatitis B vaccine is being added gradually, with the target date of 1997 for incorporation of this vaccine in the immunization programme in all countries.

Titles of the nine modules in this series are listed inside the front cover of this module. They are intended to provide information on the immunological basis for WHO-recommended immunization schedules and policies. They have been prepared for the following main audiences:

- immunization programme managers, whose questions and concerns caused this series to be written,
- consultants and advisers on immunization activities,
- teachers of courses on immunization at the university level and facilitators of workshops,
- medical and nursing students as part of the basic curriculum,
- laboratory scientists providing diagnostic or research services for vaccine-preventable diseases, and
- scientists involved in basic research aimed at improving the delivery of vaccines or providing improved vaccines.

Other modules in this series and additional materials on the EPI are available from the Expanded Programme on Immunization, World Health Organization, 1211 Geneva 27, Switzerland.

Diphtheria

1. Diphtheria Toxin

Diphtheria is a bacterial disease in which the clinical manifestations result from the action of an extracellular substance (exotoxin) produced by *Coryebacterium diphtheriae,* a club-shaped bacterium.

Diphtheria toxin, a protein with a molecular weight of 62 000, is known as an A-B type toxin, and consists of two fragments designated A and B. Fragment B is necessary for binding to surface receptors and penetration into cells. Fragment A is responsible for its toxicity and exerts its action by interfering enzymatically with protein synthesis, finally producing the death of the cells. Diphtheria toxin exerts its effects on distant tissues and organs, especially the heart (myocarditis) and the peripheral and cranial nerves (weakness progressing to paralysis).

All toxigenic strains of *C. diphtheriae* produce an identical toxin. For a diphtheria strain to become toxigenic, it must be infected by a particular bacterial virus, the bacteriophage. This process is called lysogenic conversion. The introduction of a toxigenic strain of *C. diphtheriae* into a community may initiate an outbreak of diphtheria by transfer of the bacteriophage to non-toxigenic strains carried in the respiratory tracts of its inhabitants. Both toxigenic and non-toxigenic strains of *C. diphtheriae* may be isolated during such an outbreak *(Mortimer 1988).*

When treated with formaldehyde and heat, diphtheria toxin loses its ability to bind to cells and its enzymatic activity, but retains its immunogenicity. This treatment converts diphtheria toxin to a toxoid, which is commonly used to immunize against diphtheria. In some conditions that are not completely understood, the process of conversion from toxin to toxoid may be reversible. In 1948, a Japanese pharmaceutical company distributed diphtheria toxoid which reversed to toxin and many children who had been immunized with this preparation died *(Kurokawa & Murata 1961).* Since that disaster, manufacturers have instituted procedures to prevent reversion to a toxic state.

Diphtheria is acquired through personal contact; the incubation period is generally 2 to 5 days. Diphtheria is a disease affecting the tonsils, the pharynx, the larynx, and the nose. In developing countries skin diphtheria is common, with lesions indistinguishable from, or a component of, impetigo. Laryngeal diphtheria is serious, while nasal diphtheria may be mild, often chronic. Inapparent infections outnumber clinical cases. Late effects of diphtheria include cranial and peripheral motor and sensory palsies and myocarditis. The case fatality rate is 5% to 10%.

2. The Nature of Immunity to Diphtheria

Immunity against diphtheria is antibody-mediated. Because the lethality of diphtheria is almost entirely due to diphtheria toxin, immunity to diphtheria depends primarily on antibody against the toxin. This antibody, called antitoxin, is primarily of the IgG type. Antitoxin is distributed throughout the body and can pass easily through the placenta, providing passive immunity to the newborn during the first few months of life. Diphtheria antitoxin may be induced by diphtheria toxin produced by *C. diphtheriae* during the disease or the carrier state, or by diphtheria toxoid following immunization. These antibodies are identical and cannot be distinguished by any existing techniques.

3. Techniques to Measure Antibody Response

Two important properties of diphtheria toxin are utilized to determine the activity of diphtheria antibodies. The first is its distinct dermonecrotic capacity, e.g. the ability to produce an inflammatory reaction when injected intradermally into the skin of humans or animals. This property is used for the Schick test in humans and to determine neutralization antibody in *in vivo* systems in animals. The second property is the capacity of diphtheria toxin to block protein synthesis in cultured mammalian cells, thus causing cell death. This capacity is used to determine diphtheria antibody levels in an *in vitro* neutralization test using cells sensitive to diphtheria toxin. Additional *in vitro* tests to measure diphtheria

antibodies include the passive hemagglutination test and the enzyme-linked immunosorbent assay (ELISA). For ethical, economic, and practical reasons there is growing interest in *in vitro* techniques.

3.1 Schick test

In early studies the Schick test was the standard procedure. To perform the Schick test, 0.1 ml of diphtheria toxin (about l/50 of the minimal lethal dose for a guinea pig) is injected intradermally on the volar surface of the forearm of the person being tested. If the person has circulating diphtheria antitoxin at a level of 0.01 to 0.03 IU/ml, the injected toxin will be neutralized and no reaction will occur. A positive reaction signifies lack of antitoxin and is characterized by inflammation appearing after 24 to 36 hours and persisting for 4 days or longer. A control test is always performed on the opposite arm using toxin inactivated by heating to 60°C for 15 minutes. A positive reaction to inactivated toxin and a positive reaction to toxin indicates an allergic response to toxin. The Schick test is inexpensive and its results correlate well with serum antitoxin levels. However,. in addition to technical difficulties in performing an intradermal injection, the test also requires two injections (one of test toxin and the other of control toxin) and two visits (one for the injections and a second to read the results 4 to 7 days later). The results of the Schick test depend on the skin reactivity to injected toxin *(Vahlquist 1949);* skin anergy often found in newborns and young infants may result in negative results erroneously interpreted as an evidence for immunity *(Papadatos et al. 1964, Vogelsang & Krivy 1945, Wright & Clark 1944).*

3.2 Neutralization test on animals

The *in vivo* neutralization test is usually performed on the depilated skin of rabbits *(Jensen 1933)* or guinea pigs *(Glenny & Llewellyn-Jones 1931).* Different dilutions of serum mixed with fixed amounts of diphtheria toxin are injected into the depilated skin of the animal and the antitoxin concentration is estimated based on the presence or absence of an inflammatory reaction. Results of the *in vivo* neutralization test may differ depending on the avidity of the antibody tested, the concentration of toxin used in titration, and the species of laboratory animal. The test is laborious, time-consuming, expensive, and requires suitable animals. However, the *in vivo* neutralization test shows the functional capacity of antibody to neutralize toxin. In contrast, the *in vitro* tests show not only the neutralization of toxin by antibody present in the test serum, but also reactions between other antigen-antibody systems. Therefore, the *in vivo* neutralization test should be used to calibrate and verify the *in vitro* test(s) used routinely in the laboratory.

3.3 Neutralization test on microcell culture

The neutralization test on microcell culture is based on the observation that the survival of mammalian cells in culture is inhibited by diphtheria toxin. This effect is neutralized when diphtheria antitoxin is present in serum samples *(Miyamura et al. 1974a and 1974b).* The titration of the antitoxin in the serum samples is done in plastic microtissue culture plates, in which dilutions of test sera are mixed with challenge toxin. After a short incubation, Vero (green monkey renal epithelium) cell or HeLa cell suspension in a special culture medium is added. After incubation for 3 or 4 days, results are read as a change in the color of the reagents in the microtiter plate wells. The color change is due to the metabolic formation of acid, which changes the pH. Vero cells are more sensitive to diphtheria toxin since they have large numbers of binding sites (receptors) and they take up the toxin in a highly specific, time- and temperature- dependent manner *(Middlebrook et al. 1978).* When a serum dilution contains antitoxin in excess, the cells continue to grow, and the color of the medium changes from red to yellow. Recent improvements in the microcell neutralization test include spectrophotometric determination of the equivalence point between toxin and antitoxin and computer analysis of adsorption values *(Aggerbeck & Heron 1991).*

The *in vitro* neutralization test on microcell culture is highly sensitive (minimum detectable level 0.005 IU/ml), reproducible, and requires a minimum amount of serum. Up to 100 serum specimens may be titrated in one test run. The test has been used to determine the diphtheria antibody response of humans *(Palmer et al.* 1983) and animals *(Kreeftenberg et al. 1985).* For both human and guinea pig sera, there is good correlation between the results the *in vitro* neutralization test and the *in vivo* neutralization test on rabbit skin *(Kjeldsen et al. 1988, Kriz et al. 1974, Miyamura et al. 1974a and 1974b).* A modified *in vitro* neutralization test has been developed *(Padovan et al. 1991).* All cell culture tests, however, require staff with special skills in tissue culture techniques and a laboratory with special equipment.

3.4 Passive hemagglutination

The passive hemagglutination (HA) test is frequently used to test for diphtheria antibody *(Allerdist & Ehrengut-Lange 1982, Cellesi et al. 1989a, Crossley et al. 1979, Fulthorpe 1962, Galazka & Abgarowicz 1967, Galazka & Kardymowicz 1989, Koblin & Townsend 1989, Millian et al. 1967, Ruben et al. 1978, Thorley et al. 1975).* In the HA test, sheep, turkey, horse, or human red cells (previously treated with tannic acid or diazotized benzidine and sensitized with diphtheria toxoid) are agglutinated by

diphtheria antibody. The HA test is inexpensive and can be performed in a modestly equipped laboratory. The HA test is rapid (results available in one hour), reproducible, and sensitive. Results of the HA test for diphtheria correlate well with results of the neutralization test, although the HA test tends to underestimate low concentrations of diphtheria antibody *(Galazka & Abgarowicz 1967, Scheibel et al. 1962, Simonsen 1989).* This is in contrast to the HA for tetanus, which tends to overestimate antibody titers (see Module 3). The results of the HA test for diphtheria can be distorted by non-specific agglutinins in the sera directed against the antigens on the surface of the red cell. These effects can be minimized by heating the sera at 56ºC, pre-treating sera with 2-mercaptoethanol, or absorbing the sera with unsensitized erythrocytes.

3.5 ELISA

The enzyme-linked immunosorbent assay (ELISA) involves the binding of antigen to polystyrene tubes. Exotoxins, such as diphtheria toxin (or toxoid), that have a highly lipophilic moiety in their molecule, coat the tubes efficiently *(Svenson & Larsen 1977).* Results of the direct ELISA test are highly reproducible *(Camargo et al. 1984, Melville-Smith & Balfour 1988).* When the antibody level is above 0.1 IU/ml, the results of the ELISA test correlate well with results of the *in vivo* neutralization test in guinea pigs *(Knight et al. 1986)* and the results of the neutralization test in tissue culture *(Melville-Smith & Balfour 1988).* When the antibody titer is low, the results of the ELISA test correlate poorly with results of the neutralization test. Titers of 0.001 IU/ml with the neutralization test can be 10 to 100 times higher (0.01 to 0.1 IU/ml) with the direct ELISA test *(Knight et al. 1986, Melville-Smith & Balfour 1988).*

Better correlation has been reported with modified versions of the ELISA test *(Hendriksen et al. 1989, Knight et al, 1986).* One modified ELISA test, the toxin binding inhibition test (ToBI), shows good correlation (r = 0.91) with the *in vitro* neutralization test in Vero cells *(Hendriksen et al. 1989).*

The main advantage of the ELISA test is its ability to measure IgG-specific diphtheria antibodies *(Dengrove et al. 1986).*

4. Protective Level of Antibodies

It is believed that a circulating diphtheria antitoxin level of 0.01 IU/ml, as determined by the neutralization test in animals or in cell culture, provides clinical immunity against disease. This diphtheria antitoxin level corresponds to a negative Schick test. There is good correlation between clinical protection and the presence of serum antitoxin, whether this results from disease or immunization. In the 1984 diphtheria epidemic in Sweden, all seven patients who died or showed neurological complications had antitoxin titers < 0.01 IU/ml, whereas 92% of symptom-free diphtheria carriers showed high antitoxin titers, above 0.16 IU/ml *(Bjorkholm et al. 1986).* However, it has also been shown that there is no sharply defined level of antitoxin that gives complete protection from diphtheria *(Ipsen 1946).* A certain range of variation must be accepted; the same degree of antitoxin may give an unequal degree of protection in different persons. Other factors may influence the vulnerability to diphtheria including the dose and virulence of the diphtheria bacilli and the general immune status of the person infected *(Christenson & Bottiger 1986).* Thus, an antibody concentration between 0.01 and 0.09 IU/ml may be regarded as giving basic immunity, whereas a higher titer may be needed for full protection. In some studies that used *in vitro* techniques, a level of 0.1 IU/ml was considered protective *(Cellesi et al. 1989a, Galazka & Kardymowicz 1989).*

5. Development of Antibodies due to Natural Stimulation

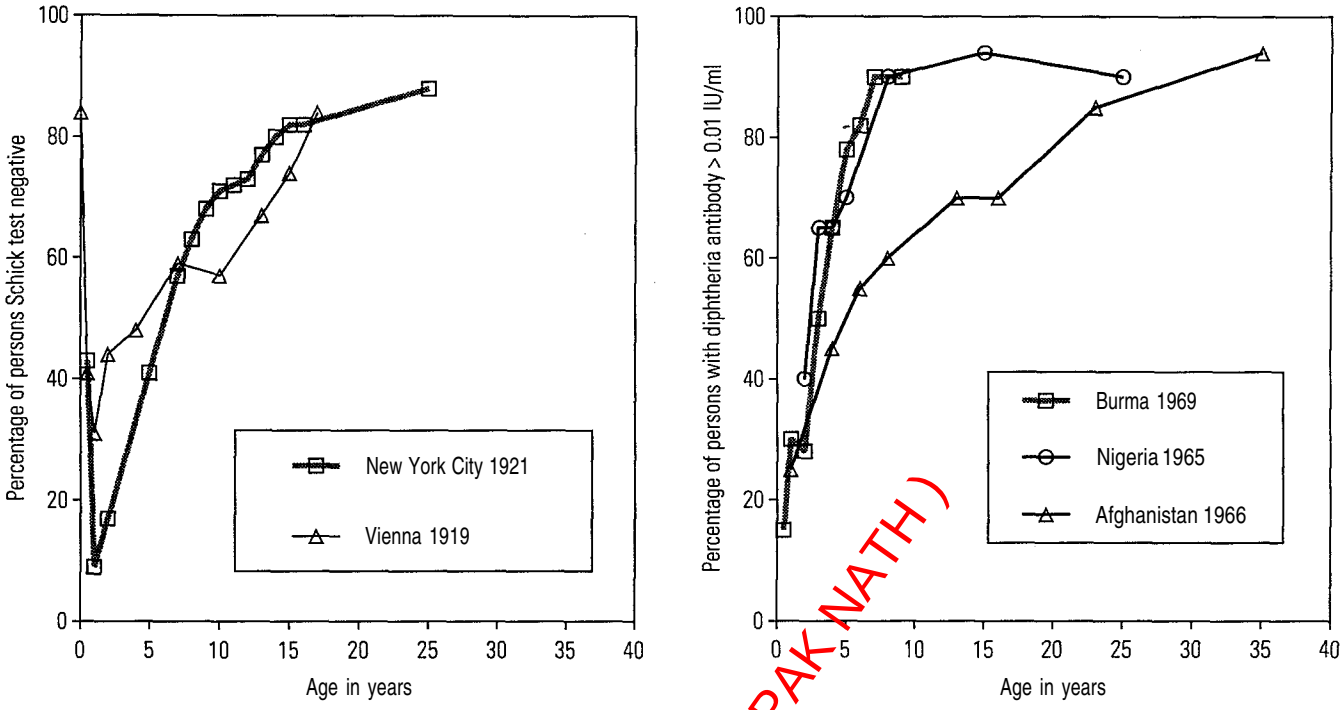
5.1 The pre-vaccine era in industrialized countries

In the pre-vaccine era, when circulation of *C. diphtheriae* organisms was frequent and the prevalence of diphtheria cases was high, natural immunity acquired by apparent infection or inapparent infection was the only mechanism of acquiring immunity. Diphtheria was primarily a disease of children. Early studies in Vienna in 1919 and New York City in 1921 showed a typical immunity pattern. Most newborn infants had antibody acquired passively from their mothers; this passive antibody waned between 6 and 12 months of age. Then immunity rose rapidly in early childhood, reflecting increasing exposure to diphtheria organisms (Figure 1). By the age of 15 to 20 years, nearly all persons had acquired natural immunity to diphtheria. This pattern was observed in the United States in 1935 *(Chason 1936),* Poland in 1954 to 1955 *(Daniel et al. 1957),* and Japan in 1955 *(Miyamura et al. 1983).*

5.2 Developing countries in the 1960s

Data from developing countries suggest that the pattern of acquiring diphtheria immunity in the 1960s resembled the pattern seen in Europe and the United States in the pre-vaccine era. Such data are

Figure 1. Natural diphtheria immunity in the pre-vaccine era in industrialized countries, 1919 to 1921, and in developing countries, 1965 to 1969. (Zingher 1923 for New York City; Stransky & Felix 1949 for Vienna; Kriz et al. 1980 for Burma, Nigeria and Afghanistan)



available from Afghanistan, Burma and Nigeria *(Kriz et al. 1980),* India *(Chakraborty & Choudhuri 1969, Robinson et al. 1964, Suri et al. 1967),* Sri Lanka *(Gunatillake & Taylor 1981),* and Zaire *(Muyembe et al. 1972).* The process of acquiring natural immunity was rapid; in some countries more than 80% of children were immune by 10 years of age (Figure 1).

5.3 Developing countries today

In developing countries, a high rate of skin infections caused by *C. diphtheriae* creates a primary reservoir of diphtheria organisms. This appears to be an important factor in the early development of natural immunity against the disease *(Baum et al. 1985, Bray et al. 1972).* Because skin infections are endemic, developing countries do not report outbreaks and epidemics of diphtheria, which were characteristic of the prevaccine era in industrialized countries. In developing countries today, the age distribution of diphtheria cases reflects the immunity status of the population and diphtheria is mostly a disease of children younger than 15 years of age.

Socioeconomic changes, especially migration from rural to urban areas, and sociocultural changes, including improved hygiene and different styles of living, may change the epidemiological patterns of diphtheria. The disease could emerge as an epidemic disease with more serious forms, including lethal laryngeal and pharyngeal diphtheria.

6. Immunity due to Immunization

6.1 Development and duration of vaccine-induced immunity

There is an age-related host response to immunization with diphtheria toxoid. The most important factor is the modifying effect of passively acquired maternal antibodies in young infants *(Halsey & Galazka 1985).* Early studies demonstrated that infants without maternal antibodies respond to diphtheria toxoid almost as well as older children *(Barr et al. 1950, Vahlquist 1949).* A recent study in Japan found that the diphtheria antibody response to diphtheria-pertussis-tetanus (DPT) vaccine containing acellular pertussis vaccine was similar in children 3 to 8 months and 24 to 30 months of age (Table 1).

A level of passive antibody higher than 0.1 IU/ml temporarily interferes with active immunization of infants, whereas a level below 0.02 IU/ml does not *(Barr et al. 1950, Vahlquist 1949).* Studies in the United States suggest that passively-acquired diphtheria antibody may influence the early response to DPT vaccine. Children with a high level of diphtheria antibody in cord serum (0.24 IU/ml), showed a decline in antibody level to 0.05 IU/ml at 2 months of age, and the first dose of DPT vaccine given at two months did not change the declining trend in antibody level *(Anderson et al. 1988).* Other studies

Table 1. Diphtheria antibody response to DPT vaccine containing acellular pertussis component in children of various ages *(Kimura et al. 1991).*

Age (months)	Geometric mean diphtheria antibody titer in IU/ml				
	Before 1st dose	Before 3rd dose*	After 3rd dose	Before booster**	After booster
3 to 8	< 0.01	0.8	1.6	0.3	6.7
9 to 23	< 0.01	0.5	1.5	0.3	10.2
24 to 30	< 0.01	0.7	1.7	0.3	8.3

** First three doses given at intervals of 6 to 10 weeks.*
*** Booster (4th) dose given 12 to 18 months after the 3rd dose.*

show that when the level of diphtheria antibody at the time of the first injection of DPT vaccine is below 0.1 IU/ml, the suppressive effect of this passively acquired antibody is less evident (Figure 2). Passive diphtheria antibody seems to show a transient suppression of the antibody response to the second injection of DPT vaccine, but no effect is seen on the response to the third injection of DPT vaccine.

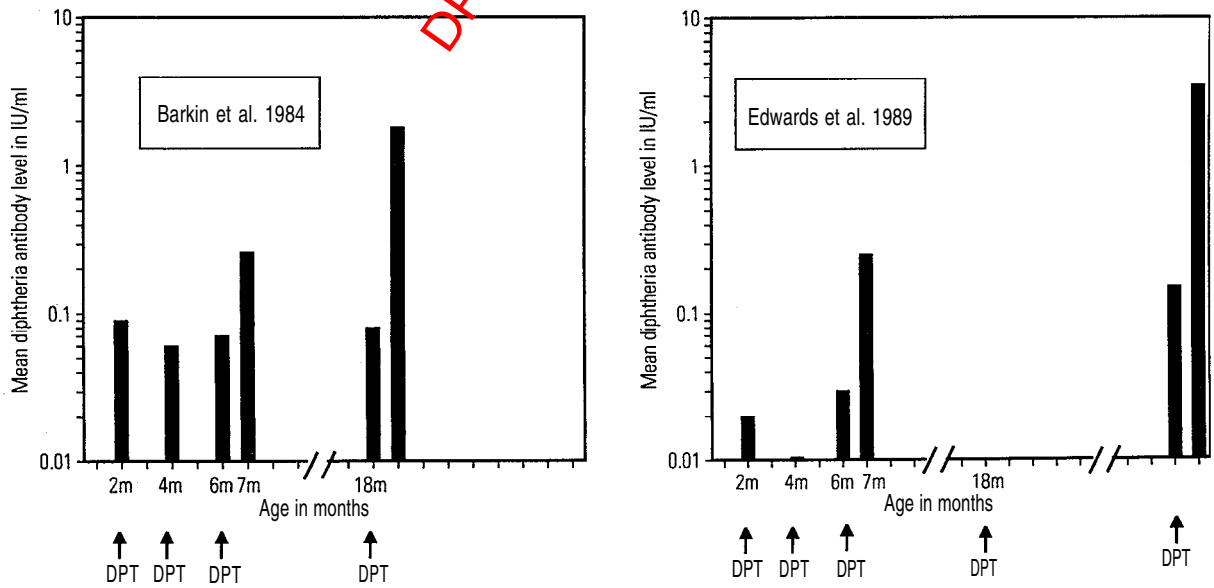
In areas where *C. diphtheriae* circulates in the population, and especially where cutaneous forms of diphtheria are common, mothers and their infants may have high diphtheria antibody titers. In Mali, 87% of cord blood samples had an antibody level of more than 0.1 IU/ml and half of them had a level of at least 1 IU/m1 *(Allerdist et al. 1981).* On the other hand, in areas where the reservoir of *C. diphtheriae* is reduced (see section 6.2), mothers are less likely to have immunity and their babies seldom acquire passive protection. There is a logarithmic rate of loss of passively-acquired antitoxin in babies, which averages about 14% per week *(Barr et al. 1949).* The half-life of diphtheria antitoxin is about 30 days *(Anderson et al. 1988).*

Primary immunization with three doses of DPT vaccine stimulates antibody levels that considerably exceed the minimum protective level (Figure 2). In the two studies presented in Figure 2, the primary series of DPT vaccine was given at 2, 4, and 6 months of age, and a booster dose was administered at 18 months of age. The antibody level starts to increase after the second dose of DPT vaccine and the level is considerably higher after the third dose. After the primary series, 94% to 100% of children have antibody levels higher than 0.01 IU/m1 *(Barkin et al. 1984, Bhandari et al. 1981, Chen et al. 1956, Guerin et al. 1988, Pichichero et al. 1986, Schou et al. 1987),* with the mean level ranging between 0.1 and 1 IU/ml *(Anderson et al. 1988, Barkin et al. 1984, Barkin et al. 1985, Edwards et al. 1989, Kimura et al. 1991),* or more *(Bhandari et al. 1981).*

The nature of the pertussis component of DPT vaccine does not seem to affect the immune response to the diphtheria component of the vaccine. Several studies show that the diphtheria antibody response following DPT containing whole cell- or acellular-pertussis components is similar *(Anderson et al. 1988, Edwards et al. 1989, Pichichero et al. 1987).*

The percentage of children with diphtheria antibody above 0.01 IU/ml following two doses of DPT vaccine administered two months apart is similar to that following three doses of DPT vaccine

Figure 2. Diphtheria antibody levels in children immunized with a primary series of DPT vaccine at 2, 4, and 6 months of age and following one or two booster doses.



administered with one month between doses. However, the mean antibody levels are significantly lower with a two-dose schedule *(Bhandari et al. 1981, Guerin et al. 1988)* and it is likely that the duration of immunity after two doses is shorter than after three doses.

The duration of immunity after the primary series of diphtheria toxoid in infancy has been studied in Denmark, where primary vaccination used DT vaccine (1950 to 1961), DPT vaccine (1961 to 1970), or DT-polio vaccine (after 1970). Except for military recruits, who receive a dose of DT vaccine, revaccinations are not routinely given. Serum antitoxin concentration, relative to time after vaccination, shows a steep decline. immediately after vaccination, followed by an exponential fall-off. Comparison of results of early and recent studies shows that the diphtheria antitoxin levels in school children have been steadily declining from the 1940s through the 1950s to 1985, although the number of doses of diphtheria vaccine administered has remained the same. Tetanus antitoxin concentration does not show such a decline. The current lower diphtheria immunity among school children in Denmark may be due to less exposure to diphtheria organisms and subsequent reduced opportunity to become naturally immune *(Schou et al. 1987, Simonsen et al. 1987, Simonsen 1989).*

The duration of post-vaccination immunity also differs in the early and recent studies performed in the United States. In the 1960s, only 10% of children had lost diphtheria immunity 7 to 13 years following primary immunization with diphtheria toxoid *(Volk et al. 1962).* In recent studies, diphtheria immunity declined more rapidly; 10% of children lost immunity by one year following the primary series *(Pichichero et al. 1987),* 67% of children lacked immunity after 3 to 13 years, and 83% after 14 to 23 years *(Crosley et al. 1979).*

In France and Taiwan, the percentage of children lacking diphtheria immunity was 25% and 37%, respectively, one year after three doses of DPT vaccine *(Chen et al. 1956, Guerin et al. 1988).* Other studies found that during the first year after the primary series of three DPT vaccine doses, the mean level of diphtheria antibody declined fourfold to fivefold *(Kimura et al. 1991, Pichichero et al. 1986).*

In contrast, studies in England and Italy showed that 96% to 100% of children immunized with three doses of DPT or DT still had protective diphtheria antibodies 4 to 8 years later *(Cellesi et al. 1989a,* Jones *et al. 1989).*

Differences in these results may be caused by different vaccines, different vaccination schedules, and different levels of exposure to *C. diphtheriae* with natural reinforcement of diphtheria immunity. The duration of active immunity in children not continually exposed to diphtheria may be shorter than in similar groups of children from communities where diphtheria is prevalent.

A booster dose administered at the end of the second year of life or at the age of 4 to 6 years stimulates abundant production of diphtheria antibody with the mean levels above 1 IU/ml *(Anderson et al. 1987, Barkin et al. 1984, Edwards et al. 1989, Kimura et al. 1991, Lewis et* al. *1986, Pichichero et al. 1987).*

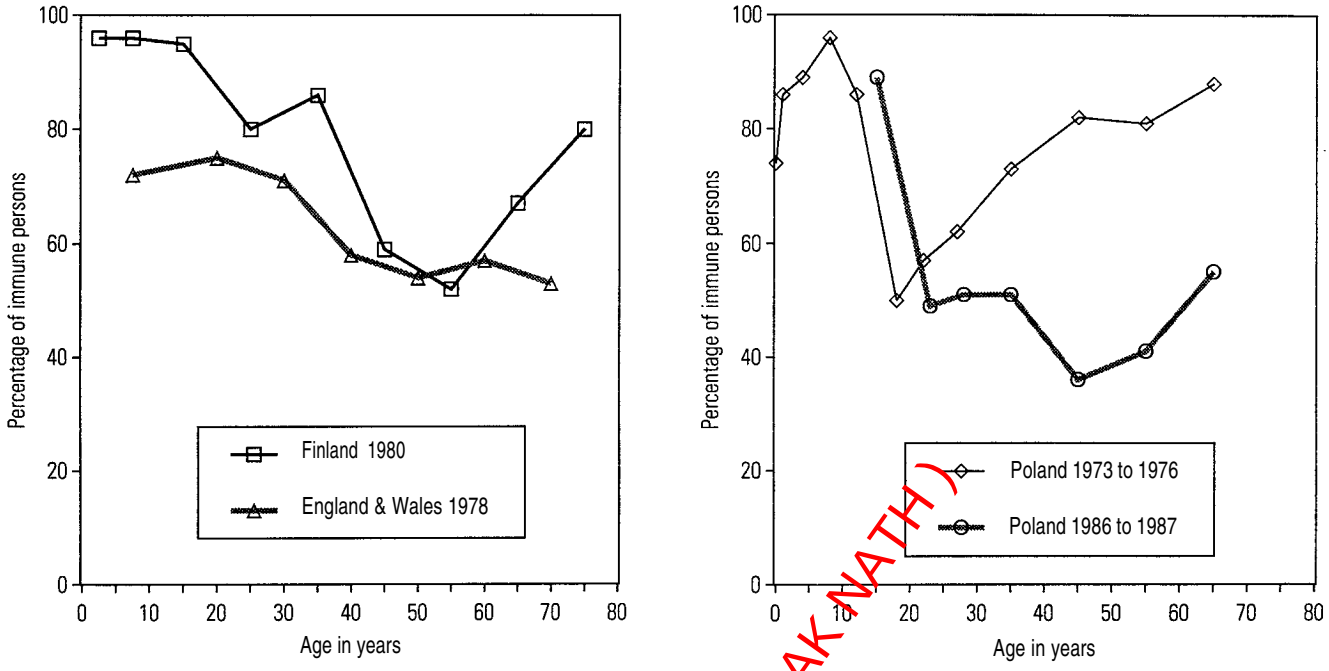
The outcome of revaccination of adults depends on several factors, including the schedule and potency of toxoids used for primary immunization, the time since the last dose of diphtheria toxoid, and the age of the vaccinees. In Denmark, toxoids with a large dose of antigen are used for primary immunization. Revaccination of Danish adolescents, military recruits, or adults with Td vaccine containing a reduced amount of diphtheria toxoid stimulated rapid and vigorous production of diphtheria antitoxin with the mean level exceeding 1 IU/ml *(Simonsen et al. 1986a and 1986b, Volk et al. 1962).* Revaccination response decreased with increasing time from primary vaccination, but even if more than 20 years had elapsed, safe individual protection could be obtained by a single booster dose *(Simonsen 1989).* However, results of other studies showed that 35% to 88% of adults seroconverted following a single dose of vaccine with a reduced amount of diphtheria toxoid. *(Allerdist &1Ehrengut-Lange 1982, Palmer et al. 1983, Ruben et al. 1978).* A small amount of diphtheria toxoid was effective in inducing a secondary response in already primed school-children or adults, but it is insufficient to stimulate an effective immune response in those who had never been actively immunized or who had not acquired basic immunity by natural means *(Feery et al. 1981, Galazka & Olakowski 1962, Trinca et al. 1975).* The effective course of primary immunization should include three doses of toxoid of reduced potency with an interval of 4 to 6 weeks between the first and second dose and 6 to 12 months between the second and third dose *(Feery et al. 1981).*

6.2 Changes in the immune profile of various age groups following mass immunization

Mass immunization programmes result in considerable reduction of diphtheria incidence. They also result in profound and important changes in the immune status of different age groups. Although direct comparison of the immunity levels in different countries is complicated by different methods used to determine diphtheria immunity, some common characteristics may be noted.

Children acquire a high level of diphtheria immunity as the result of childhood immunization. The level of immunity declines in late childhood and

Figure 3. Diphtheria immunity in the post-vaccine era in Finland, England and Wales, and Poland *(Ketrulla et al. 1980, PHLS 1978, Galazka & Sporzynska 1979 and Galazka & Kardymowicz 1989).*



adolescence, depending on the schedule of immunization with diphtheria toxoid and the incidence of diphtheria (Figure 3). High levels of immunity in children result in reduced incidence of disease. As diphtheria has become rare, opportunities for acquiring or reinforcing natural immunity have also been reduced.

Adults might again become susceptible to diphtheria due to reduced opportunities to boost immunity through subclinical infections. The likelihood of having protective antibody levels decreases with age and in some industrialized countries less than 50% of adults may be immune to diphtheria. The age group with the lowest level of diphtheria antibodies is 20 to 40 year olds in Germany *(Nauman et al. 1983),* various areas of the ex- USSR *(Dalmatov et al. 1986, Maksimova et al. 1984, Schwartz et al. 1987),* and Japan *(Miyamura et al. 1974a); 40* to 50 year olds in Poland *(Galazka & Kardymowicz 1989),* Australia *(Forsell 1972),* and England *(PHLS 1978);* and persons older than 50 years in Denmark *(Kjeldsen et al. 1988),* Finland *(Kerttula et al. 1980),* Sweden *(Christenson & Bottiger 1986),* and the United States *(Sargent et al.* 1984). In some countries, elderly persons are still immune to diphtheria, probably due to their natural immunity developed during past epidemics of diphtheria. In one province of China where diphtheria incidence has been considerably reduced following immunization the lowest levels of immunity were noted in persons aged 10 to 20 years *(Expanded Programme on Immunization 1989b).*

In some countries, there is a difference between the immune status in males and females. In Denmark and Sweden (but not in Poland and the United States), the immunity among men older than 20 years of age is higher than among women older than 20 years of age *(Christenson & Bottiger 1986, Galazka & Kardymowicz 1989, Sargent et al. 1984, Simonsen* 1989). This may be explained by the fact that many men in these countries served in the military (in Denmark 26% to 83% in various age groups — *Simonsen et al. 1987)* and received booster injections of diphtheria-tetanus toxoid *(Christenson & Bottiger 1986; Kjeldsen et al. 1988, Simonsen 1989).* In other countries, males appear to be significantly less protected than females; this fact is difficult to explain *(Cellesi et al. 1989a).*

A large pool of susceptible persons creates an epidemic potential. Recently, an increased incidence of diphtheria has been noted in several European countries. During the early and mid-1980s, small outbreaks of diphtheria were reported from Sweden, Germany, and Portugal *(Bjorkholm et al., 1986, Expanded Programme on Immunization 1988a, Galazka & Keja 1988, Rappuoli et al. 1988).* In-the eastern part of Germany, 6 cases of diphtheria occurred in 1986 to 1989 after 12 years of freedom from indigenous diphtheria *(Expanded Programme on Immunization 1991).* In the former Soviet Union, diphtheria incidence started to increase in the early 1980s, reached its first peak in 1983 to 1985 and its second peak in 1990 to 1991. A total of 1876 and 3897 diphtheria cases were reported in Russia in 1991 and 1992, respectively *(Expanded Programme on Immunization 1993, Galazka 1992).* The epidemic spread to Ukraine, where 1552 cases were reported in 1992. In

many of these outbreaks, adolescents and adults were mainly affected.

Changes in the age distribution of diphtheria cases have also been seen in developing countries. In Jordan, children below 10 years of age were the most affected group but in an outbreak in 1982 and 1983, adolescents and adults were largely involved *(Khuri-Bulos et al. 1988).* In Indonesia, the reported morbidity rates for the age group 0 to 4 years fell below the reported rates for the age group 5 to 9 years *(Kim-Farley et al. 1987).* These observations may reflect changing immunity status in various age groups caused by increasing coverage rates with DPT vaccine. An outbreak of diphtheria primarily involving adults has been reported from China; of 103 reported cases, 80 were over 16 years of age. This epidemic occurred in an area where diphtheria had been successfully controlled (no cases reported in the previous 3 years) and where the immunity among adults had declined *(Youwang et al. 1992).*

6.3 Strategies for immunization against diphtheria

There is no simple and universal schedule for immunization against diphtheria. The choice of an appropriate schedule depends on the epidemiological pattern of diphtheria and on the level of development of immunization services.

In developing countries where the reservoir of *C. diphtheriae* is still large and natural immunity plays a significant role in protection against the dangerous, pharyngeal form of the disease, the first priority is to ensure high coverage of infants with the primary series of three doses of DPT vaccine. Priority should be given to achieving at least 90% coverage.

In developing countries which have already achieved high coverage with three doses of DPT vaccine in children under one year of age, the policy of using a booster dose of DPT vaccine at the end of the second year of age and/or a dose of DT or Td at school entry should depend on the pattern of diphtheria and the availability of the vaccines. If diphtheria poses a significant health problem in preschool or school-age children, supplementary doses of diphtheria toxoid may be warranted. Data from serological studies which show declining antibody levels may serve as a valuable guide in deciding when booster doses are warranted. The main issue may be whether or not the child. would otherwise visit the health center for preventive health activities. If the child is present in any case, administering a fourth DPT dose (and perhaps a fifth dose of OPV) may be appropriate.

The use of DT or Td vaccine at school entry or leaving be important for providing anti-tetanus immunity for these age groups and will be discussed in the module on tetanus. Health authorities need to consider the time required to deliver these additional vaccine doses and balance this against the time needed for other services. The cost of additional doses should also be considered.

In developed countries, primary immunization usually-consists of three doses of DPT vaccine, given at intervals of 4 or more weeks, beginning at 2 or 3 months of age, and reinforced by a fourth dose given in the second year of life. The policy of using booster doses of vaccines containing diphtheria (and tetanus) toxoid varies considerably. In some countries, booster doses of DPT vaccine are given above the age of 3 years (Czechoslovakia, Hungary, United States). In many other countries, booster doses of DT vaccine are given at primary school entry and at school leaving. Many countries, however, give only monovalent tetanus toxoid to older school children.

The immunity level acquired in infancy and early childhood should be maintained through properly timed booster doses of DT or Td vaccine. Td vaccine should be used for older children or adolescents leaving primary or secondary schools.

Since the existence of a pool of susceptible adults creates an epidemic potential, the introduction of Td vaccine for high risk groups of adults should be considered. Some controversy surrounds this recommendation. Some authors propose immunizing adults with adult-type Td vaccine every 10 years and giving Td vaccine whenever tetanus toxoid is indicated, e.g. in treating wounds in emergency rooms *(Karzon & Edwards 1988).* Other authors recommend using Td vaccine for high risk groups, especially persons vulnerable to the acquisition of virulent *C. diphtheriae,* such as those travelling to developing countries, military personnel, medical staff, kindergarten and creche personnel, teachers, and alcohol and drug abusers *(Edwards. 1990, Galazka & Kardymowicz 1989).* Authors from Canada question the need for general revaccination of adults against diphtheria and tetanus *(Mathias & Schechter 1985),* arguing that the mortality from diphtheria remains at a relatively low level in Canada and that there is no obvious decrease in immunity against diphtheria after primary immunization. In Canada, however, hundreds of diphtheria cases were reported in the 1970s and naturally-acquired immunity may have contributed to present high levels of immunity.

An important issue in the immunization of adults against diphtheria is the reactogenicity of diphtheria toxoid. It is believed that diphtheria toxoid can induce local and, occasionally, general reactions in previously sensitized (immune) individuals. This problem may be largely overcome by using a low-dose, adult-type preparation of vaccine (Td) containing highly purified toxoids. However, even with Td vaccine, a significant proportion of vaccinees have mild

local reactions *(Allerdist & Ehrengut-Lange 1982, Trinca 1975)* and some vaccinees experience moderately severe local reactions and systemic symptoms *(Palmer et al. 1983).* Some authors have suggested pre-immunization screening for susceptibility by performing a Schick test or determining a diphtheria antibody level *(Palmer et al. 1983).* However, screening is cumbersome and time-consuming, and is not practical.

7. Implications for Immunization Programmes

The primary series of three doses of DPT vaccine given in infancy provides immunity against diphtheria for several years. The duration of the diphtheria immunity following primary immunization may differ considerably, depending on the epidemiological situation and the frequency of natural stimulation and reinforcement of immunity against diphtheria.

The global recommendation for diphtheria immunization is to apply an effective primary immunization in infancy and to maintain immunity throughout life. The immunization schedule should be tailored to specific conditions in a given country, taking into consideration the actual epidemiological pattern of diphtheria and the level of development of the immunization services.

In all countries, priority should be given to efforts to reach at least 90% coverage with three doses of DPT vaccine in children below one year of age.

In developing countries where diphtheria is endemic, the three primary doses will be enough to prevent diphtheria emerging as an epidemic disease. In these countries the process of maintaining immunity operates through natural mechanisms, including frequent skin infections caused by *C. diphtheriae.*

In other developing countries where a high immunization coverage rate has been achieved in children under one year of age, the policy of using further doses of vaccines containing diphtheria toxoid should depend on the epidemiology of diphtheria. If diphtheria poses a significant health problem in preschool or school-age children, supplementary doses of diphtheria toxoid should be considered. A fourth dose of DPT vaccine at the end of the second year of life and/or a dose of DT vaccine at school entry are the most frequently selected options.

In countries where diphtheria has been successfully controlled, the immunity level acquired through immunization in infancy and early childhood should be maintained through properly timed booster doses of DT or Td vaccines. These vaccines should be used for older children or adolescents leaving primary or secondary schools.

In many recent diphtheria outbreaks in the developed world, adolescents and adults were mainly affected. Serological investigations in several developed countries showed a low level of diphtheria immunity among adults aged 20 to 50 years. Such studies can serve as tools for reviewing existing immunization schedules. Since the existence of a pool of susceptible adults creates an epidemic potential, countries with low levels of diphtheria immunity in adults should consider the introduction of the adult type Td toxoid for high risk groups in the adult population.

Abbreviations

DPT	diphtheria-tetanus-pertussis vaccine
DT	diphtheria-tetanus vaccine, child type
ELISA	enzyme-linked immunosorbent assay
HA	passive hemagglutination test
IU	international units
Td	adult preparation of diphtheria and tetanus toxoids with a low amount of diphtheria toxoid
ToBi	toxin-binding inhibition test

References

Aggerbeck H, Heron I. Improvement of a Vero cell assay to determine diphtheria antitoxin content in sera. Biologicals 1991;19:71-76.

Allerdist H, Ehrengut W, Fofana Y. Diphtheria immunity in Mali (mothers and their neonates and children under two years of age). Tropenmed Parasit 1981; 32:274-275.

Allerdist H, Ehrengut-Lange J. Serokonversion nach Diphtherieschutzimpfung bei Jugendlichen und Erwachsenen. Eine Studie am Hamburger Krankenhauspersonal. Dtsch Med Wochenschr 1982;107:1755-1760.

Anderson EL, et al. Clinical and serological responses to acellular pertussis vaccine in infants and young children. Am J Dis Child 1987;141:949-953.

Anderson EL, Belshe RB, Bartram J. Differences in reactogenicity and antigenicity of acellular and standard pertussis vaccines combined with diphtheria and tetanus in infants. J Infect Dis J 1988;157:731-737.

Barkin RM, Samuelson JS, Gotlin LP. DTP reactions and serologic response with a reduced dose schedule. J Pediatr 1984;105:189-194.

Barkin RM, et al. Primary immunization with diphtheria-tetanus toxoids vaccine and diphtheria-tetanus toxoids-pertussis vaccine adsorbed: comparison of schedules. Pediatr Infect Dis J 1985;4:168-171.

Barr M, Glenny AT, Randall KJ. Concentration of diphtheria antitoxin in cord blood and rate of loss in babies. Lancet 1949;2:324-326.

Barr M, Glenny AT, Randall KJ. Diphtheria immunisation in young babies. A study of some factors involved. Lancet 1950;1:6-10.

Baum KF, et al. Corynebacterium diphtheriae in sickle cell leg ulcers in Jamaica. West Indian Med J 1985;34:24-28.

Bhandari B, Pamecka RK, Mandowara SL. Seroconversion following primary immunization with D.P.T. vaccine: two versus three doses. Indian J Pediatr 1981;18:41-47.

Bjorkholm B, et al. Antitoxin antibody levels and the outcome of illness during an outbreak of diphtheria among alcoholics. Stand J Infect Dis 1986;18:235-239.

Bray JP, et al. Epidemic diphtheria and skin infections in Trinidad. J Infect Dis 1972;126:34-40.

Camargo ME, et al. Immunoenzymatic assay of anti-diphtheric toxin antibodies in human sera. J Clin Microbial 1984;20:772-774.

Cellesi C, et al. Immunity to diphtheria in a sample of adult population from central Italy. Vaccine 1989a;7:417-420.

Cellesi C, et al. Immunity to diphtheria, six to 15 years after basic three-dose immunization schedule. J Biol Stand 1989b;17:29-34.

Chakraborty SM, Choudhuri AB. Isolation of toxinogenic strains of C diphtheriae from skin lesion of children in a rural West Bengal community. Indian J Med Res 1969;57:1651-1658.

Chason OL. Diphtheria immunity in rural Alabama. Am J Hyg 1936;23: 539-557.

Chen B-L, et al. Studies on diphtheria-pertussis-tetanus combined immunization in children. II. Immune responses after primary vaccination. J Immunol 1956;77:39-45.

Christenson B, Bottiger M. Serological immunity to diphtheria in Sweden in 1978 and 1984. Scand J Infect Dis 1986;18:227-233.

Crossley K, et al. Tetanus and diphtheria immunity in urban Minnesota adults. JAMA 1979;242:2298-2300.

Dalmatov VV, et al. Serological surveillance of diphtheria infection (in Russian). J Microbial Epidemiol Immunol 1986;no 12:43-47.

Daniel E, et al. The assessment of the potency of diphtheria toxoid on the basis of the Jensen test (in Polish). Medyk i Medycyna 1957;2:5-12.

Dengrove J, et al. IgG and IgG subclass specific antibody responses to diphtheria and tetanus toxoids in newborns and infants given DTP immunization. Pediatr Res 1986;20:735-739.

Edwards KM, et al. Evaluation of a new highly purified pertussis vaccine in infants and children. J Infect Dis 1989;160:832-837.

Edwards KM. Diphtheria, tetanus, and pertussis immunization in adults. Infect Dis Clin North Am 1990;4:85-103.

Expanded Programme on Immunization. Reported trends of EPI diseases in Europe. Wkly Epidemiol Rec 1988a;63:81-85.

Expanded Programme on Immunization. Diphtheria and measles control, China. Wkly Epidemiol Rec 1988b;63:225-227.

Expanded Programme on Immunization. Outbreak of diphtheria, USSR. Wkly Epidemiol Rec 1991;66:181-184.

Expanded Programme on Immunization. Outbreak of diphtheria, update, Russian Federation. Wkly Epidemiol Rec 1993;68:134-138.

Feery BJ, et al. Diphtheria immunization in adolescents and adults with reduced doses of adsorbed diphtheria toxoid. Med J Aust 1981;1:128-130.

Forsell P. Diphtheria immunity in Victoria. Med J Aust 1972;1:1023.

Fulthorpe A. Multiple diphtheria antigen-antibody systems by passive haemagglutination techniques. Immunology 1962;5:30-45.

Galazka A. Diphtheria in adults. Presented at the International Symposium on Working Towards New and Improved Vaccines, Madrid, Fundacion Ramon Areces, 12-14 February 1992.

Galazka A, Abgarowicz A. Assays of diphtheria and tetanus antibodies by the passive hemagglutination method. Epidemiol Rev (Warsaw) 1967;21:237-252.

Galazka A, Kardymowicz B. Immunity against diphtheria in adults in Poland. Epidemiol Infect 1989;103:587-593.

Galazka A, Keja J. Diphtheria: incidence trends and age-wise changes of immunity. Stand J Infect Dis 1988;20:355-356.

Galazka A, Olakowski T. Immunization of school-age children against diphtheria. Przegl Epidemiol 1962;16:431-442.

Galazka A, Sporzynska Z. Immunity to tetanus and diphtheria in various age groups of the Polish population. Arch Immunol Ther Exper 1979;27:715-726.

Glenny AT, Llewellyn-Jones M. The intracutaneous method of testing diphtheria toxin and antitoxin. J Path Bacteriol 1931;34:143-156.

Guerin N, et al. Evaluation de l'immunite diphterique chez les enfants. Pediatrie 1988;24:77-80.

Gunatillake PDP, Taylor C. The role of cutaneous diphtheria in acquisition of immunity. J Clin Microbiol 1981;13:83-88.

Halsey N; Galazka A. The efficacy of DPT and oral poliomyelitis immunization schedules initiated from birth to 12 weeks of age. Bull WHO 1985;63:1151-1169.

Hendriksen CFM, van der Gun JW, Kreeftenberg JG. Combined estimation of tetanus and diphtheria antitoxin in human sera by the *in vitro* Toxin-Binding Inhibition (ToBI) test. J Biol Stand 1989;17:191-200.

Ipsen J. Circulating antitoxin at the onset of diphtheria in 425 patients. J Immunol 1946;54:325-347.

Jensen C. Die intrakutane Kaninchenmethode zur Auswertung von Diptherietoxin und Antitoxin, Copenhagen, 1933.

Jones AE, et al. Durability of immunity to diphtheria, tetanus and poliomyelitis after a three dose immunization schedule completed in the first eight months of life. Vaccine 1989;7:300-302.

Karzon DT, Edwards KM. Diphtheria outbreaks in immunized populations. N Engl J Med 1988;318:41-43.

Kerttula Y, et al. Immunity to diphtheria in Helsinki in 1975. Scand J Infect Dis 1980;12:37-39.

Khuri-Bulos N, et al. The changing epidemiology of diphtheria in Jordan. Bull WHO 1988;66:65-68.

Kim-Farley R, et al. Assessing the impact of the Expanded Programme on Immunization: the example of Indonesia. Bull WHO 1987;65:204-206.

Kimura M, et al. A comparative trial of the reactogenicity and immunogenicity of Takeda acellular pertussis vaccine combined with tetanus and diphtheria toxoids: outcome of 3- to 8-month-old infants, 9- to 23-month-old infants and children, and 24- to 30-month-old children. Am J Dis Child 1991;145:734-741.

Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria and tetanus in the age group 30-70 years. Scand J Infect Dis 1988;20:177- 185.

Knight PA, Tilleray J, Queminet J. Studies on the correlation of a range of immunoassays for diphtheria antitoxin with the guinea-pig intradermal test. Dev Biol Stand 1986;64:25-32.

Koblin BA, Townsend TR. Immunity to diphtheria and tetanus in inner- city women of childbearing age. Am J Public Health 1989;79:1297.

Kreeftenberg JG, et al. An investigation of a mouse model to estimate the potency of the diphtheria component in vaccines. J Biol Stand 1985;13:229-234.

Kriz B, et al. Determination of diphtheria antitoxin in guinea-pig sera by the Jensen and tissue-culture methods. J Biol Stand 1974;2:289- 295.

Kriz B, et al. Immunological surveys of diphtheric antitoxin antibodies in some African and Asian countries. J Hyg Epidemiol Microbiol Immunol 1980;24:42-62.

Kurokawa M, Murata R. On the toxicity of the "toxoid" preparation responsible for the Kyoto catastrophe in 1948. J Med Sci Biol 1961;14: 249-256.

Lewis K, et al. A double-blind study comparing an acellular pertussis-component DTP vaccine with a whole-cell pertussis-component DTP vaccine in 18-month-old children. Am J Dis Child 1986;140:872-876.

Maksimova NM, et al. State of immunity to diphtheria and tetanus in some administrative regions of the RSFSR (in Russian). J Microbiol Epidemiol Immunol 1984;4:58-63.

Mathias RG, Schechter MT. Booster immunization for diphtheria and tetanus: no evidence of need in adults. Lancet 1985;1:1089-1091.

Melville-Smith M, Balfour A. Estimation of Corynebacterium diphtheriae anti-toxin in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralization test. J Med Microbiol 1988;25:279-283.

Middlebrook JL, Dorland RB, Leppla SH. Association of diphtheria toxin with Vero cells: demonstration of a receptor. J Biol Chem 1978;253: 7325-7330.

Millian SJ, et al. A serologic survey of tetanus and diphtheria immunity in New York City. Arch Environ Health 1967;15:776-781.

Miyamura K, et al. Micro cell culture method for determination of diphtheria toxin and antitoxin titers using Vero cells. I. Studies on factors affecting the toxin and antitoxin titration. J Biol Stand 19 74a;2:189-201.

Miyamura K, et al. Micro cell culture method for determination of diphtheria toxin and antitoxin titers using VERO cells. II. Comparison with the rabbit skin method and practical application for seroepidemiological studies. J Biol Stand 1974b;2:203-209.

Miyamura K, Murata R, Kono R. Status of antitoxic immunity against diphtheria in Japan: a report of the National Epidemiological Surveillance for Important Communicable Diseases. Jpn J Med Sci Biol 1983;36:177-190.

Mortimer EA. Diphtheria toxoid. In: Vaccines (SA Plotkin and EA Mortimer Jr, editors). Philadelphia: W.B. Saunders Company, 1988.

Muyembe JJ, et al. L'epidemiologie de la diphterie in Republique du Zaire: le role de l'infection cutanee. Ann Sot Belg Med Trop 1972;2: 141-152.

Naumann P, Hagedorn HJ, Paatz R. Diphtherie-Immunitat und ihre epidemiologische Bedeutung. Dtsch Med Wochenschr 1983;108:1090-1096.

Padovan E, et al. Determination of diphtheria toxin neutralizing antibody titers with a cell protein synthesis inhibition assay. Med Microbiol Immunol Berl 1991;180:29-35.

Palmer SR, Balfour AH, Jephcott AE. Immunisation of adults during an outbreak of diphtheria. Br Med J 1983;286:624-626.

Papadatos C, Papavangelou G, Koukou D. Diphtheria antitoxin levels and the Schick reaction in mothers and their newborns. Acta Paediatr Scand 1967;172 (suppl):181-185.

Pichichero ME, Barkin RM, Samuelson JS. Pediatric diphtheria and tetanus toxoids-adsorbed vaccine: immune response to the first booster following the diphtheria and tetanus toxoids vaccine primary series. Pediatr Infect Dis J 1986;5:428-430.

Pichichero ME, et al. Acellular pertussis vaccine; immunogenicity and safety of an acellular pertussis vs. a whole cell pertussis vaccine combined with diphtheria and tetanus toxoids as a booster in 18- to 24- month old children. Pediatr Infect Dis J 1987;6:352-363.

Public Health Laboratory Services (PHLS). Susceptibility to diphtheria: a survey by an ad hoc working group. Lancet 1978;1:428-430.

Rappuoli R, Perugini M, Falsen E. Molecular epidemiology of the 1984-1986 outbreak of diphtheria in Sweden. N Engl J Med 1988;318:12-14.

Robinson P, Lalitha Bai M, Narendran S. A Schick test survey in Bangalore with a discussion on the problem of diphtheria in India and other tropical countries. J Trop Pediatr 1964;10:39-46.

Ruben FL, Nagel J, Fireman P. Antitoxin responses in the elderly to tetanus-diphtheria (Td) immunization. Am J Epidemiol 1978;108:145-149.

Sargent RK, et al. Diphtheria immunity in Massachusetts: A study of three urban patient populations. Am J Med Sci 1984;287:37-39.

Scheibel I, et al. Duration of immunity to diphtheria and tetanus after active immunization, with mention of non-conformity between haemagglutinating and neutralizing diphtheria antitoxin titers in some human sera. Acta Path Microbiol Scand 1962;55:483-495.

Schou C, Simonsen O, Heron I. Determination of tetanus and diphtheria antitoxin content in dried samples of capillary blood: a convenient method applied to infants. Scand J Infect Dis 1987;19:445-451.

Schwartz SA, Bukova VE, Pichushkov AV. Time course of diphtheria morbidity and population immunity (in Russian). J Microbiol Epidemiol Immunol 1987;no 2:26-32.

Simonsen O. Vaccination against tetanus and diphtheria. Evaluation of immunity in the Danish population, guidelines for revaccination, and the methods for control of vaccination programs. Dan Med Bull 1989;36: 24-47.

Simonsen O, et al. Revaccination of adults against diphtheria. I: Responses and reactions to different doses of diphtheria toxoid in 30- 70-year-old persons with low serum antitoxin levels. APMIS (Copenhagen) 1986a;94(C):213-218.

Simonsen O, et al. Revaccination of adults against diphtheria. II: Combined diphtheria and tetanus revaccination with different doses of diphtheria toxoid 20 years after primary vaccination. APMIS (Copenhagen) 1986b;94(C):219-225.

Simonsen O, et al. Susceptibility to diphtheria in populations vaccinated before and after elimination of indigenous diphtheria in Denmark. APMIS (Copenhagen) 1987;95(C):225-231.

Stransky E, Felix NS. Susceptibility of Filipini children to diphtheria. J Philipp Med Assoc 1949;25:491-495.

Suri JC, et al. Schick test survey in Punjab. Indian J Med Res 1967; 55:179-184.

Svenson SB, Larsen K. An enzyme-linked immunosorbent assay (ELISA) for the determination of diphtheria toxin antibodies. J Immunol Methods 1977; 17:249-256.

Thorley JD, et al. Passive transfer of antibodies of maternal origin from blood to cerebrospinal fluid in infants. Lancet 1975;1:651-653.

Trinca JC. Combined diphtheria-tetanus immunization of adults. Med J Aust 1975;2:543-546.

Vahlquist B. Response of infants to diphtheria immunisation. Lancet 1949:1;16-18.

Vogelsang TM, Krivy BO. Schick reactions in recently confined women and their new-born infants. J Hyg 1945;44:437-441.

Volk VK, et al. Antigenic response to booster dose of diphtheria and tetanus toxoids seven to thirteen years after primary inoculation of noninstitutionalized children. Public Health Rep 1962;77:185-194.

Wright PG, Clark WM. Schick reactions in recently confined women and their infants. Br Med J 1944;2:146-148.

Youwang Y, et al. Epidemiological features of an outbreak of diphtheria and its control with diphtheria toxoid immunization. Int J Epidemiol 1992;21:807-811.

Zingher A. The Schick test performed on more than 150 000 children in public and parochial schools in New York (Manhattan and the Bronx). Am J Dis Child 1923;25:392-405.