JUKKA LUMIO

Studies on the Epidemiology and Clinical Characteristics of Diphtheria during the Russian Epidemic of the 1990s

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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of Building B,
Medical School of the University of Tampere,
Medisiinarinkatu 3, Tampere, on September 26th, 2003, at 13 o'clock.

Acta Universitatis Tamperensis 954
University of Tampere
Tampere 2003

ACADEMIC DISSERTATION
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Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 954
ISBN 951-44-5749-8
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 278
ISBN 951-44-5750-1
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2003

To my Russian colleagues

ABSTRACT

Background: Diphtheria in an infectious disease caused by a toxin produced by *Corynebacterium diphtheriae*. It is one of the biggest killers in the history of mankind. Diphtheria disappeared from Europe after the World War II along with increasing standard of living and immunization campaigns. An epidemic of respiratory tract diphtheria started in Russia in 1990. All together 150,000 persons fell ill and 5,000 died. This was a tragedy but gave a possibility to study scientifically respiratory tract diphtheria first time in half of a century.

Aims: This series of studies was designed to investigate the mode of transmission of diphtheria and its potency to spread in the population as well as the clinical characteristics of the disease.

Subject and methods: The subjects were on the one hand Finnish persons infected as a consequence of the Russian epidemic and on the other hand patients treated in St. Petersburg for diphtheria. Finnish patients were identified from the registers of the National Public Health Institute. The Russian patients were enrolled among adults treated in Botkin Hospital with a suspicion of diphtheria. Data on the clinical characteristics were collected retrospectively from patient hospital records. The incidence and the risk factors of diphtheritic cardiac damage were studied in a prospective trial.

Results: Ten Finnish persons were infected after a trip to the epidemic area in Russia between years 1990 and 2000. This was the consequence of more than 6 million trips from Finland to Russia. In addition to the 10 cases, two children were infected in Finland after a visit of Russian friends to their home. Four on the altogether 12 infections were severe and two patients died. All persons with a severe disease had a direct saliva contact to Russian persons. In Finland 91 health care workers were exposed to the respiratory tract secretions of diphtheria patients. Using throat cultures, none were found infected. Two thirds of 1 860 adult diphtheria patients treated in hospital in St. Petersburg had a mild non-membraneous infection. Eight percent of the patients had a severe toxic disease and 2% died. In a prospective trial of 122 patients, a quarter of the patients acquired diphtheritic heart complications observed by serial electrocardiographic recordins. The risk factors for cardiac damage were a severe acute respiratory tract inflammation and an age of 40 years or more. Two percent of patients admitted to the hospital with the clinical diagnosis of diphtheria had only a non-toxin producing strain *C. diphtheriae* isolated. Three out of these 112 patients died. In autopsy, in all three organ damage typical for toxic diphtheria were observed.

Conclusions: Respiratory tract diphtheria is not especially contagious. It has low potency to spread in countries like Finland with good coverage of childhood immunizations against diphtheria but no regular booster vaccinations for adults. The most important mode of transmission of diphtheria is probably direct contact to respiratory tract secretions of those carrying the bacillus. The risk of nosocomial diphtheria is low if the recommended general hygienic measures in hospitals are complied with. As a clinical disease diphtheria is today very similar to what it was 50 years ago. However, in a nation wide epidemic the mortality is lower. The bases of the specific diagnosis of diphtheria is the isolation of a toxin producing strain of *C. diphtheriae.* The production of toxin is, however, not always demonstrated by in vitro methods. The decision whether to threat the patient with diphtheria antitoxin has to be done with the knowledge of patient history and the clinical findings without waiting for microbiological results.

LYHENNELMÄ

Tausta: Kurkkumätä eli difteria on *Corynebacterium diphtheriae* -bakteerin aiheuttama vakava infektiotauti. Sen oireet aiheuttaa bakteerin tuottama myrkky. Yleisin kurkkumädän muoto on ylähengitysteiden tulehdus. Vakavaksi taudin tekee tukehtumisvaara ja sydänlihasvaurio. Difteria on historia suurimpia tappajia. Euroopasta se hävisi toisen maailmansodan jälkeen elintason nousun ja rokotusten myötä. Suomen naapurissa Venäjällä alkoi vuonna 1990 difteriaepidemia, jossa sairastui 150.000 ja kuoli 5.000 henkilöä. Tämä tragedia oli samalla mahdollisuus tutkia hengitystiedifteriaa ensimmäisen kerran puoleen vuosisataan.

Tavoitteet: Tutkimussarjalla pyrittiin selvittämään kurkkumädän tartuntatapoja ja leviämiskykyä sekä taudin oireita ja taudin vakavuuteen vaikuttavia tekijöitä.

Aineisto ja menetelmät: Aineistona olivat toisaalta kaikki Suomessa Venäjän epidemian seurauksena sairastuneet henkilöt, toisaalta Pietarissa difterian takia sairaalassa hoidetut aikuiset. Suomalaiset kurkkumätäpotilaat saatiin tietoon Kansanterveyslaitokseen tehdyistä tartuntatauti-ilmoituksista. Pietarilaiset potilaat tavoitettiin Botkinin sairaalasta, jossa hoidetaan kaikki pietarilaiset difteriatartunnan saaneet aikuiset. Taudinkuvaa tutkittiin taannehtivasti sairauskertomustietoja käyttäen. Sydänvaurioiden yleisyyttä ja riskitekijöitä selvitettiin ennakkoon suunnitellulla (prospektiivisella) tutkimuksella.

Tulokset: Vuosina 1990–2002 kaikkiaan 10 henkilön todettiin saaneen kurkkumätätartunnan matkustettuaan Suomesta Venäjän epidemia-alueelle. Tänä ajanjaksona tehtiin yli 6 miljoonaa matkaa Suomesta Venäjälle. Lisäksi kaksi lasta sai tartunnan Suomessa Venäjältä kyläilemään tulleelta henkilöltä. Neljä tartunnan saaneista sairastui vakavasti ja kaksi heistä kuoli. Kaikilla vakavasti sairastuneilla oli ollut suora sylkikontakti venäläiseen henkilöön. Suomessa 91 terveydenhoitotyöntekijää altistui hoidon aikana difteriapotilaiden hengitystie-eritteille. Viljelyin ei yhdelläkään työntekijällä todettu tartuntaa. Venäjällä tutkittiin 1.860 difteriapotilasta. Kahdella kolmasosalla nielussa ei ollut difterialle tyypillisinä pidettyjä katteita. Vakava toksinen tautimuoto oli 8 %:lla. Potilaista kuoli 2 %. Neljännes difteriapotilaista sai sydänlihasvaurion. Sydänvaurion riskitekijät olivat vaikea nielutulehdus ja yli 40 vuoden ikä. Kahdella prosentilla lääkärin mielestä oireista päätellen difteriaa sairastaneista oli nielussa vain sellainen difteria-bakteerikanta, joka ei tuottanut myrkkyä. Näistä potilaista kolme (2 %) kuoli. Ruumiinavauksissa kaikilla kuolleista todettiin elinmuutoksia, jotka ovat tyypillisiä toksiselle difterialle.

Johtopäätökset: Hengitysteiden kurkkumätä ei ole historiallisesti huonosta maineestaan huolimatta erityisen tarttuva tauti. Sen tärkein tartuntatien on todennäköisesti suora kontakti bakteeria kantavan henkilön hengitystie-eritteisiin. Tartunta väentungoksessa tai epäsuorasti saastuneiden astioiden tai muiden esineiden välityksellä on hyvin harvinainen. Sairaalassa työntekijöiden infektioriski on vähäinen, jos noudatetaan tavallisia sairaalahygieenisiä käytäntöjä, joita suositetaan muutenkin kaikkien potilaiden hoidossa. Difteria on tautina hyvin saman kaltainen kuin edellisissä epidemioissa puoli vuosisataa sitten. Kuolleisuus väestön laajuisessa epidemiassa on kuitenkin nykyään pienempi. Kurkkumädän diagnoosi perustuu myrkkyä tuottavan difteria-bakteerin eristämiseen. Bakteerikannan toksiinintuottoa ei kuitenkaan aina kyetä osoittamaan laboratoriossa. Päätös siitä annetaanko sairastuneelle hoidon alussa difteriavastamyrkkyä (antitoksiinia) on tehtävä tapahtumatietojen ja taudinkuvan perusteella odottamatta tietoa bakteeriviljelystä ja bakteerikannan toksiinin tuotosta.

CONTENTS

LIST OF ORIGINAL STUDIES

The thesis is based on the following original communications, referred to in the text by their Roman numerals.

I Lumio J, Jahkola M, Vuento R, Haikala O, Eskola J. Diphtheria after visit to Russia. Lancet 1993; 342:53-4.

II Rakhmanova AG, Lumio J, Groundstroem K, Valova E, Nosikova E, Tanasijchuck T, Saikku J. Diphtheria outbreak in St. Petersburg: clinical characteristics of 1860 adult patients. Scand J Infect Dis 1996; 28:37-40.

III Rakhmanova AG, Lumio J, Groundstroem KWE, Taits BM, Zinserling VA, Kadyrova SN, Goltsova EY, Melnick OB. Fatal respiratory tract diphtheria apparently caused by nontoxigenic strains of *Corynebacterium diphtheriae*. Eur J Clin Microbiol Infect Dis 1997; 16:816-820.

IV Lumio J, Ölander R-M, Groundstroem K, Suomalainen P, Honkanen T, Vuopio-Varkila J. Epidemiology of three cases of severe diphtheria in Finnish patients with low antitoxin antibody levels. Eur J Clin Microbiol Infect Dis 2001; 20:705-710.

V. Lumio JT, Groundstroem KWE, Melnick OB, Huhtala H, Rakhmanova AG. The incidence and risk factors for electrocardiographic abnormalities in diphtheria – a prospective study. Am J Med, in press.

VI Lumio J, Suomalainen P, Ölander R-M, Saxén H, Salo E. A fatal case of diphtheria in an unvaccinated infant in Finland. Pediatr Infect Dis J 2003;22:844-6.

ABBREVIATIONS

AD	Anno Domini, after the birth of Christ
ADP	adenosine diphosphate
A-V	atrio-ventricular
BC	before the birth of Christ
CAMP	a diffusible protein typical for streptococci
$CD4^+$	helper T cells
C. diphtheriae	*Corynebacterium diphtheriae*, the bacillus causing diphtheria
CI	confidence interval
CIA	Confidence Interval Analysis
CSF	cerebrospinal fluid
DTP	diphtheria-tetanus-pertussis vaccine with high dose of diphtheria toxoid
DT	diphtheria-tetanus vaccine with high dose of diphtheria toxoid
ECG	electrocardiography
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization (by WHO)
IU	international units
l	litre
Lf	flocculation units, a measure of antigen content in a vaccine
ml	millilitre
PCR	polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
Td	tetanus-diphtheria vaccine with low dose of diphtheria toxoid
tox	gene coding for toxin production in the bacterium
WHO	World Health Organization

I INTRODUCTION

The Russian poet and country doctor Anton Pavlovich Chekhov (1860–1904) was a master of minimalist expression. In his letters he expressed the view that "the gift of writing is the gift of reduction" and "brevity is the sister of talent". No wonder, one of his short stories "The enemies" starts: "On a dark September evening, around ten o'clock, six-year-old Andrei, the only son of doctor Kirilov from Zemstvo, died of diphtheria". This sentence led the reader right into the centre of a drama familiar to everyone in the late 19th century. Diphtheria was one of the biggest baby killers of all times. More peculiar and more frightening than the life threatening infection of an individual was the recurring epidemic nature of the disease. When diphtheria entered the neighbourhood it could kill half the children, only to return with equal severity a generation later.

The first valuable remedy against diphtheria was discovered in the 1890s, diphtheria antitoxin produced in animals. The case fatality of diphtheria decreased from 40% to 10% in a few years. If the remedy was available and you could afford it. Immunization with diphtheria toxoid vaccine was implemented in the 1920s in the United States and two decades later in Europe. About one decade after the population-wide childhood immunizations were started, the ongoing epidemics of diphtheria faded out and have not returned to the Western nations.

In 1892, nearly 1% of all articles in the world's medical literature, listed in *Index Medicus*, addressed diphtheria. This was more than 100 times the proportion in 1982. Our knowledge of how to manage diphtheria dates mainly to the times of World War II, when the epidemics were last seen in Europe. When there was diphtheria, there was no evidence-based medicine, no controlled trials, no statistics. Our current textbooks tell the story as told by careful and considering practitioners rather than by clinical scientists. This state of affairs is acceptable, as most doctors practising today in Western Europe or in North America have never seen a case of diphtheria.

Then, in the late 1980s came "glasnost" that eventually broke the Soviet Union into Russia and a number of so called Newly Independent States. This development, with all the social and economical perplexity it brought, saw a pronounced decrease in mortality among middle-aged men with the lowest level of education [Plavinsli et al. 2003]. It also saw an epidemic of respiratory tract diphtheria flare up quite at the gates of Western Europe. The epidemic hit Russia hardest and especially its biggest cities, St. Petersburg and Moscow. Between the years 1990 and 1997, there were more than 150,000 cases of diphtheria with more than 5,000 deaths in the Russian Federation. Many concurrent events were needed for the resurgence of the "historical" disease;

decreased coverage of childhood immunization, large scale population movements from areas where skin and wound diphtheria were still endemic, and a partial collapse of the infrastructure of the society.

Now the epidemic has subsided in Russia. Contrary to many pessimistic predictions, the epidemic never really entered the West. The vaccination coverage as well as the standard of living and standard of hygiene were evidently good enough to keep diphtheria out. The Russian epidemic permitted the epidemiological and clinical aspects of the disease to be investigated with the tools of modern science. What we learned during the epidemic of the 1990s can be of benefit today in the developing countries, where diphtheria is endemic, and to us, when diphtheria strikes again.

II REVIEW OF THE LITERATURE

1 Epidemiology

1.1 Historical perspective

The clinical presentation of diphtheria is not distinctive enough to be easily differentiated from other causes of "croup" (concept launched by Francis Home, 1719–1813), like the whooping cough and streptococcal angina [Feldman 2001]. Instead, it was rather the dramatic epidemic nature of the disease that drew physicians' attention to diphtheria already in the dawn of written history. Some of the plagues of the Middle Ages may have been epidemics of diphtheria, such as one described by Saint Denis in 580 Anno Domini (AD) or another by Baronius in 1004 AD [Metaxa Quiroga 1990]. However, the credit for describing the epidemic nature of diphtheria is usually given to 16th century Spain, where it was called "morbus suffocans" or "el garrotillo" [English 1985].

Before the 19th century, diphtheria was strictly an epidemic disease. It came from "nowhere", and in a few years it disappeared, only to return 20 to 25 years later. Diphtheria was mainly a rural disease. In a few weeks after the first case in the district it was everywhere, taking its toll mainly among the poor. The largest outbreak among North American settlers raged from 1735 to 1740 in the New England colonies; one person in forty died of diphtheria. At the same time, there were similar outbreaks in Great Britain, France and the West Indies [English 1985]. The vast majority of the deceased were children [English 1985, Kleinman 1992].

Still, in the beginning of the 20th century, diphtheria was predicted to be "the scourge of America in the future" [Kleinman 1992]. At that time, in many places the mortality rates were 100 to 200 / 100,000 population [English 1985, Kleinman 1992]. It was not until 1935 that diphtheria ceased to be the leading infectious killer of children in the United States [Kleinman 1992]. In the "Old World", the dreadful killer ruled longer. It made so far its last attack in the 1940s, during World War II [Collins 1946].

1.2 Developed countries

Diphtheria is notifiable in all countries of Europe and North America [Efstratiou and George 1996]. Widespread immunizations led to the virtual elimination of diphtheria in the developed countries, although minor outbreaks among special risk groups still occur.

North America

The incidence of diphtheria started to diminish at the turn of the 20th century evidently without the influence of any active measures, probably due to the rise in standard of living and to better general hygiene [Collins 1946]. The wide use of active immunization in the general population started in Canada and the United States around 1925 [Collins 1946] and a few years later in Australia [Hooker and Bashford 2002]. It is obvious that vaccinations greatly catalysed the subsidence of diphtheria [Collins 1946]. After the implementation of childhood immunization, the great epidemics did not reappear. The yearly number of cases of diphtheria fell from 106/100,000 population in 1924 to about one-tenth of that figure, to 10.7/100,000 in 1944. At the same time the proportion of adults (over 30 years of age) among cases of diphtheria increased from 8% to 40–50% [Brainerd and Bruyn 1951].

In the last half of the 20th century only small outbreaks of respiratory tract diphtheria among special risk groups, such as alcoholics, intravenous drug abusers, residents of camps and in tight communities of ethnic minorities have occurred [Lyman et al. 1956, Jellard 1972, Munford et al. 1974, Hennekens and Saslaw 1976, Dobie and Tobey 1979, Chen et al. 1985, Harnisch et al. 1989, Centers for Disease Control and Prevention 1997, Golaz et al. 2000]. Travel to areas where diphtheria is endemic has brought individual cases or clusters of a few cases to North America [Farizio et al. 1993]. From 1980 to 1994, a total of only 41 cases of diphtheria were reported in the United States [Golaz et al. 2001].

Europe

Wide-scale population immunizations against diphtheria were started in Europe in the early 1940s, twenty years later than in North America [Collins 1946]. The trigger for the implementation of vaccination was the epidemic of diphtheria which swept over Europe during and after World War II. In 1943, the annual incidence of diphtheria per 100,000 population was as high as 760 in Norway, 622 in the Netherlands and 212 in

Germany [Collins 1946, Eskola et al. 1998]. These incidences were ten times higher than a few years earlier. In 1943, there were approximately 1 million cases and 50,000 deaths in Europe [Galazka 2000]. In Finland, 1945 was the year with the highest incidence, 500 cases / 100,000 population [Official Statistics of Finland 1974]. The Finnish epidemic died out in 1956. The European epidemic of the 1940s largely spared the southern parts of the continent.

Prior to the wide use of diphtheria toxoid vaccine, at least 40% of diphtheria cases occurred in children below five years of age, and altogether some 70% of cases were in children younger than 15 years [Galazka and Robertson 1995]. In the Netherlands, Norway and Denmark there was a sharp shift towards older age groups during the 1940s, i.e. already before widespread immunizations had been started [Galazka and Robertson 1995]. In Copenhagen, Denmark, an epidemic started in 1944 and amounted to 2200 cases, of which 1500 (68%) were adults [Ipsen Jr. 1954]. This shift of diphtheria to older age groups may have been the result of a documented drop in diphtheria immunity among adults in Copenhagen due to a long period of low diphtheria incidence.

Since the vaccination campaigns in Europe started, the development in diphtheria in Europe has been similar to that of North America [Kwantes 1984, Galazka and Robertson 1995, Gilbert 1997, Gomez et al. 1999, Hasselhorn 2001]. A median 1100 cases of diphtheria were reported yearly in Europe in the 1980s [Gilbert 1997]. Individual cases and small outbreaks of diphtheria have been mainly among people in special risk groups [Christensson et al.1989] and among those who have travelled to an endemic area [Antos et al. 1992, Anonymous 1993].

1.3 Developing countries

In the pre-vaccination era, skin diphtheria was extremely common in tropical countries during the first years of life. It led to relatively few complications but resulted in immunity to diphtheria toxin [Golaz et al. 2001]. Immunization with diphtheria toxoid was introduced in most developing countries by the late 1970s within the Expanded Programme on Immunization (EPI) [Galazka and Robertson 1995]. The success rates have varied greatly from country to country. During an outbreak of diphtheria in Yemen in 1981–1982 the vaccination coverage was 10% [Jones et al. 1985, Galazka and Robertson 1995]. Sixty-seven per cent of the cases in this epidemic were in children below the age of five years. At the same time, there was an epidemic in Jordan, where the vaccination coverage among children was 70% [Khuri-Bulos et al. 1988]. Contrary to the epidemic in Yemen, in Jordan 65% of the cases involved persons older than 15

years. In recent years, outbreaks of diphtheria have been described in China, India, Ecuador, India, Jordan, Leshoto, Sudan, Yemen and Algeria [Eskola et al. 1995, Galazka and Robertson 1995]. With better vaccination coverage, the developing countries are going through the same development that occurred a few decades earlier in the developed countries [Youwang et al. 1992, Tharmaphornpilas et al. 2001]. The endemic rate of diphtheria is decreasing. In outbreaks, the victims are mainly unvaccinated elderly persons with no natural immunity.

Skin and wound diphtheria is widely endemic in the tropics and this clinical form is the prevailing type of diphtheria. Cutaneous diphtheria is difficult to differentiate clinically from other skin infections, and the causative bacillus, *Corynebacterium diphtheriae,* is hard to eradicate from skin lesions. Therefore, total diphtheria eradication in tropical and developing countries will be difficult, if not impossible. Good vaccination coverage will, however, prevent severe epidemics.

1.4 Russia and its neighbours

In the Russian Federation, physicians are obliged to report cases of diphtheria to the State Sanitary-Epidemiological Service, an office specialized in epidemiological surveillance of infectious diseases [Stratchounski et al. 2001]. It is, and it was in the Soviet era, a well functioning and reliable data collection system [Vitek et al. 2000].

The latest population-wide diphtheria epidemic in Russia occurred during and after World War II, with around 100 yearly cases per 100,000 population still in the late 1950s (Figure 1) [Vitek et al. 2000]. This epidemic was milder than the epidemic in Finland at the same time (Figure 2). A small temporary rise in the number of cases of diphtheria occurred in 1983-1985.

Since 1990, a massive epidemic started in Eastern Europe, first in the Russian Federation. The epidemic later extended to all the Newly Independent States of the former Soviet Union: Ukraine, Lithuania, Latvia, the Republic of Moldova, Belarus, Armenia, Azerbaijan, Georgia, Tajikistan, Kazakhstan, the Kyrgyz Republic and Uzbekistan. In Russia the epidemic resulted in more than 157,000 infections and 5,000 deaths [Korzenkova et al. 1991, Galazka et al. 1995, Maurice 1995, Centers for Disease Control and Prevention 1996, Rey et al. 1996, World Health Organization 1993, Hardy et al. 1996, Leon et al. 1997, Galazka 2000]. This was more than 80% of all cases of diphtheria reported in the whole world in 1990 to 1997 [Dittman et al. 2000]. The Russian epidemic peaked in 1994 to 1995. Especially in the large cities, the rates were high (St. Petersburg, 52.5/100,000 and Moscow, 47.1/100,000). In St. Petersburg, there were more than 2,500 cases annually in 1994 and 1995 (Figure 1). Of the cases in

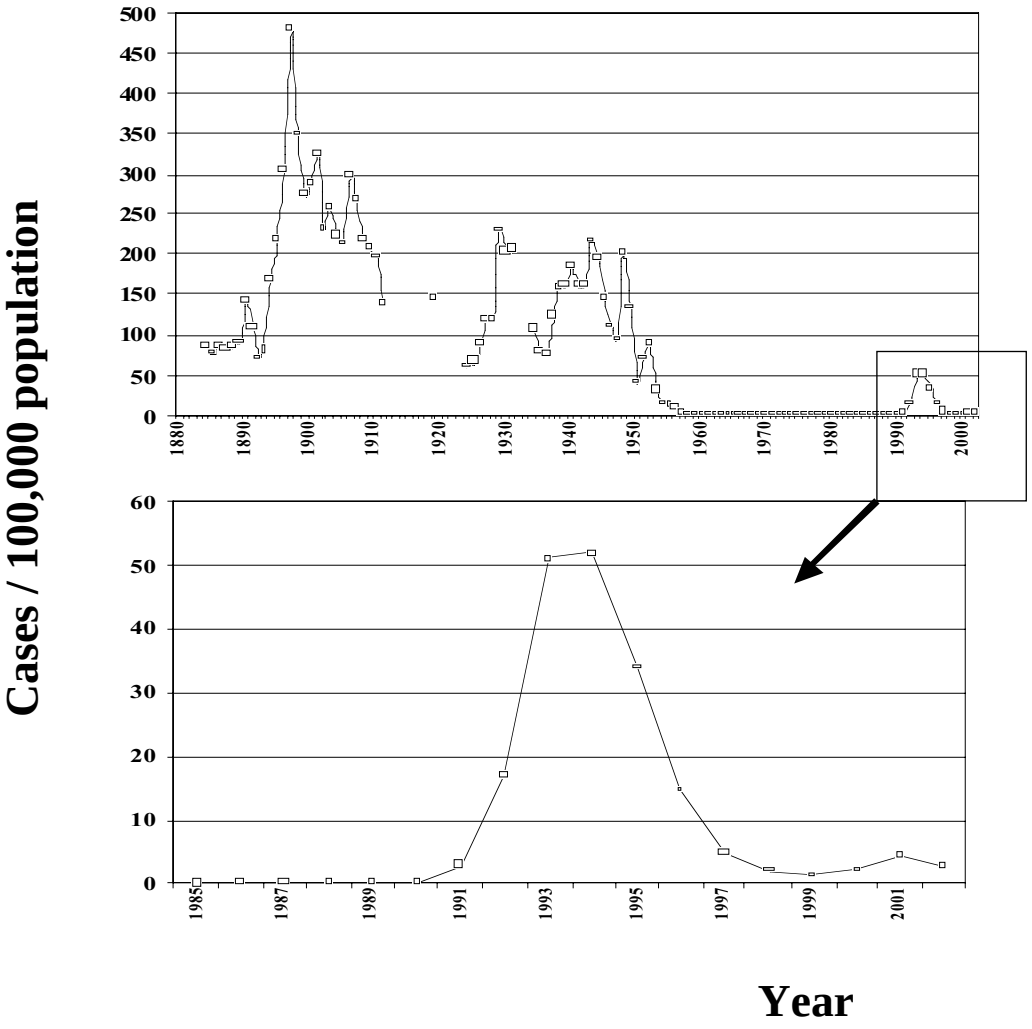


Figure 1. The incidence of diphtheria in Russia and in Soviet Union in 1882 to 2002 (official statistics).

Russia in 1990–1997, 58–68% occurred among adults, 6–8% among adolescents 14–17 years of age and 27–32% among children <14 years of age [Markina et al. 2000].

Many appraisals of the possible reasons for the resurgence of diphtheria after 30 years absence in a country with at least a reasonable vaccination policy have been published [Ryan 1993, Maurice 1995, Leon et al. 1997]. As favourable general conditions for a diphtheria outbreak the following have been mentioned: a sufficiently large susceptible population, a sufficient quantity of toxin producing *C. diphtheriae* circulating in the society and subpopulations with low economic status, inadequate hygiene and reluctance to seek early medical care. A World Health Organization (WHO) list enumerates the following reasons for the epidemic in the Russian Federation: fall in childhood vaccination coverage, large-scale population movements, socioeconomic instability, partial deterioration of the health care infrastructure, delay in implementing

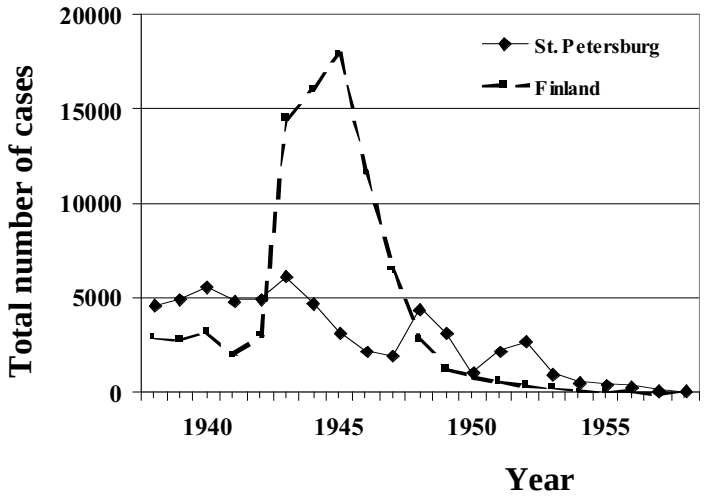


Figure 2. The incidence on diphtheria in St. Petersburg and in Finland in the 1940s and 1950s (official statistics).

aggressive measures to control the epidemic and inadequate information about the epidemic for physicians and the public [Dittman et al. 2000].

The vaccination coverage of children in Russia decreased significantly in the 1980s. A study of three-year-old children in Moscow in 1980-1981 found that 28% of the children were unvaccinated and another 44% had not received the recommended fourth dose of diphtheria-tetanus-pertussis vaccine [Markina et al. 2000]. Medical contraindications were the reason for failure to vaccinate in two-thirds of the unvaccinated children, parental refusal accounted for less than 5%. The vaccine itself was adequate. In the late 1980s, there were major population movements in Russia. There was a return of hundreds of thousands ethnic Slavs from Central Asian and Caucasian countries and a massive flight of refugees from fighting in Georgia, Armenia, Azerbaijan and the northern Caucasus [Dittman et al. 2000]. In addition to that, a hundred thousand soldiers returned from Afghanistan [Galazka 2000]. The crisis of infrastructure is illustrated by news in Russian daily newspapers reporting about changes in the society (Table 1). Reading these, no scientific analysis is needed to imagine the force of the social changes that followed the breaking up of Soviet Union.

Nearly 90% of the cases reported in the Russian epidemic were bacteriologically proven [Dittman et al. 2000]. At the height of the outbreak, the prevalent strain (79% of all isolates) was a toxin-producing strain of *C. diphtheriae* biotype *gravis*. In the late 1990s, when the epidemic was subsiding, the prevailing strain was of biotype *mitis*. (A.

Table 1. The turmoil in Russia, as reflected in the situation and changes in everyday life in St. Petersburg (population 4.3 million) in the first half of the 1990s [Various Russian newspapers, press releases by the Finnish Consulate in St. Petersburg, Olli Kuukasjärvi, personal communication].

- There are 1 million alcoholics
- The number of intravenous drug abusers has doubled
- Male life expectancy has fallen to 59 years
- The number of abortions is 100,000 per year
- Four out of every 100 babies are abandoned by parents
- There are 100,000 legal and 200,000 illegal refugees
- Vaccination coverage has fallen to 60%
- Salary of an infectious disease specialist is $40 a month

Efstratiou, personal communication). According to gene typing, the strains isolated recently in other countries such as France [Patey et al. 1997] and Rumania [Damian et al. 2002] have not been similar to those in the Eastern European epidemic.

The epidemic did not spread to neighbouring Western European countries. Only a few cases of diphtheria linked to Eastern Europe were reported from 1990 to 1997 in other countries; in Finland (3 cases), in Germany (3), in Belgium (3) and in the United States (2) [Anonymous 1995, Editorial 1997]. St. Petersburg was the city that suffered the worst outbreak. In 2001 there were still about 250 cases of respiratory tract diphtheria reported, double the incidence of the previous year, but since then there has been a decreasing tendency again [Dr. O. Parkov, State Sanitary-Epidemiological Service, St. Petersburg, personal communication].

1.5 Bacterial and molecular epidemiology

In 1931, Anderson and co-workers discovered that the species *C. diphtheriae* consisted of more than one biotype [Anderson et al. 1931]. In an endemic situation as well as during epidemics several biotypes circulate simultaneously [Gibbard et al. 1945, Carter 1947, Coyle et al. 1989, Marston et al. 2001]. In the 1930s and 1940s in Glasgow the proportions of the biotypes *gravis*, *mitis* or *intermedius* varied year by year [Carter 1947]. Nowadays biotyping is generally of limited value in epidemiological work. The application of molecular typing for *C. diphtheriae* has been rewarding and is becoming increasingly important [Efstratiou and George 1995].

There are many examples of the benefits of molecular epidemiology in real situations. In an outbreak in two localities in Sweden with an occasional case in Denmark in the 1980s, 36 isolates representing *mitis* and *gravis* biotypes were available for genetic typing [Rappuoli et al. 1988]. Although the strains represented 17 different

groups of *C. diphtheriae*, all the clinical and fatal cases were caused by isolates from the same genetic group. This knowledge made it possible to analyze the probable mode of spread of the infection [Larsson et al. 1987]. Recently it has been shown by random amplified polymorphic DNA analysis that strains with distinct molecular subtypes have persisted in the United Stated and Canada for at least 25 years [Marston et al. 2001]. The strains are endemic rather than imported from countries with current endemic or epidemic diphtheria. Gene typing has also shown that the *C. diphtheriae* strains isolated from recent outbreaks in Rumania [Damian et al. 2002] and France [Patey et al. 1997] were not identical to the epidemic strains isolated at the same time in Russia and Moldova. The strains in France probably originated from Guyana and New Caledonia.

In the aftermath of a diphtheria epidemic it is commonly observed that the biotypes of *C. diphtheriae* in circulation tend to change, and the bacteria tend to lose the gene coding for toxin production (*tox*) before *C. diphtheriae* ceases to circulate. Toxin production enhances person-to-person transmission of the bacterium by causing symptoms facilitating the spread [Karzon and Edwards 1988]. It has been postulated that the loss of converting phage or the toxin gene is caused by an increasing proportion of persons who have acquired antitoxin antibodies by vaccination or natural infection and will therefore not contract a clinical disease after infection [Groman 1984, Karzon and Edwards 1988]. Theoretically, in such a situation the *tox* gene would not offer any advantage to the host bacterium and this would lead to the loss of the gene.

1.6 Transmission

Reservoir

Although many warm-blooded animals are susceptible to diphtheria in experimental conditions, man is the only reservoir of the disease in nature [Efstratiou and George 1996]. Worldwide, the main reservoir of *C diphtheriae* is the human skin [Funke et al. 1997]. Infected skin and wounds are making diphtheria endemic in many developing countries [Koopman and Campbell 1975, Harnisch et al. 1989]. The relative lack of respiratory tract infections in endemic areas may be due to better natural immunity in the populations of areas where *C. diphtheriae* is endemic [Jellard 1972].

In most epidemics of diphtheria respiratory tract disease predominates [Brainerd and Bruyn 1951, Naiditch and Bower 1954, Jellard 1972, Dobie and Tobey 1979, Salih et al. 1981, Youwang et al. 1982, Chen et al. 1985, Coyle et al. 1989, Harnisch et al. 1989, Grunner et al. 1994, Galazka et al. 1995]. However, in epidemics of respiratory

tract infections, an important role for the cutaneous forms has been suggested [Koopman and Campbell 1975, Harnisch et al. 1989]. In cutaneous diphtheria the diagnosis is commonly late, the convalescent carrier state may be long and transmission from the wounds is more efficient than from the respiratory tract [Hewlett 1985].

In respiratory tract diphtheria, bacterial carriage lasts for a median of 2 to 3 weeks if antibiotics are not given [Hartley and Martin 1920, Weaver 1921]. With antibiotic treatment 84–96% of the patients are rendered culture-negative within 2 to 3 days [McCloskey et al. 1974, Miller et al. 1974]. Even during high incidence of diphtheria, the rate of chronic or convalescent carriers of *C. diphtheriae* is low, between 0.3% and 4.4% [Taylor et al. 1962, Damian et al. 2002]. Only during school epidemics have high carrier rates, up to 29%, been reported for children [Miller et al. 1972].

Diphtheria toxoid vaccine induces antibodies only against the diphtheria toxin. It does not prevent the carrier state nor does it stop the spread of infection [Miller et al. 1972]. The vaccine is not immunogenic for all persons vaccinated [Karelitz and Moloshok 1944]. The level of protective antibodies wanes in time, making the person vulnerable to a second episode of diphtheria. In many epidemics, 5–10% of all cases have been second cases for the individual [Brainerd and Bruyn 1951, Naiditch and Bower 1954] and a good proportion of all patients, up to 30%, have been adequately vaccinated [Gibbard et al. 1945, Grant 1945, Naiditch and Bower 1954]. There is evidence that vaccination may give better protection against clinical disease than the immunity acquired after natural infection [Grant 1945].

Transmission in different settings

Clusters of multiple cases of diphtheria and asymtomatic infections have been common in families and in schools, but not in workplaces [Magdei et al. 2000, Vitek et al. 1999]. In a prospective survey conducted in a boarding school, throat cultures were taken every 2 to 4 days from several types of contacts [Favorova 1969]. Of those sharing a room in a dormitory with an infected student 9.6% tested positive for *C. diphtheriae* as compared to 5% of those attending the same class and 1.3% of those only sharing the same table at meals. In a prospective case-control study in the Republic of Georgia, risk factors for acquiring diphtheria were found to be household exposure to diphtheria, sharing a bed, sharing cups and glasses and taking a bath less than once a week [Quick et al. 2000b]. Kissing was not found to be a risk factor, but this kind of information may not have been shared completely with the investigators. Attending crowded places like churches, clubs, movies and celebrations was not a risk factor for diphtheria. The risk of a secondary clinical case in the same household seems to be low, a few per cent. The risk

is obviously dependent on the vaccination status of the individuals and the hygienic conditions [Farizio et al. 1993].

There is anecdotal data on the nosocomial risk for diphtheria for health care personnel during the recent epidemic in Belarus [Filonov et al. 2000]. In some other studies no infections in health care workers were observed [Larsson et al. 1987, Farizio et al. 1993]. In an epidemic in a mental institution in the United Kingdom 7% of patients and 0.6% of employees with close contact to index cases became infected [Gray and James 1973]. During a recent minor outbreak among alcoholics in Sweden, no transmission to health care workers was observed [Larsson et al. 1987, Christensson et al. 1989]. In Botkin's Hospital in St. Petersburg over 5,000 patients were treated for respiratory tract diphtheria between 1992 and 2002 with no reported nosocomial clinical cases of diphtheria among the 2,500 health care workers [Professor A. Rakhmanova, personal communication].

In Germany, persons having professional contact other than health care with persons coming from an endemic area for diphtheria, especially employees in refugee centres, seem to have an increased risk for the disease [Hasselhorn 2001]. In the Swedish epidemic, it was concluded that diphtheria might have spread from a kitchen worker to the clients by food [Christenson et al. 1989]. There are also reports of diphtheria being transmitted by milk or other dairy products from a *C. diphtheriae* carrier handling the milk [Goldie and Maddock 1943, Jones et al. 1985].

2 Aetiology

2.1 History

The devastating diphtheria epidemic in New England in 1735 to 1740 made physicians ask important questions like, where does the disease come from, how is it spread, is it contagious. Members of the clergy, in many cases more influential than the medical faculty, offered their explanations. According to them the disease was a manifestation of God's retribution for original sin [Kleinman 1992]. Those who became ill were viewed as somehow predisposed to illness because of certain unholy habits.

Medical opinion was spread among several points of view. There were two problems to solve; where did the originator of the disease come from and how did it cause the disease. In the late 18th century, "tellurism" had a strong hold. In "tellurism", the source of the disease was "miasma", a noxious emanation from the soil or earth [English 1985]. In pathogenetic considerations, "humoralism" dominated. According to this ancient complex of ideas, disease in an individual resulted from an imbalance or

"disequilibrium" in the fluids of the body rather than something attacking from outside [English 1985]. In the early 19th century a rival notation, later called the "anatomical idea" by the German pathologist Rudolf Virchow (1821–1902), gained ground. This dogma argued that disease was located in the organs rather than the fluids of the body. Virchow was especially interested in diphtheria, and with the help of new methods, microscopes, microtomes, stains and lighting, he precisely described the cellular nature of the diphtheritic membrane [English 1985].

In the late 19th century the door was open for the germ theory. Again, diphtheria was at the centre. Actually the germ theory had been "in the air" for several centuries and, when it entered the arena, it became one of the scientific theories held by the public at large [English 1985]. Even after accepting that diphtheria was contagious, American physicians especially continued to argue for a "miasmatic" or "zymotic" origin [Metaxa Quiroga 1990, Hooker and Bashford 2002]. Others argued that inclement weather or filth, poverty, poor diet, overcrowding or inadequate clothing caused diphtheria and no other explanations were needed [English 1985]. Adoption of the germ theory was helped by several preceding "epidemiological" discoveries. Oliver Wendell Holmes (in 1843) in Boston and Ignaz Semmelweis (in 1847) in Budapest had shown that obstetricians spread childbed fever from patient to patient, and John Snow (in 1849) had shown the relationship between water from the Broad Street pump in London and an outbreak of Cholera [English 1985].

That diphtheria was of contagious character, was demonstrated conclusively by Max Oertel (1835–1897) in 1871 when he produced a membrane in a rabbit's throat after swabbing it with secretions from a human with diphtheria [English 1985]. The definite proof that a bacillus caused diphtheria came from Friedrich Loeffler (1852–1915) in 1884 [English 1985]. Loeffler was a student of Virchow and had joined Robert Koch (1843–1910) in Berlin. In order to make this breakthrough he had to develop a new stain, methylene blue, and a new culture medium, blood agar [English 1985]. In his original report he already had shown in animal experiments that the bacillus was not invasive and postulated that the bacillus produced a toxin [Loeffler 1884]. Four years later, Pierre Paul Emil Roux (1853–1933), an assistant of Louis Pasteur, proved Loeffler's hypothesis that the bacillus produced toxin resulting in distant effects [English 1985]. Soon after these discoveries, in 1893 in New York, routine clinical laboratory testing for diphtheria was introduced [Metaxas Quiroga 1990].

The biological heterogeneity of the diphtheria bacillus became evident in 1931 when Anderson and co-workers discovered two forms, which he named *Bacillus diphtheriae gravis* and *Bacillus diphtheriae mitis* [Anderson et al. 1931]. Already in the beginning of the 20th century it had been observed that both toxin-producing and nontoxigenic strains of *C. diphtheriae* are circulating both during epidemics and between

epidemics [Harnish et al. 1989]. In 1948, Stephen Elek presented a cheap and practical test for demonstrating the toxin production of *C. diphtheriae* strains on an agar plate [Elek 1949]. Elek's test soon replaced the cumbersome animal tests of virulence. In Elek's classical article there was total agreement in "virulence" between the guinea-pig test and the plate test for 153 *C. diphtheriae* strains of various biotypes [Elek 1949]. The *in vitro* test was not merely a valuable tool for clinical work, it also gave scientists the means to study more accurately the clinical disease and the epidemiology of diphtheria.

2.2 The Klebs-Loeffler bacillus today

The etiological agent of diphtheria is *Corynebacterium diphtheriae.* It is an aerobically growing gram-positive rod. It is one of the nearly 100 species in the genera *Corynebacteriacae* [Funke et al. 1997]. Key reactions for the differentiation of coryneform bacteria are catalase, fermentative or oxidative metabolism and motility [Funke et al. 1997]. The initial screening reactions should also include nitrate reduction, urea production, esculin hydrolysis, acid production from glucose, maltose, sucrose, mannitol and xylose, and the CAMP reaction with a β-haemolysin-producing strain of *Staphylococcus aureus* [Funke et al. 1997].

Recent 16S rRNA gene sequence analysis and DNA-DNA hybridisation studies have shown that the *C. diphtheriae* group of organisms includes not only *C. diphtheriae* (with the four biotypes *gravis, mitis*, *belfanti* and *intermedius*, which are closely related) and *C. pseudotuberculosis* but also *C. ulcerans* as a valid independent species [Funke et al. 1997]. Of these, only *C. diphtheriae* biotype *intermedius* can be easily distinguished on the basis of colony morphology. According to old observations, *C. diphtheriae gravis* would associate with severe disease forms and *C. diphtheriae mitis* with a milder disease [Anderson et al. 1931]. Now, when it is known that the property of producing toxin lies in the genes of a lysogenic bacteriophage that infects *C. diphtheriae* strains, this observation, although made in many epidemics, is being re-examined [Collier 2001].

There are many useful techniques for strain tracking or for the determination of the clonal origin of different *C. diphtheriae* isolates that are more discriminatory than colony type, biotype or toxin testing [Funke et al. 1997]. Using the older methods, phage typing or serotyping, many strains remain untypeable. These methods have been superseded by tests using molecular techniques. Analysis of whole-cell peptide, multilocus enzyme electrophoresis and pulsed field gel electrophoresis [Riegel et al. 1997, Marston et al. 2001, Damian et al. 2002], genomic DNA tests like restriction length polymorphism [Rappuoli et al. 1988, Riegel et al. 1997, Damian et al. 2002],

random amplified polymorphic DNA [Kombariva et al. 2001] and ribtyping [Riegel et al. 1997, Marston et al. 2001, Damian et al. 2002] have been tested with success in several diphtheria outbreaks.

2.3 Toxin

A key issue of diphtheria is the demonstration of toxin production by the *C. diphtheriae* strain. Iron limitation is an essential factor in the expression of the gene for diphtheria toxin. The agar plate test, described originally by Elek [Elek 1949], is reliable and cheap. However, it is slow for clinical practice, needing at least 48 hours, and even after that it is prone to misinterpretation. Newer modifications of this test can give the result in 16 hours [Engler et al. 1997]. Other methods that have been employed for detection of toxin from bacterial cultures are *Vero* cell bioassay, western blotting and capture enzyme-linked immunosorbent assay (ELISA) [Efstratiou and George 1996]. There are also tests that determine diphtheria toxin from bacterial culture supernatants by agglutination of *Staphylococcus aureus* [Jalgaonkar and Saoji 1993], by agglutination of latex particles [Toma et al. 1997] or by an ELISA using monoclonal antibodies [Hallas et al. 1990]. Although they seem to work well, they are not a solution to the need for early diagnosis. *In vivo* toxin testing in guinea pigs is not to be recommended except in exceptional circumstances [Efstratiou and George 1997].

Polymerase chain reaction (PCR) has been used to detect the diphtheria toxin gene and its regulatory element in culture supernatants. Several groups of investigators have published applications of this procedure [Michailovich et al. 1995, Nakao et al. 1996, Funke et al. 1997, Marston et al. 2001, Damian et al. 2002]. Michailovich and co-workers [1995] found a 100% agreement between a PCR test and the Elek test for 250 *C. diphtheriae* isolates. On rare occasions the gene tests can give false-positive results, when the organism possesses the *tox* gene but is nontoxigenic because it is not able to express the gene. A gene test [Nakao and Popovic 1997] and an immunochromatographic strip test [Engler et al. 2002] to detect toxigenicity directly from clinical specimens (throat swabs) have performed well in two studies. Comparing the performance of the immunochromatographic test directly on throat swabs and on broth cultures of throat swabs, a 99% concordance was seen in 850 specimens in two field trials [Engler et al. 2002].

Of some concern is the result in a recent report of national and international external quality assessment schemes [Snell et al. 1984, Engler et al. 2001]. Twenty-three national diphtheria reference laboratories from 20 countries were made to identify *C. diphtheriae* and its toxin production from six simulated clinical specimens [Engler et al.

2001]. Only three laboratories obtained correct biochemical and toxigenecity results for all six specimens. Three laboratories failed to isolate any corynebacteria from four or more specimens.

2.4 Laboratory diagnosis

The diagnosis of diphtheria in clinical practice rests on both the isolation of *C. diphtheriae* from a clinical sample and the demonstration of toxin production either by the isolated bacterium or *in situ* directly from a clinical sample. However, laboratory diagnosis must be regarded as complementary to, and not a substitute for, clinical diagnosis. Detailed typing methods of the isolated strain serve only epidemiological purposes.

C. diphtheriae will not be identified from throat or pus culture, unless especially requested. If specific laboratory methods are not used, missing the diagnosis of diphtheria is an evident risk, as a third of the patients with exudative diphtheritic throat infection harbour group A β-haemolytic streptococci in their throat [McCloskey et al. 1971] and most patients with skin or wound diphtheria have also *Staphylococcus aureus* or pyogenic streptococci in their lesions [Grunner et al. 1994].

For laboratory diagnosis, throat and nose or nasopharyngeal swabs need to be taken [Efstratiou and Maple 1994, Efstratiou and George 1996, Efstratiou and George 1999]. After collection the swabs must be sent to the laboratory immediately as rapid inoculation on special culture media, e.g. Hoyle's, Downie's or Tinsdale's media, is most important. Isolation of *C. diphtheriae* is facilitated by planting on a selective medium such as cysteine tellurite blood agar or by picking multiple coryneform colonies from a semiselective medium such as colistine-nalidixic acid blood agar [Funke et al. 1997]. If specimens cannot be taken to the laboratory immediately, a transport medium (e.g. Amies) should be used. The growing coryneform bacteria can be identified by commercial kits such as the API CORYNE® (bioMerieux).

The most important test for the microbiological diagnosis of diphtheria is the detection of toxigenicity of the isolated strain. Currently the only *in vitro* method readily available to the diagnostic laboratory is the Elek test, a method very prone to misinterpretation [Brooks and Joynston 1990, Funke et al. 1997]. The PCR method is simple and rapid; the final result can be obtained within 5–6 hours from the selection of colonies. The gene tests for determination of diphtheria toxin directly from clinical samples, if available, can be of considerable help in choosing early antitoxin therapy. To be efficacious, antitoxin treatment should be given during the first three days of illness.

The decision whether to give it or not still depends in the first place on the characteristics of the clinical disease aided by the history of the patient.

3 Pathogenesis

3.1 "Classical" diphtheria

Diphtheria is caused by a toxin-producing strain of *C. diphtheriae*. The bacterium is usually not invasive but remains in the superficial layers of the respiratory mucosa or in skin lesions. Both the acute local inflammation in diphtheria and the late manifestations (myocarditis and polyneuropathy) are regarded as caused by the exotoxin produced by *C. diphtheriae* [Pappenheimer Jr. 1982, Pappenheimer Jr 1983, Pappenheimer Jr 1984, Burch et al. 1968, Groman 1984, Solders et al. 1989]. The toxin is produced at the site of infection (usually in the respiratory tract or in wounds) and is absorbed and transported by the blood circulation. The cells of practically all organs in humans have receptors for the exotoxin [Pappenheimer Jr 1982, Morris and Sealinger 1983]. Localization of the diphtheria toxin has been demonstrated in patients with myocarditis *in situ* in the myocardium by immunofluorescent antibodies [Burch et al. 1968].

The structural gene for diphtheria toxin (*tox*) resides within the genome of a bacteriophage β. Nontoxigenic *C. diphtheriae* strains can become toxigenic if exposed to a tox^+ phage [Collier 2001]. On rare occasions, also *C. ulcerans* [Wong and Groman 1984, deCarpentier et al. 1992, Hust et al. 1994, Ahmad et al. 2000, Wagner et al. 2001] and *C. pseudotuberculosis* [Wong and Groman 1984] have carried the *tox* gene, have produced a toxin identical to diphtheria toxin and have caused toxic diphtheria-like disease in humans [Wong and Groman 1984, Wagner et al. 2001].

The diphtheria exotoxin consists of two polypeptide chains, A and B, linked by a disulfide bond. Fragment B binds to the cellular receptor and is involved in the entry of the catalytic A chain to a susceptible cell [Kaneda et al. 1984]. Fragment A causes a complete cessation of protein synthesis in mammalian cells in 24 to 48 hours by inactivating the cytosolic elongation factor 2 by ADP-ribosylation [Pappenheimer Jr. 1982, Morris and Sealinger 1983, Groman 1984, Pappenheimer Jr. 1984, Sunner and Pullen 1995, Collier 2001]. For sensitive animal species such as guinea pigs, rabbits and primates, the lethal dose of diphtheria toxin is 0.1 μg/kg or less [Pappenheimer Jr. 1982]. Due to its fusogenic and cytotoxic properties, diphtheria toxin has been tested with promising results in cancer therapy [van der Spek et al. 1994, Mizuguchi et al. 1996, Kreitman 1999] and with some success to impair human immunodeficiency virus production in infected cells [Harrison et al. 1992].

Severe diphtheria can lead to disseminated intravascular coagulation and bleeding tendency (described in the early literature as "haemorrhagic diphtheria"), to renal damage and to multiorgan failure [Carter 1943, Isaak-Renton et al. 1981, Centers for Disease Control 1982, Havaldar 1992]. It is not known whether these complications are direct consequences of toxin action or triggered by tissue damage and secondary infections.

3.2 Other infections by *Corynebacterium diphtheriae*

C. diphtheriae probably has additional virulence factors besides the exotoxin as nontoxigenic strains are also associated with significant invasive disease. Nontoxigenic and toxigenic strains of *C. diphtheriae* can cause invasive pyogenic infections. There are reports of single cases and small outbreaks of exudative tonsillitis [Jepchott et al. 1975, Wilson et al. 1990, Wilson et al. 1992, Havaldar and Shanthala 1993, Reacher et al. 2000], of purulent tracheitis [Suresh et al. 1992], of septic arthritis [Afghani and Stutman 1993, Barakett et al. 1993, Damade et al. 1993, Hogg et al. 1996], of osteomyelitis [Poilane et al. 1995], of abscesses [Brooks et al. 1974, Isaac-Renton et al. 1981, Chen et al. 1985, Patey et al. 1997] and of septicaemia and endocarditis [Pike 1951, Isaac-Renton et al. 1981, Barakett et al. 1992, Tiley et al. 1992, Damade et al. 1993, Lin et al. 1994, Hogg et al. 1996, Patey et al.1997, Durandy and Hulin 1999]. Most of these infections have been caused by nontoxigenic strains. The reported incidence of bacteremia in diphtheria varies immensely from a few percent to 90% [Naiditch and Bower 1954, Patey et al. 1997]. Bacteremia, when it occurs, seems to be an early phenomenon even preceding the local respiratory tract symptoms. Except in cases with endocarditis, the clinical role of bacteremia remains obscure.

4 Clinical picture

Diphtheria is an acute infection of the respiratory tract, of skin and wounds or rarely of the gastrointestinal, genital or urinary tract. The etiological agent is a bacillus, *Corynebacterium diphtheriae*. Signs and symptoms of both the acute infection and its late manifestations, myocarditis and polyneuropathy, are considered consequences of the toxin produced by the bacillus.

4.1 Acute local disease

Historical perspective

The first to describe diphtheria may have been the Indian physician who in the 6th century before Christ (BC) described the symptoms of an incurable disease that closed the throat [Metaxa Quiroga 1990]. Equally justified, the earliest possible accounts of diphtheria as a specific disease are found in the Hippocratic (460–377 BC) works (Epidemics III, case 7, 5th century BC) or in a description fitting diphtheria with even greater accuracy in the text "Ulcers about the Tonsils" by Aretaeus, the Cappodocian (81–138 AD) [English 1985]. After the ancient Greek scientists, not much was added to our understanding of diphtheria for one and a half millennia.

A decisive step was attributed to the devastating epidemic in 1735 to 1740 among New England settlers [Kleinman 1992]. Children fell acutely ill with sore throat, there was a thick membrane in the throat, and many soon died through suffocation [English 1985]. The peculiar epidemic nature after a long absence of this kind of clinical syndrome led doctors and lay observers to ask the question, is this throat distemper a "new disease".

Francis Home (1719–1813) in Edinburgh described in 1765 twelve cases of "croup" with membranes, obvious cases of diphtheria [Feldman 2001]. The etymology of diphtheria extends back only to 1826, when Pierre Bretanneau (1778–1862), a French pathologist and clinician, coined the term "diphtherite" [English 1985, Metaxas Quiroga 1990, Hardy 1992]. He chose this Greek root, which meant skin or hide, because of the pharyngeal membrane of diphtheria often looked like a piece of leather. Pierre Bretanneau gave diphtheria the first clinical case definition.

Do we know the nature of diphtheria?

Problems with clinical definition

There is no uniformly accepted clinical case definition for diphtheria. Nor is there general agreement on how to divide the infection into clinical categories (clinical forms) [Carter 1943, Brainerd 1951, Naiditch and Bower 1954]. The World Health Organization has given one case definition [World Health Organization 1994]. This definition stresses a positive culture for *C. diphtheriae*. It leaves out patients with no pseudomembrane. WHO does not demand the demonstration of toxin production by the

bacterial isolate for the clinical diagnosis of diphtheria. The WHO definition is obviously intended for epidemiological work and early detection of cases of diphtheria in conditions of limited resources. It is not suitable for scientific work aiming to investigate the clinical characteristics and the prognosis of diphtheria.

Relevance of old observations

The large published clinical studies of respiratory tract diphtheria in population-wide epidemics date to the first half of the 20th century. All the studies are retrospective (see Table 4). When trying to apply the results to the current situation, many problems arise: (1) are the data really from patients with diphtheria, (2) how did the wider availability of antibiotics starting in the early 1950s affect the possibility for microbiological diagnosis of diphtheria, (3) are the clinical case definitions in different studies sufficiently similar, (4) how does the varying share of vaccinated and unvaccinated persons influence the observations, (5) how to interpret data from studies with varying proportions of infants and the elderly if the data is not reported by age group, and (6) are data from epidemics among special risk groups applicable for the whole of society. In conclusion, there are two types of concerns: are the observations really made from diphtheria patients and to what kind of population the results apply.

In the early studies the diagnosis of diphtheria was based almost exclusively on typical clinical presentation with a positive throat culture for *C. diphtheriae*. Toxin production by the isolated strain was not required (see Table 4). During an epidemic, a carrier of *C. diphtheriae* with a respiratory tract infection caused by some other organism may have been mistakenly regarded as having diphtheria. As the rate of asymptomatic carriers is usually low, a positive culture is fairly specific in recognizing true cases of diphtheria, but during outbreaks a good share of carriers of *C. diphtheriae* with other throat infections may have been included in the analysis. In most clinical studies the enrolment criterium has been a positive throat culture [Carter 1943, Naiditch and Bower 1954]. Diphtheria cultures are not used routinely for patients with upper respiratory symptoms. Patients are likely to get empirical antibiotic treatment early in the disease, hampering the possibilities of later microbiological diagnosis.

These pitfalls in the specific diagnosis of diphtheria underline the importance of the clinical case definition. Even in unselected series of hospital patients, a varying share of the patients, 16–70%, with a positive throat culture or clinical suspicion of diphtheria have had a non-typical, in other words a non-membraneous, non-complicated infection [Johnson Jr. 1947, Brainerd and Bruyn 1951, Naiditch and Bower 1954, Dobie and Tobey 1979, Singh et al. 1999]. In many reports, in addition to bacteriologically proven

cases, patients with typical peseudomembranes or typical complications (myocarditis or polyneuropathy) have been included without bacteriological diagnosis. This, evidently, gives a bias for the observations. In some, even recently published clinical reports the diagnosis has been based on clinical diagnosis alone, thus including only cases regarded as typical [Singh et al. 1997].

The non-vaccinated, small children, the elderly and alcoholics, are known to be at special risk for diphtheria and they have an increased risk for a more severe form of the disease. Since the 1920s in the United States and the 1940s in Europe, a good proportion of the population has been immunized [Collins 1946]. Still in the 1950s there were no reliable data on vaccination coverage in the population [Naiditch and Bower 1954]. The most comprehensive studies are from the period when the proportion of vaccinated persons was varying and often unknown [Carter 1943, Collins 1946, Naiditch and Bower 1954]. In the published clinical reports the proportion of small children and persons over 40 years of age has been varying, and in many publications the data is not reported separately for different age groups [Carter 1943, Collins 1946, Brainerd 1951, Naiditch and Bower 1954, Hardy 1992, Youwang et al. 1992, see Table 4]. All outbreaks in developed countries since the 1970s have occurred among special groups such as alcoholics and intravenous drug abusers [Munford et. 1974, Hennekens and Saslav 1976, Nemes and Westhoff 1983, Chen et al. 1985, Cristensson et al. 1989, Harnish et al. 1989, Grunner et al. 1994] or social, cultural or ethnic minorities [Jellard 1972, Chen et al. 1985, Harnish et al. 1989, Hogg et al. 1996, Centers for Disease Control and Prevention 1997]. These groups do not represent the general populations in respect to hygiene, risk factors for severe outcome and opportunities to get early (antitoxin) treatment.

In conclusion, our understanding of diphtheria as a clinical disease, as presented in the textbooks, is not based on adequate controlled studies. From the available data, it is difficult to predict what would be the fate of individual patients and the population if we should now face an epidemic of respiratory tract diphtheria in a developed country.

Clinical presentation

Diphtheria is an acute exudative or membraneous inflammation of the upper respiratory tract. [World Health Organization 1994]. In more than 90% of cases the primary focus is in the tonsils or in the pharynx [Brainerd and Bruyn 1951, Naiditch and Bower 1954]. At the outset, the membranes are firmly adherent and they often extend to the uvula and soft palate, and sometimes to the esophagus and the stomach [Honey 1947]. In 1–3% of patients the primary site is either the nostrils, the conjunctiva, the ears or the larynx

[Brainerd and Bruyn 1951, Naiditch and Bower 1954]. These sites are affected mainly in small children. Respiratory tract diphtheria and cutaneous diphtheria can coexist in a patient [Shenoy et al. 2002].

Signs and symptoms

After an incubation period of usually 2 to 5 days (occasionally up to 10 days) the onset of diphtheria is insidious with a sore throat and modest fever [Brainerd and Bruyn 1951, Naiditch and Bower 1954, McCloskey et al. 1971]. The pain and difficulties in swallowing may be intense. Nausea, vomiting and headache are more common in diphtheria than in other throat infections, e.g. in streptococcal tonsillitis [McCloskey et al. 1971] (Table 2). In a one-sided infection the oedema is often mistaken for a peritonsillar abscess, and an unnecessary tonsillectomy may be performed.

The classical local finding in diphtheria is an exudative or membraneous inflammation with or without submucosal and subcutaneous swelling ("bull neck"). The membranes can be initially patchy, but become thicker in a few days. The membranes, more correctly pseudomembranes, are thick and they are composed of fibrin, decaying epithelial cells, bacteria and polymorphonuclear cells (Figure 3a). The pseudomembranes are often greyish or rusty brown in colour and firmly adherent. Attempts to remove the pseudomembrane leave a raw, bleeding surface (Figure 3b).

The diagnosis of diphtheria should not be based on the presence of a pseudomembrane. Even in unselected series of hospital patients a varying share of the patients, usually a quarter to three quarters, have had a non-membraneous infection [Brainerd and Bruyn 1951, Naiditch and Bower 1954, Dobie and Tobey 1979, Singh et al. 1999]. There may never have been membranes, or the membranes may have been lost early in disease.

Table 2. The symptoms and signs in 134 patients with respiratory tract diphtheria at the time of initial examination [McCloskey et al. 1971].

Feature	Total number of patients	Rate (%)
Fever	114	86
Sore throat	115	85
Nausea or vomiting	34	25
Headache	24	18
Oedema of the neck	24	18
Chills	13	10

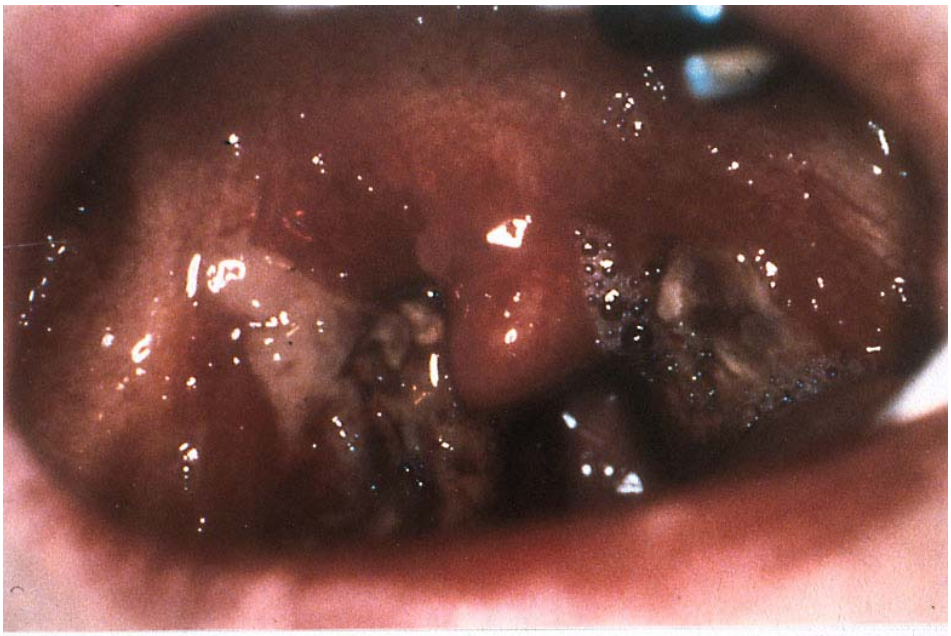
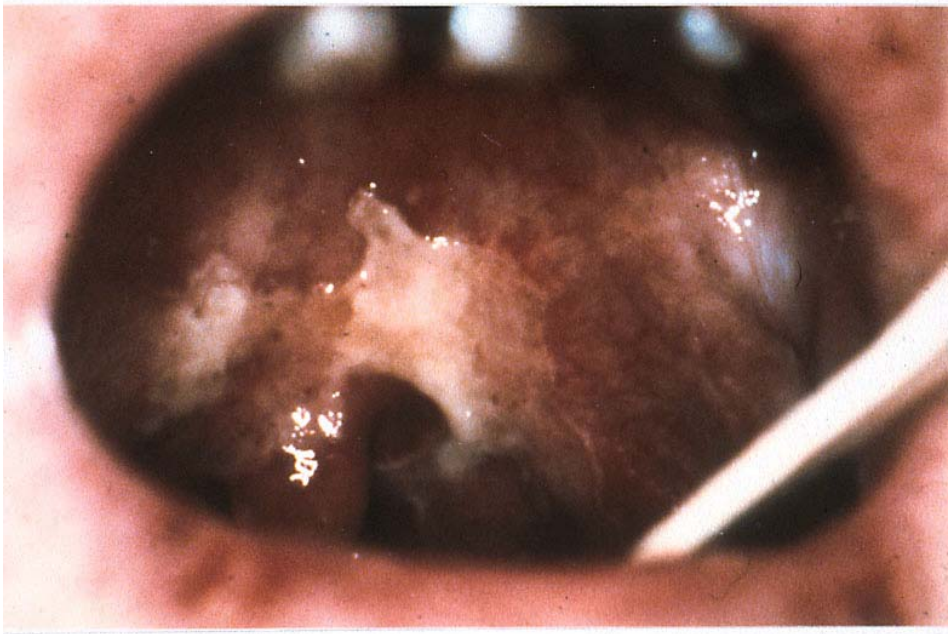


Figure 3. Acute tonsillo-pharyngeal diphtheria with thick brownish membranes (a).



Throat after detachment of the diphtheritc membrane (b).

There is convincing evidence that respiratory tract diphtheria can present as a mild inflammation of the tonsils or the pharynx without pseudomembranes ("catarrhal form") [Paley and Truelove 1948, Jones et al. 1985, Grigoryev 1995, Grinchuck 1996]. Even deaths in late sequels of such mild local forms of diphtheria have been described [Paley and Truelove 1948].

There is no uniformly accepted way of dividing diphtheria into clinical groups. Diphtheria is usually divided according to the anatomical localization of exudate or pseudomembrane (Table 3). In severe cases, the membranes extend from their usual sites, the tonsils and the pharynx ("faucial form"), to the larynx, the trachea and even to the bronchi and bronchioles ("extended form") [Naiditch and Bower 1954]. A case of diphtheria is called "toxic" when there is visible subcutaneous oedema and regional lymphadenopathy giving a bull neck appearance. The toxic forms are often anatomically extended forms [Naiditch and Bower 1954]. Severe toxic diphtheria ("malignant diphtheria") may lead to a bleeding tendency due to disseminated intravascular coagulation ("haemorrhagic form") and to renal failure or to multiple organ failure [Carter 1943, Centers for Disease Control and Prevention 1982, Sing et al. 1999].

Table 3. The division of 1,433 cases of diphtheria into clinical forms in a series of patients collected from 1940 to 1950 [Naiditch and Bower 1954]. Of the patients, 439 were 5 years old or younger and 122 were 40 years old or older.

Clinical form	Total number of patients	Mortality rate (%)
Tonsillopharyngeal	879	1.8
Tonsillopharyngeal and nasal	102	8.9
Tonsillopharyngeal and nasal and laryngeal	22	18.2
Tonsillopharyngeal and nasal and laryngeal and tracheobroncial	15	86.8
Tonsillopharyngeal and laryngeal	123	7.3
Tonsillopharyngeal laryngeal and tracheobroncial	51	54.8
Tonsillopharyngeal with bull neck[1]	98	16.3
Tonsillopharyngeal and nasal with bull neck[1]	36	36.1
Tonsillopharyngeal and nasal and laryngeal with bull neck[1]	7	42.9
Tonsillopharyngeal and nasal and laryngeal and tracheobroncial with bull neck[1]	11	90.9
Tonsillopharyngeal and laryngeal with bull neck[1]	10	40.0
Tonsillopharyngeal and laryngeal and tracheobroncial with bull neck[1]	11	90.9
Primary nasal	27	0
Primary laryngeal	20	0
Primary laryngeal and tracheobronchial	6	33.3
Primary tracheobronchial	1	0

[1] oedema of the neck

The early symptoms depend on the localization of inflammation. In faucial diphtheria there is often sequestration of saliva in the mouth due to pain in swallowing and early pharyngeal paralysis. In nasal diphtheria, there is a serous, followed by serosanguineous discharge from the nostrils. Typical of primary laryngeal diphtheria is gradually increasing hoarseness and later stridor and difficulty in breathing [Naiditch and Bower 1954, McCloskey et al. 1971, World Health Organization 1994, Grigoryev et al. 1995]. For the clinical diagnosis of the localized laryngeal form of the disease an indirect laryngoscopy is needed to show the swollen epiglottis and subglottis with pseudomembranes.

Laboratory findings

There is nothing characteristic of diphtheria in the findings of clinical chemistry. Leucocytosis is usually moderate [Naiditch and Bower 1954]. In patients with a clinical bleeding tendency, low thrombocyte values, high amounts of fibrinogen degradation products and an elevated D-dimer level in the blood are regularly observed. Abnormal renal function tests, proteinuria and haematuria are seen in the 3% of patients who acquire renal damage as a complication of severe diphtheria [Branierd and Bruyn 1951].

4.2 Late disease

Myocarditis and polyneuropathy are the two main complications of diphtheria. They manifest late in the disease; myocarditis on the second week of disease and polyneuropathy one month after the onset of respiratory tract symptoms. They are considered to be caused by the exotoxin of *C. diphtheriae*.

Cardiac disease

Sudden vascular collapse is a striking consequence of diphtheria. Cardiac damage is the second most common cause of mortality in diphtheria, next only to asphyxia with suffocation. Death due to myocarditis comprises not less than a third of all deaths [Dobie and Tobey 1979, Naiditch and Bower 1954]. The risk factors for cardiac involvement and the incidence and the pathogenesis of this complication are not well established. Although newer studies indicate that the heart involvement is not

predominantly inflammation, for historical reasons the term “myocarditis” is used throughout this review.

History

As cited by Warthin [1924], it seems to have been Werner, a German physician who connected pathology of the heart to diphtheria and sudden death. The report was published in 1842. This observation was helped by the invention of tracheostomy (Bretonneau, in 1825) to treat diphtheria patients, as the severely sick patients survived long enough to manifest myocarditis [English 1985, Metaxa Quiroga 1990, Hardy 1992]. At the time of Werner’s observation, only palpation of the heart and the arteries, auscultation of the heart and necropsy were available to study further the causes of diphtheritic circulatory catastrophe. The great interest in the diphtheritic heart is illustrated by the development of theories of its pathogenesis from 1860 to 1920. In order, the following theories were put forward: (1) cardiac thrombosis, (2) infectious endocarditis, (3) endarteritis of cardiac vessels, (4) infectious parenchymatous myocarditis, (5) infectious interstitial myocarditis, (6) injury to cardiac nerves, ganglions, vagus nerve and abdominal sympathetic and vasomotor system, resulting in cardiac paralysis, (7) toxic myocarditis and (8) a special affinity of diphtheria toxin for the impulse-conducting system of the heart (bundle of His) [Warthin 1924]. The still prevailing toxin theory was made possible by identification of the diphtheria bacillus (Klebs, Loeffler and Darier in 1883–1885) and diphtheria toxin (Roux in 1988) [English 1985, Metaxa Quiroga 1990, Hardy 1992, Hooker and Bashford 2002]. These observations led to animal experiments. Living cultures of diphtheria bacilli or their toxin were injected into guinea pigs, rabbits and dogs, confirming the role of diphtheria toxin in the pathogenesis of diphtheritic myocarditis [Marvin 1925].

Pathogenesis

At the turn of the 20th century, it was the pathologists performing autopsies who could best study diphtheritic heart damage. In the 1920s, after numerous published series of autopsies, knowledge of the findings on the diphtheritic heart was almost what we know now. The most constant and characteristic findings in a microscopic examination of the heart are cloudy swelling, fatty or waxy degeneration and necrosis of myofibres [McCullogh 1920, Warthin 1924, Marvin 1925]. The damage is most marked in the myocardium. Contrary to earlier beliefs, the damage has no predilection for the heart

ganglia or the conduction system. Hyaline degeneration and inflammatory reaction, as indicated by mononuclear cell and eosinophile cell infiltration, are usually a late finding and not very prominent [Gore 1948, Riley Jr. and Weaver 1952]. These phenomena may indicate regeneration.

Later, in the 1960s, the localization of the diphtheria toxin was demonstrated *in situ* in the myocardium by immunofluorescent antibodies [Burch et al. 1968]. Electron microscope investigation has shown damage of mitochondria associated with depletion of glycogen and accumulation of lipid droplets in the damaged myofibers [Burch et al. 1968]. These observations are in accordance with the finding of interference by diphtheria toxin with the synthesis and function of the cytochrome system [Pappenheimer Jr. and Murphy 1984]. There are no reports describing findings on endomyocardial biopsy specimens taken from a living person. In conclusion, the heart damage in diphtheria seems to be more of an acute toxic cardiomyopathy than myocarditis. It seems to be more prominent in heart muscle cells than in the cells of the conduction system.

Electrophysiology

Diphtheria was one of the first diseases where the "polygraphic method", the predecessor of modern electrocardiography (ECG), was used. The first studies using this method on diphtheria patients were published in Germany in early 20th century [Rohmer 1912]. In diphtheria, the changes in ECG recordings are more often those found in myocardial damage (T-wave and ST-segment changes and extrasystoles) than those found in conduction abnormalities (intraventricular conduction defects and total atrio-ventricular (A-V) dissociation) [Begg 1937, Boyer and Weinstein 1948, Naiditch and Bower 1954, Morgan 1963, Ledbetter et al. 1964]. A fairly recent Russian report indicates that echocardiography may be superior to ECG in detecting early myocarditis [Kots et al. 1991].

The ECG abnormalities seen in diphtheria do not differ from those seen in other acute infections with myocarditis [Boyer and Weinstein 1948, Morgan 1963, Morgera et al. 1992]. In a ten-year material of 1,433 unselected hospital patients (28% of them adults) 70 patients had an ECG recorded [Naiditch and Bower 1954]. Sixty-one had T-wave or ST-segment changes, 7 had an A-V block of first degree, 8 had a bundle branch block and 9 had a complete A-V block. A more recent material describes the findings of 229 children treated in one hospital in the United States for respiratory tract diphtheria in a 7-year period [Ledbetter et al. 1964]. Of 47 children with clinically proven myocarditis, 11 had T-wave or ST-segment changes only, 11 had A-V block of first

degree, 9 had intraventricular block and 14 had complete bundle branch block or complete A-V block. The findings in these two studies could indicate that conduction abnormalities are associated especially with more severe forms of diphtheria. However, the data is conflicting. In a large study with serial ECG recordings from each patient, no obvious correlation was found between the severity of acute diphtheria and the severity of ECG changes [Altshuler et al. 1948].

According to published reports, changes in ECG recordings of diphtheria patients emerge between day 3 and day 47 from the onset of the disease, with a median delay of approximately 11 days [Stecher 1928, Altshuler et al. 1948, Boyer and Weinstein 1948, Ledbetter et al. 1964, Vichitbandha et al. 1969, Salih et al. 1981, Araujo et al. 1990]. There is some evidence that the changes emerge earlier, during the first week in severe forms of diphtheria [Wesselhoeft 1940, Boyer and Weinstein 1948, Brainerd and Bruyn 1951, Morgan 1963]. The electrophysiological abnormalities usually last for several weeks but finally return to normal in most patients [Altshuler et al. 1948, Brainerd and Bruyn 1951, Tahernia 1969, Salih et al. 1981].

Laboratory findings

There are four reports in which aminotransferase values have been followed in patients with diphtheritic myocardits [Chesler 1958, Choremis and Leonidas 1962, Bethell et al. 1995, Havaldar et al. 2000]. Aspartate aminotransferase was more sensitive than alanine aminotransferase in recognizing patients with myocarditis. In one study, the amount of aspartate aminotransferase was elevated in all 19 patients with myocarditis and in none of the 20 without this complication [Choremis and Leonidas 1962]. The elevation of aspartate aminotransferase was less pronounced but of longer duration (median 2 weeks) than in myocardial infarction. The level correlated to the severity of changes in the ECG recording. However, in this study the patients with myocarditis had very severe diseases, and the origin of the aspartate aminotransferase remained obscure. There are no studies in which the values of enzymes more specific for the heart muscle, like MB fraction of creatine kinase or troponin I, have been measured in diphtheria patients.

Incidence and risk factors

Population-wide epidemics of respiratory tract diphtheria have not occurred in the era of modern scientific methodology. The true incidence and risk factors for myocarditis have not been clearly established in prospective clinical trials with strict criteria for what

constitutes an abnormal ECG. However, since the introduction of ECG in clinical practice, about 30 studies reporting on cardiac involvement in respiratory tract diphtheria have been published (Table 4). Only twelve studies have included more than 10 adult patients [Ball 1945, Carter 1947, Altshuler et al. 1948, Gore 1948, Weinstein 1948, Brainerd and Bruyn 1951, Naiditch and Bower 1954, Boyer and McCloskey et al. 1971, Dobie and Tobey 1979, Harnisch et al. 1989, Kadirova et al. 2000, Quick et al. 2000b]. The reported risk of clinically evident cardiac involvement has ranged from 2% [Havaldar et al. 1989] to 33% [Nathanson 1928], typically from 10% to 20% [Honey and Welford 1934, Grant 1945, Carter 1947, Naiditch and Bower 1954, Ledbetter et al. 1964, Harnisch et al. 1989]. The incidence of heart involvement as determined by an ECG analysis has varied even more, from 7% [Vichitbandha and Honghathai 1969, Dobie and Tobey 1979] to 89% [Alstead 1932]. The highest reported incidence (65%) for myocarditis in a relatively unselected patient population comes from a study by Boyer and co-workers in the 1940s where serial ECG recordings were performed on 93 paediatric and adult patients with diphtheria [Boyer and Weinstein 1948]. However, in this study the diagnostic criteria for diphtheria were not given, very transient T-wave and ST-segment alterations were regarded as indicative of diphtheritic myocarditis, and the results for the children and the 33 adult patients were not reported separately. In a study by Altshuler and co-workers on 574 United States soldiers with serial ECG follow-up, a 24% incidence of ECG changes was reported [Altshuler et al. 1948].

There are only three studies, none of them prospective, where any factors related to the risk of cardiac involvement have been reported. In a study where serial ECG recordings were used to identify cardiac involvement, the patients with myocarditis had a higher average age than patients without myocarditis (25 vs. 12 years) [Boyer and Weinstein 1948]. A small study using only clinical criteria for complications showed that patients older than 60 years had the most complications [Harnisch et al. 1988].

Prognosis

A great difficulty in studies focusing on the prognosis of diphtheritic myocarditis is a reliable diagnosis for cardiac involvement. Mild T-wave changes and slight prolongation of the A-V conduction time are non-specific and observed in various infections. Another pitfall in interpreting data from the early studies is that the clinical criteria for the diagnosis of diphtheria are varying and poorly documented, and the bacteriological diagnosis is either not mentioned or defectively performed (Table 4). Therefore, it is not surprising that there is huge variability between different studies on the risk of a lethal outcome for patients with diphtheritic myocarditis. There are two larger fairly unbiased

old studies. The death rate for those with cardiac involvement was 21% in the study by Boyer and Weinstein [1948] and 1.4% in the study by Altshuler and co-workers [Altshuler et al. 1948]. In some studies, death rates of more than 50% have been reported [Havaldar et al. 1989, Araujo et al. 1990].

High serum aspartate aminotransferase values have been reported to be associated with increased risk of death [Havaldar et al. 2000]. Abnormal conduction in the heart seems to give a considerably worse prognosis than changes indicating myocardial involvement. In one series of 100 patients with faucial diphtheria, 11% of patients with only T-wave changes died in contrast to 52% of those with intraventricular or complete A-V block [Begg 1937]. Patients with A-V dissociation still have a high mortality in spite of modern intensive care and electric pacing of the heart [Salih et al. 1981, Havaldar et al. 1989, Araujo et al. 1990, Havaldar 1992, Stockins et al. 1994, Bethell et al. 1995, Stastny et al. 1999, Perles et al. 2000]. In a study on 15 children using 24-hour ECG recording, even modest ventricular ectopia (extrasystoles) at the time of presentation predicted a fatal outcome [Bethell et al. 1995].

If the patients survive, even severe changes in the ECG recordings usually disappear in three months and the clinical recovery is complete [Begg 1937, Thompson et al. 1937, Boyer and Weinstein 1948, Brainerd and Bruyn 1951, Salih et al. 1981]. According to old observations, heart failure during convalescence is very rare [Marvin 1925]. However, autopsy findings show that diphtheritic myocardial lesions heal at least in some cases by fibrosis, so some permanent damage is left even if the ECG returns to normal [Wesselhoeft 1940]. The elevation of aspartate aminotransferase values in patients with even severe diphtheritic myocarditis is modest compared to the elevation in patients with myocardial infarction [Chesler 1958, Choremis and Leonidas 1962, Tahernia 1969, Bethell et al. 1995]. This could indicate smaller permanent myocardial damage in diphtheria. There is still debate on whether an episode of diphtheritic myocarditis brings an increased risk of heart failure later in life.

Polyneuropathy

Diphtheritic polyneuropathy is a demyelinating process affecting cranial, peripheral and autonomic nerves. Both sensory and motor disturbances occur. Cranial nerves are affected earlier, median 21 to 30 days after the throat inflammation. At least partly the cranial nerve pareses can be caused by locally absorbed diphtheria toxin. The peripheral neuropathy is a late complication of diphtheria emerging during convalescence, median one to two months after the onset of acute infection. The damage to peripheral nerves is regarded as being direct damage by circulating diphtheria toxin.

Table 4. Reported incidence and death rate of diphtheritic myocarditis in the earlier studies.

Reference	Method	Basis of diagnosis[1]	Patient material	Proportion of adults (%)[2]	Criteria for taking ECG	Number of patients	Proportion with a clinical diagnosis of myocarditis (%)	Proportion with ECG-verified myocarditis (%)	Death rate (%)
McCulloch 1920	Retrospective	NM[3]	Hospital patients	0/ 80 (0)	By clinical need	80	NM	19 / 80 (21)	NM
Smith 1921	Prospective ?[4]	NM	Hospital patients, unselected	NM	Serial for all patients	242	NM	(28)	NM
Marvin 1925	Prospective ? [4]	Most culture positive, toxin NM	Faucial and laryngeal forms excluded	23/ 90 (26)	Serial for selected patients	150	NM	37 / 90 (41)	(27), of all patients
Alstaed 1932	Retrospective	NM	Selection of different severities	0/ 100 (0)	Serial for all patients	100	NM	89/100 (89)	(19), of myocarditis patients
Honey et al. 1934	Retrospective	NM	Hospital patients, unselected	37/ 496 (7), of myocarditis patients	ECG not taken ?	4,671	486/ 4,671 (11)	NM	(62), of myocarditis patients
Begg 1937	Prospective	NM	Severe forms only	NM	Serial for all patients	100	(27)	(84)	(28), of myocarditis patients
Burkahard et al. 1938	Retrospective	NM	Only toxic cases with membrane	9/ 37 (24), of myocarditis patients	Daily for all patients	140	NM	37 / 140 (26)	(38), of myocarditis patients
Bower 1941	Retrospective	NM	Survivors who had an ECG taken	NM	At least one for each patient	278	NM	28 / 278 (10)	(0), only survivors included
Grant 1945	Retrospective	Culture positive, toxin NM	Hospital patients, Unselected	0/ 927 (0)	NM	927	122/ 927 (13)	NM	52/ 927 (19), of all patients
Carter 1947	Retrospective	Culture positive, toxin NM	Population based	(20-22), estimated	NM	1,270	133/ 1,270 (10)	NM	NM
Altshuler et al. 1948	Retrospective	Culture-positive, toxin NM	Male soldiers, hospital patients, unselected	(100)	Serial for a minimum of 5 weeks	574	NM	139/ 574 (24)	2/ 139 (1.4), of myocarditis patients
Boyer et al. 1948	Prospective ? [4]	Culture positive, toxin NM	Mild and severe, selected	(36), over 19 years of age	Serial for all	93	NM	(65,5)	(21)
Gore. 1948	Retrospective	NM	US Army personnel, only fatal cases	150/ 187 (80), over 19 years of age	For 36%, not systematic	205	NM	(100) of those 52 with ECG	(100), only fatal cases included
Brainerd et al. 1951	Retrospective	Most culture positive, toxin NM	Those treated at hospital	(40), over 14 years of age	For some patients, not systematic	273	NM	(37)	NM
Naiditch et al. 1954	Retrospective	Culture positive, toxin NM	Cases with membrane	(28), over 20 years of age	For unknown proportion	1,433	(9)	NM	(52), of those with clinical myocarditis

Reference	Method	Basis of diagnosis[1]	Patient material	Adults (%)	Criteria for taking ECG	Number of patients	Proportion with clinical diagnosis of myocarditis (%)	Proportion with ECG-verified myocarditis (%)	Death rate (%)
Morgan 1963	Retrospective	Culture positive, toxin NM	Hospital patients, unselected	(0), over 17 years of age	At least one for each	95	4/ 95 (4.2)	31/ 95 (32)	3/ 95 (3.2)
Ledbetter et al. 1964	Retrospective	NM	Hospital patients, unselected	(0)	For 226/229 at least one	229	NM	47/ 226 (21)	(4.4), of all patients (21), of those with yocarditis
Tahernia 1969	Retrospective	NM	Hospital patients, unselected	(0)	At least one for each patient	46	21/ 46 (46)	10/ 46 (21)	10 / 21 (48) , of those with clinical myocarditis
Vichitbandha et al. 1969	Retrospective	20% cuture positive, toxin NM	Hospital patients, unselected	(0)	At least one for each patient	521	NM	37/ 521 (7)	11/ 37 (30), of those with myocarditis
McCloskey et al. 1971	Retrospective	75% culture and toxin positive	Hospital patients, unselected	34/ 148 (23)	At least one for each patient ?	148	59/ 148 (40)	NM	1/ 59 (1.7) of those with myocarditis
Munford et al. 1974	Retrospective	All culture and toxin positive	Population based	(15), over 20 years of age	NM	2,547	NM	NM	NM
Dobie et al. 1979	Retrospective	35/ 42 (83%) culture and toxin positive	Hospital patients, unselected	(84), over 19 years of age	Not systematic	42	(2.4)	(7.1)	(33)
Salih et al. 1981	Prospective	38% culture positive, toxin NM	Complicated cases only	(0)	For all, by clinical need	29	6/ 29 ? (21)[4]	6/ 29 ? (21)[4]	5/ 6 (83), of those with myocarditis
Jones et al. 1985	Retrospective	56/ 149 (38%) culture and toxin positive	Hospital patients, unselected	(1.3), over 14 years of age	NM	149	NM	NM	NM
Harnish et al. 1989	Retrospective	40% culture and toxin positive	Population based	(82)	NM	153	NM	8/ 72 (11)	(25), of those with myocarditis
Havaldar et al. 1989	Retrospective	NM	Hospital patients, unselected	(0)	NM	228	5/ 228 (2.2)	NM	2/ 5 (60), of those with myocarditis
Araujo et al. 1990	Prospective	Culture positive, toxin NM	Severe forms only	(0)	Serial for all patients	14	(100)	(100)	(50)
Stockins et al. 1994	Retrospective	Culture positive, toxin NM	Hospital patients, unselected	(0)	Not systematical	167	NM	46/ 167 (27)	11/ 46 ? (24)[4]

[1] Culture means a positive respiratory tract culture for *Corynebacterium diphtheriae*, toxin means that the isolated strain is producing toxin *in vitro*

[2] Adult if a person over 15 years of age, unless otherwise stated

[3] Not mentioned

[4] ? means that the data is not unambiguously stated in the article

Historical perspective

After recognition of the diphtheria as a disease entity in the 1820s, for several decades not much attention was paid to the neurological complications. There was a high mortality to the acute infection and problems of treating the severe local complications, and cardiac complications predominated [Metaxas Quiroga 1990]. The first clinical article focusing especially on neurological complications of diphtheria was published by Ernest Remak (1849–1911) in 1900 in German [reviewed by Walshe 1918–1919]. Already Remak regarded polyneuropathy as a consequence of damage to neurons by circulating diphtheria toxin. In the beginning of the 20th century, several outbreaks of skin diphtheria occurred in soldiers stationed in the tropics. Observations on these outbreaks led to a number of articles about "diphtheritic paralysis" [Walshe 1918–1919]. Most studies behind our current knowledge about the incidence, risk factors and histopathology of diphtheritic polyneuropathy were published from the big epidemics of diphtheria in Europe during and after World War II [Burkhard et al. 1938, Carter 1943, Gaskill and Korb 1946, Naiditch and Bower 1954]. Since the 1970s, mainly electrophysiological investigations have added to our knowledge on diphtheritic polyneuropathy [Kurdi and Abdul-Kader 1979].

Pathogenesis

Practically every human cell type, including the autonomic and peripheral nerve cells, has receptors for the diphtheria toxin [Morris and Sealinger 1983, Pappenheimer Jr. 1982]. *In vitro*, and when injected into experimental animals, diphtheria toxin produces motoneuron damage in eight days by inhibiting neurofilament transport [Sunner and Pullen 1995]. The early occurring palatal paralysis is regarded as a consequence of locally absorbed toxin [Walshe 1918–1919, Gaskill and Korb 1946]. This theory is supported by the observation, that the manifestation is rare in patients with skin and wound diphtheria [Gaskill and Korb 1946]. The late neuropathy affecting the cranial nerves and the peripheral nerves of limbs and trunk is thought to be caused by toxin being spread by the blood circulation. There is no explanation for the long delay between the acute local infection and the onset of polyneuropathy.

According to old necropsy findings, the pathological changes in the peripheral nervous system are concentrated in the region of the dorsal root ganglia and adjacent parts of the dorsal, ventral and mixed spinal nerve roots [Fisher and Adams 1956]. The typical findings are patchy demyelination with thin and short internodes in the presence

of preserved axonal continuity. In a case report on findings in a living person's sural nerve biopsy, a reduced number of myelinated nerve fibres and no inflammatory cells were described [Solders et al. 1989]. In electron microscopy, normal but demyelinated nerve fibres have been observed [Solders et al. 1989]. These observations are consistent with mixed sensory/motor demyelinating non-inflammatory neuropathy.

Electrophysiological investigations show slowed conduction velocities, multiple conduction block and prolonged F response latencies [Solders et al. 1989, Ghanem 1993]. Prolonged distal motor latencies are more common and a more pronounced finding than slowing of motor conduction [Logina and Donaghy 1999]. The abnormalities are maximal seven weeks after the onset of neuropathy. Autonomic effector organ tests show early and prominent impairment in the parasympathetic vagal functions, in the variation of the R-wave to R-wave interval in ECG and in the Valsalva manouvre [Solders et al. 1989]. The electrophysiological abnormalities are very similar to those seen in the Guillain-Barré syndrome, but they deteriorate longer (for a median of 49 days vs. 10 days) and start to improve later (after a median of 73 days vs. 21 days) [Logina and Donaghy 1999]. The possible involvement of autoimmunity in the pathogenesis of diphtheritic neuropathy has not been tested with determinations of antibodies against neural antigens.

Clinical presentation

Cranial or bulbar neuropathy

There are two distinct types of neurological complications; cranial (bulbar) neuropathy that affects predominantly the IX and X cranial nerves and peripheral neuropathy [Walshe 1918–1919, Dobie and Tobey 1979]. In severe cases of cranial neuropathy all cranial nerves from III to XII can be affected [Piradov et al. 2001]. The cranial neuropathy emerges two days to two months (median 10–20 days) after respiratory tract symptoms [Walshe 1918-1919, Burkhard et al. 1938, Brainerd and Bruyn 1951, Naiditch and Bower 1954, Dobie and Tobey 1979, Salih et al. 1981, Solders et al. 1989, Logina and Donaghy 1999, Piradov et al. 2001]. Palatal paralysis can be very early and may already be present when the patient first contacts health care. Typical symptoms of cranial nerve involvement are dysphagia, aspiration, nasal regurgitation, nasal voice, numbness of the gums and the tongue and diminished taste [Gaskill and Korb 1948, Dobie and Tobey 1979, Piradov et al. 2001]. The improvement of bulbar symptoms starts 30 days (range 3–98 days) after the onset of neurological symptoms [Logina and Donaghy 1999]. In a few patients a secondary deterioration in convalescence can occur [Logina and Donaghy 1999].

Peripheral neuropathy

Peripheral neuropathy with sensory and motor paralysis of the muscles of the trunk and limbs manifests later. The delay from onset of respiratory tract symptoms to onset of paralysis varies from 12 to 91 days (median 37–61 days) [Walshe 1918–1919, Brainerd and Bruyn 1951, Dobie and Tobey 1979, Soders et al. 1989, Roche et al. 1990, McAuley et al. 1999, Logina and Donaghuy 1999, Piradov et al. 2001]. In most cases the motor dysfunction is more prominent. In about a third of the patients the sensory disturbances manifest first or dominate [Walshe 1918–1919, Gaskill and Korb 1946, Piradov et al. 2001].

Typically, the motor dysfunction starts from the periphery of the limbs. It can lead slowly, in a few weeks or months, to total quadriplegia and paresis of respiratory muscles [Piradov et al. 2001]. Maximal motor disturbances are reached in 6 to 9 weeks [Piradov et al. 2001]. Even in mild cases the patients have hypo- or areflexia. Sensory dysfunction starts usually with numbness or tingling in the periphery of extremities, but sometimes pain in these areas can dominate [Walshe 1918-1919, Gaskill and Korb 1946, Piradov et al. 2001]. Paresis of the oculomotor nerve is not rare in diphtheria. It appears late in the disease and can, rarely, be the only manifestation of neuropathy [Dobie and Tobey 1979, Piradov et al. 2001]. It brings blurred vision and difficulties in accommodation. The peak severity of peripheral neuropathy is reached two to 14 weeks (median 49 days) after the onset of diphtheria, and improvement begins in 20 to 115 days (median 73 days) [Logina and Donaghuy 1999].

Autonomic nerve dysfunction has been observed in up to 50% of the patients with diphtheritic neuropathy. A short-lived impairment of the parasympathetic reflex arch leading to cardiac vagal dysfunction is a well documented consequence [Brainerd and Bruyn 1951, Naiditch and Boyer 1954, Solders et al. 1989, Indiaquez 1992]. Sinus tachycardia, postural hypotension, hyperhidrosis and retention of urine are typical manifestations of autonomic nerve disturbances [Piradov et al. 2001].

Laboratory findings

The cerebrospinal fluid (CSF) analysis typically gives normal results even in patients with marked headache or meningeal signs. In patients with peripheral neural disease, the CSF protein value is moderately increased (on rare occasions up to 4 g/l) in most patients and remains elevated until the recovery of the neurological signs [Gaskill and Korb 1946, Brainerd and Bruyn 1991, Roche et al. 1990]. The CSF cell count is usually normal.

Incidence and risk factors

The true incidence of diphtheritic neuropathy is hard to extract from the literature. The few clinical materials focusing on this aspect are retrospective, they have variable proportions of patients representing the different age groups and the proportion of immunized persons is unknown or not given. None of the studies report how actively and how long the patients were screened for neurological problems.

Mild paralysis of the palate and the pharynx has been reported in 5-20% of patients with acute pharyngeal diphtheria in fairly unselected patient populations [Brainerd and Bruyn 1951, Naiditch and Bower 1954, Salih et al. 1981, Havaldar 1992]. Nearly all patients with a severe form of diphtheria have this complication. The incidence of peripheral neuropathy is reported in 1–10% of patients after respiratory tract diphtheria [Carter 1943, Brainerd and Bruyn 1951, Naiditch and Boyer 1954, Dobie and Tobey 1979, Salih et al. 1981, Harnisch et al. 1989, Havaldar 1992]. Of patients with diphtheritic neuropathy, about 60% have cranial neuropathy only, 15% have peripheral neuropathy only, and 25% have both [Brainerd and Bruyn 1951, Naiditch and Boyer 1954, Salih et al. 1981]. Diphtheritic neuropathy is more common in patients with a severe form of acute diphtheria [Walshe 1918–1919]. The risk for this complication seems to be especially high in alcoholics, in persons over 40 years of age and in infants [Naiditch and Bower 1954, Harnish et al. 1989].

Diphtheritic neuropathy seems to be more common after skin and wound diphtheria than after respiratory tract diphtheria. Incidences from 20% to 40% have been reported after wound infection [Gaskill and Korb 1946, Naiditch and Boyer 1954, Dobie and Tobey 1979].

Prognosis

A report from Latvia describing 50 fairly unselected patients with diphtheritic peripheral polyneuropathy was published recently [Logina and Donaghuy 1999]. All but one patient also had cranial neuropathy. Thirty-two per cent of the patients needed nasogastric tube nutrition at some point, 48% became unable to walk, and 20% needed artificial ventilation for an average of 27 days. The observed severity of the outcomes may be explained by the fact that patients with cranial neuropathy only were not included in the analysis.

Both the cranial and the peripheral polyneuropathy usually resolve completely [Gaskill and Korb 1946, Piradow et al. 2001]. The time for total resolution of the

symptoms is usually 2 to 12 weeks in cranial paralysis and 7 weeks to 6 months in peripheral paralysis [Walshe 1918-1919, Gaskill and Korb 1946, Brainerd and Bruyn 1951, Salih et al. 1981, Ghanem 1993, Piradow et al. 2001]. Isolated palatal paresis resolves quicker, in 2 to 4 weeks. There are no reports of permanent paralysis after diphtheria. In one series of 109 cases with diphtheritic polyneuropathy, all recovered completely in an average of 100 days [Gaskill and Korb 1946]. In contrast to earlier reports, the recent Latvian study found that after one year 80% of patients with peripheral diphtheritic neuropathy still had limb symptoms and 6% could not walk [Logina and Donaghuy 1999].

Two inventions have changed the prognosis of diphtheric polyneuropathy fundamentally; ventilator therapy and antibiotics. In a recent series of 32 patients with severe polyneuropathy, 24 needed ventilator therapy for a median 2 weeks [Piradov et al. 2001]. Only two of them died. In the preantibiotic era the prognosis for diphtheritic bulbar paralysis was less good. Up to 15% of patients with this paralysis developed aspiration pneumonia with 60% mortality [Naiditch and Bower 1954].

4.3 Mortality

Diphtheria is one of the biggest killers in history. Even after such inventions as antitoxin therapy, antibiotics and intensive care with mechanical ventilators the overall mortality of diphtheria remains high, around 5%.

History and the present

Throughout the 19th century the vast majority of diphtheria victims were children. Of those who contracted the disease 40–50% died. [Collins 1946, Chen et al. 1985, English 1985, Kass 1993]. The only real change in the prognosis of diphtheria took place late in the 19th century with the invention of diphtheria antitoxin [Grundbacher 1992]. The case fatality ratio fell in a few years to 5-15% and has changed little since then (Figure 4 and Figure 5) [Naiditch and Bower 1954, Singer and Underwood 1962, Munford et al. 1974, Nemes and Westhoff 1983, Chen et al. 1985, English 1985, Kass 1993, Lyman et al. 1994]. The role of antitoxin therapy for the decreaced mortality rate of diphtheria is open to the objection that about the time of the introduction of antitoxin many more cases of diphtheria, diagnosed as such, were established by bacteriological diagnosis. Conequently, mild cases which would previously have been called something else, were now included as diphtheria and automatically lowered the death-rate of the whole group [Singer and Underwood 1962].

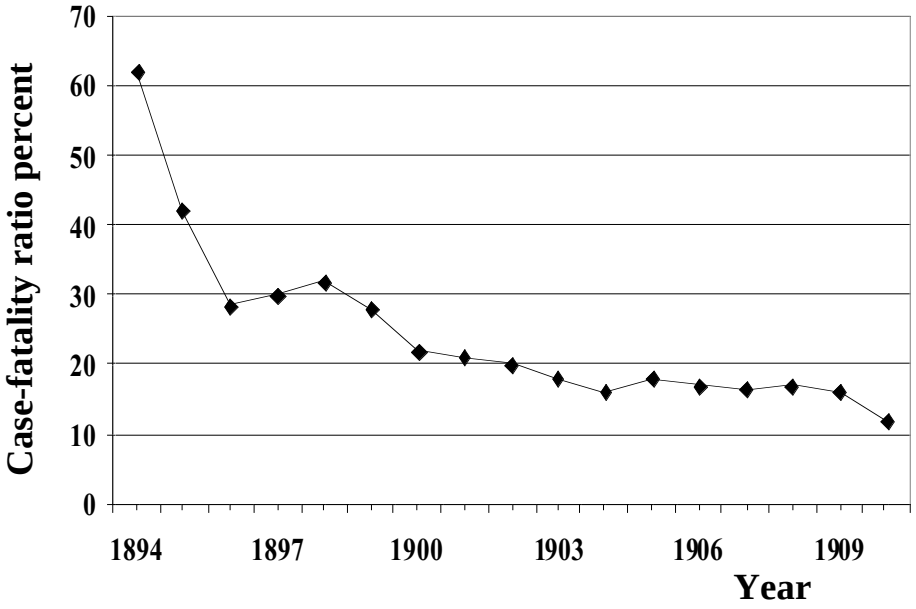


Figure 4. Case mortality rate of laryngeal diphtheria in London fever hospital, 1894-1910.

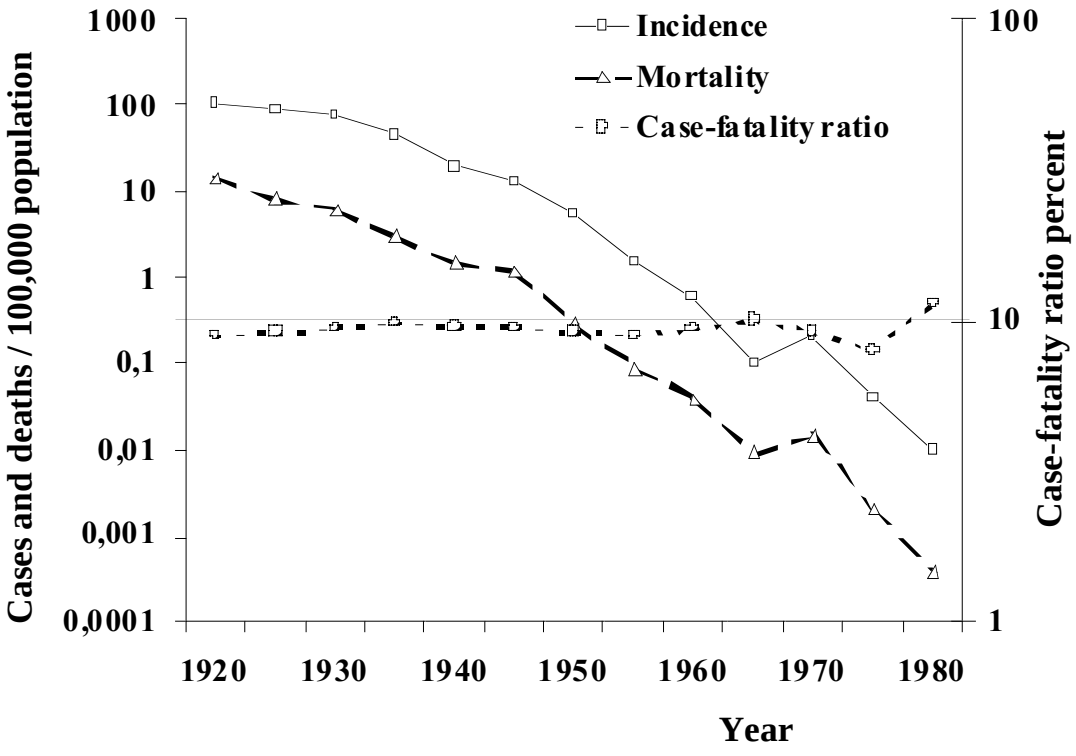


Figure 5. The incidence, the mortality, and the case-fatality ratio of diphtheria in United States in the 20th century.

Antitoxin treatment did not, however, influence the epidemiology of diphtheria. Therefore, even though the case fatality ratio fell markedly, diphtheria remained a common cause of child death between 1900 and 1920. As a matter of fact, during the same period the incidence as well as the case fatality ratio of scarlet fever, a disease for which there was no efficient remedy, declined with equal rapidity in the United States [Collins 1946]. When active immunization against diphtheria was implemented in the 1920s, the decline in diphtheria mortality was considerably accelerated [Collins 1946, Munford et al. 1974, Chen et al. 1985]. The availability of antibiotics did not make an obvious change in the overall case fatality ratio of diphtheria [Naiditch and Bower 1954, McCloskey et al. 1971, Chen et al. 1985].

Risk factors for death

The risk of death is determined by the severity of the acute respiratory tract infection (Table 2) [Naiditch and Bower 1954]. Death rates of 33% to 91% have been observed in patients with a membrane extending to the tracheobronchial tree, and death rates of 16% to 43% in patients with toxic disease without an extensive membrane. In local nasal and laryngeal diphtheria mortality is negligible [Naiditch and Bower 1954].

Fourteen studies have focused on risk factors for death in diphtheria [Honey and Welford 1934, Burkhard et al. 1938, Carter 1943, Brainerd and Bruyn 1951, Naiditch and Bower 1954, Taylor et al. 1962, Miller et al. 1974, Munford et al. 1974, Dobie and Tobey 1979, Jones et al. 1985, Harnisch et al. 1989, Havaldar et al. 2000, Khetsuriani et al. 2000, Quick et al. 2000a]. Only one study is prospective [Quick et al. 2000a]. Of demographic and socio-economic factors, age below 5 years or over 40 years, low socio-economic status and alcoholism have emerged as risk factors in almost all studies. The other commonly discovered risk factors are non-vaccination, late admittance to hospital, late antitoxin therapy, myocarditis and severe infection of the respiratory tract (toxic or extensive disease).

An interesting observation was made by Munford and co-workers [1974]. In reviewing diphtheria deaths in the United States from 1959 to 1970, they found that the relative risk of death was greater for persons involved in small outbreaks. This could explain why the case fatality ratio has not been lowered in the industrialized countries in recent decades (Figure 5). A low incidence might lead to late diagnosis and late antitoxin treatment. In contrast, the lower mortality observed in diphtheria patients serving in the United States Army compared to the mortality in other United States citizens in the 1940s (0–2.3% vs. 6.0–9.5%) could be explained by early access to therapy [Riley and Weaver 1952]. In addition to that, soldiers are mostly non-alcoholic young healthy men. Diphtheria immunization gives about 90% protection

against clinical disease and the clinical disease in vaccinated persons is milder. The risk of cardiological and neurological complications and death is 50–80% lower in immunized persons compared to non-immunized [Carter 1943, Collins 1946, Ipsen Jr. 1954, Naiditch and Bower 1954, Taylor et al. 1962, Glinyenko et al. 2000, Khetsuriani et al. 2000, Quick et al. 2000a, Quick et al. 2000b]. There is still controversy over whether different biotypes of *C. diphtheriae* carry different risks for severe disease, late complications and death. There is some evidence that infection by a bacillus belonging to the biotype *gravis* carries a worse prognosis [Anderson et al. 1931, Carter 1943, Munford et al. 1974]. In epidemics where the same strains of *C. diphtheriae* cause both skin and respiratory tract infections, the death rate of patients with skin infections is lower (around 1%) than that of patients with respiratory tract infections (around 10%) [Dobie and Tobey 1979, Harnisch et al. 1989].

The most common causes of death in diphtheria are asphyxia and suffocation by the pseudomembranes (about two thirds) and myocarditis (about one third) [Naiditch and Bower 1954]. Suffocation is relatively more common in children and fatal myocarditis in adults [Brainerd and Bruyn 1951, Naiditch and Bower 1954]. Before the antibiotic era, secondary aspiration pneumonia due to cranial nerve neuropathy was common in diphtheria. It was a contributory factor in about half the deaths from diphtheria. In a material from Los Angeles in the 1940s, when about half the patients received antibiotics, aspiration pneumonia was involved in 69 out of 139 deaths [Naiditch and Bower 1954]. Nowadays death from secondary bacterial pneumonia is rare [Piradov et al. 2001].

5 Treatment

5.1 Historical perspective

At the end of the 19th century there were still very few specific remedies for any disease. The main objective was usually to support the patient's general strength. For "croup" and diphtheria, many measures and pharmaceutical products were recommended; bed rest, warm baths, cleanliness, effective ventilation, light diet, herring roe, emetics, bloodletting by venipuncture or leeches, the practice of blistering and various stimulants like alcohol, caffeine, strychnine, Coramine and dextrose [Wesselhoeft 1940, English 1985, Metaxas Quiroga 1990, Hardy 1992, Kass 1993, Feldman 2001]. For local relief and for destruction of the pseudomembrane, various modes of treatment were tried; gauzes with Calomel (mercurous chloride) on the breast, gargling, spraying, irrigating, vaporizing and swabbing of various substances, such as silver nitrate and iron perchloride, on the throat [English 1985, Metaxas Quiroga 1990,

Hardy 1992, Feldman 2001]. There is no evidence of the benefit from any of these measures or substances.

Suffocation by the membranes was by far the most common cause of death from diphtheria in the 19th century. The invention of tracheostomy to alleviate this complication was a small step forward. This procedure had been performed successfully by the Greek physicians Claudius Galen (130–200 AD) and Aretaeus, the Cappodocian (81–138 AD) and had been known since then [Hardy 1992]. It had mainly been used in cases where foreign bodies had been inhaled. There is some dispute over who first used tracheostomy for a patient with diphtheria. Most investigators give the credit to the French physician Pierre Bretanneau (1778–1862), who in 1825 carried out this procedure on a 4-year-old girl with diphtheria [English 1985]. Tracheostomy was not a great success. The survival rate of diphtheria patients after tracheostomy varied from physician to physician. In many hospitals it was 24% to 32% [English 1985, Hardy 1992].

Intubation, introduced in 1885 especially for the treatment of patients with severe diphtheria, was more successful [Hardy 1992]. The first to perform the procedure was a young practitioner, Joseph O'Dwyer (1841–1898) who was employed in New York [Hardy 1992, Wiedeman 1992]. Already two years after the introduction of this procedure 20,000 intubations for diphtheria patients had been reported [Hardy 1992]. By 1900 it had replaced tracheostomy in many hospitals in the United States. England was the last stronghold for tracheostomy and there intubation was not adopted until 1929 [Hardy 1992, Kass 1993].

Diphtheria antitoxin was a big step forward in the treatment of diphtheria. Antitoxin therapy became available at a time when in Switzerland, for instance, half of all child deaths were due to diphtheria [Grundbacher 1992]. In 1888, Pierre Paul Emil Roux (1853–1933) isolated the diphtheria toxin [English 1985]. In 1890 the German bacteriologist Emil Adolpf von Behring (1854–1917) published two articles about diphtheria antitoxin in "Deutsche Medizinische Wochenschrift" [Grundbacher 1992, Hansen 1999, Winau and Winau 2002]. To produce the antitoxin, Behring and the Japanese investigator Shibasaburo Kitasato (1852–1931) used bacterial toxin rather than the bacterium itself to immunize animals [Grundbacher 1992]. The hypothesis was that the cause of diphtheria was not an invasive bacillus but the toxin produced by the bacillus. The first child was successfully treated with 50 ml (presumably around 500 international units) of "immune serum" on 25 December, 1891 [Grundbacher 1992]. A few years after diphtheria antitoxin became widely available, the mortality of diphtheria decreased from 40–50% to 5–10% [Naiditch and Bower 1954, Chen et al. 1985]. Already in the first years of the 20th century, the results of antitoxin treatment were so convincing that controlled studies did not seem necessary and were regarded as unethical

[English 1985](Figure 4). The discovery of antitoxin immediately received enormous publicity, and later Behring was awarded the first Nobel Prize for medicine and physiology in 1901 [Hansen 1999, Winau and Winau 2002]. The dose initially used, with obviously good but poorly documented results, was 20 ml to 50 ml of animal "immune serum" per patient, probably containing approximately 2,000 international units [Grundbacher 1992]. The dose was chosen on the basis of neutralization studies in animals. Local injections of antitoxin to peritonsillar tissues were also used earlier, but the benefit of this practice has not been documented.

The discovery of penicillin in the 1940s (Sir Alexander Fleming 1881-1955) did not seem to change much the fate of diphtheria victims. In a report on 1,433 patients with respiratory tract diphtheria in the United States in 1940 to 1950, approximately half the patients received penicillin [Naiditch and Bower 1954]. The investigators did not note any difference in the clinical course of diphtheria between patients who received penicillin and those who did not.

5.2 Antitoxin

When antitoxin therapy was adopted, the case-fatality ratio for diphtheria soon decreased from 30–50% to 5–15% [Collins 1946, Neiditch and Bower 1954, Lyman et al. 1956a, English 1985, Kass 1993]. In three large published materials the death rate was 0% to 5% when antitoxin was given at the latest two days after the onset of the illness as compared to 9% to 30% when it was given later [Brainerd and Bruyn 1951, Naiditch and Bower 1954]. Antitoxin treatment is probably not useful after the third day of the disease. The results of one old retrospective study that looked at the efficacy of antitoxin in decreasing the risk of diphtheritic polyneuropathy, are perplexing [Gaskill and Korb 1946]. In a series of 109 patients with cutaneous diphtheria, the incidence of diphtheritic neuropathy was 14% if antitoxin was given at the latest on day 32 after the onset of wound infection, 31% if antitoxin was given later than that, and 61% if no antitoxin was given. This observation has not been confirmed by other investigators. The benefit of antitoxin treatment for patients with nasal diphtheria or skin and wound diphtheria has not been documented, and antitoxin is not routinely recommended for these forms of diphtheria [American Association for Pediatrics 1997].

No comparative trials on different doses or different preparations of antitoxin are available. The doses recommended by WHO for the treatment of different forms of diphtheria are based on empirical observations alone (Table 5). Considering the scarce evidence, the dosage recommendations in different western guidelines are amazingly uniform [Farizio et al. 1993, World Health Organization 1994, American Academy for

Table 5. Recommended doses of horse diphtheria antitoxin in the western world and in the Russian Federation [Ministry of Health of USSR 1986, Farizio et al. 1993, World Health Organization 1995, Grinchuck 1996, American Academy for Pediatrics 1997, Bonnet and Begg 1999].

Clinical form	Recommended dose of antitoxin	
	World Health Organization[1]	Russia
Nasal	10,000– 20,000	20,000– 40,000
Tonsillar	15,000– 25,000	20,000–200,000
Pharyngeal	20,000– 40,000	20,000–200,000
Laryngeal	20,000– 40,000	20,000– 40,000
Combinations of the above	40,000– 60,000	50,000–300,000
Severe form (toxic, extensive) or delayed therapy[2]	40,000– 100,000	80,000–500,000

[1] In nasal form intramuscularly, in combined and severe forms intravenously, in other forms either intramuscularly or intravenously. For children younger than 10 years, the dose may be reduced to a half.
[2] Treatment delayed to day 3 of disease or later

Pediatrics 1997]. Only one injection is recommended. Antitoxin should be given either intramuscularly or, especially in severe forms, intravenously. In the Russian Federation much higher doses of antitoxin given over 2 to 3 days are recommended for severe forms of diphtheria [Ministry of Health of the USSR 1986, Grinchuck 1996].

For treatment, antitoxin of human and horse origin are available. The human antitoxin is expensive and not readily available in most countries [Wilde et al. 1996]. It is usually recommended only for infants with previous severe allergic reactions to horse proteins. If allergy to horse proteins is suspected, for testing a drop of 1:10–1:100 saline dilution of antitoxin is instilled onto the conjunctiva or 0.02–0.1 ml (enough to raise a small wheal) is injected intradermally [Bonnet and Begg 1999]. For allergic persons a short desensitisation schedule using rising doses of antitoxin is recommended [World Health Organization 1994, American Academy for Pediatrics 1997, Bonnet and Begg 1999]. Horse antitoxin seems to be fairly safe. In a series of 1,433 diphtheria patients treated in the 1940s, 8.8% got an immunological reaction [Naiditch and Bower 1954]. About 4% of the patients had a serum sickness type reaction and the same proportion got a febrile reaction. Only 8 patients had an anaphylactic ("immediate type", type I) reaction and none died.

As antitoxin should be given early, the decision on treatment must be made on the basis of the patient history and the clinical findings without waiting for microbiological confirmation. The recently developed PCR test to detect the gene coding for diphtheria toxin production directly from clinical specimens is promising for the rapid diagnosis of diphtheria [Michailovic et al. 1995, Popovic et al. 1995, Nakao et al. 1997]. Once it is

validated in a clinical setting, a more specific approach to determining the need for antitoxin therapy may become possible.

5.3 Antibiotics

Antibiotic treatment is recommended for patients with suspected or proven diphtheria. *C. diphtheriae* is sensitive *in vitro* to most antibiotics commonly used in the empiric treatment of acute upper respiratory infections (Table 6). An erythromycin-resistant strain of non-toxigenic *C. diphtheriae* has been isolated in an epidemic in Seattle [Harnish et al. 1989] and rifampicin-resistant strains have been found in France [Patey et al. 1997] and in northwestern Russia [Maple et al. 1994].

In studies done in the pre-antibiotic era, *C. diphtheriae* was spontaneously eradicated from 50% of the patients in two to three weeks and from 90% in four weeks after the first positive culture [Hartley and Martin 1920, Weaver 1921]. Over 90% eradication rates in two to five days have been obtained with erythromycin and clindamycin treatment and 84% to 89% eradication rates with benzathine penicillin treatment [Wood and Gorman 1957, Zalma et al. 1970, McCloskey et al. 1974]. In one study in which diphtheria patients were treated with erythromycin for 6 days, there was a 96% bacterial eradication rate in 48 hours but 21% received a bacteriological relapse two weeks after therapy [Miller et al. 1974]. This rate of permanent eradication was not much different from that what one would expect without antibiotic therapy. According to one old uncontrolled study, erythromycin combined with tetracycline was superior to either drug alone in treating chronic carriers of *C. diphtheriae* [Kiselev 1964].

Table 6. The sensitivity of *Corynebacterium diphtheriae* to various antibiotics [McCloskey et al. 1971, Maple et al. 1994, Wilson 1995].

	Minimal inhibitory concentration (mg/l)	
	MIC_{90}	MIC, range
Erythromycin	≤ 0.06	≤ 0.06
Chloramphenicol	1.0	1.0
Clindamycin	0.5	0.025 – 0.5
Penicillin	0.23	0.02 – 0.4
Ampicillin	0.46	0.25 – 1.0
Ciprofloxacin	≤ 0.06	< 0.01– ≥ 2
Azithromycin	0.01	0.016 – 0.03
Tetracyclin	0.5	0.5
Cefuroxime	0.9	0.5 – 2.0
Gentamycin	0.24	≤ 0.12 – 0.5
Trimethoprim	0.25	0.12 – 2.0

Table 7. The recommended antibiotic treatment for patients with diphtheria and their close contacts [Centers for Disease Control and Prevention 1991, Farizio et al. 1993, World Health Organization 1995, American Academy for Pediatrics 1997, Bonnet and Begg 1999].

Patients (for 14 days)	
Peroral choice	
Erythromycin[1]	40-50 mg/kg/d, maximal dose 2 g/d
Penicillin V	125-250 mg four times daily
Parenteral choice	
Procaine penicillin	25,000 IU/kg/d, maximal dose 1,2 million IU/d, intramuscularly
Benzathine penicillin	600,000 IU for children < 6 years of age, 1 injection, intramuscularly
	1.2 million IU for persons > 6 years of age, 1 injection, intramuscularly
Erythromycin	40-50 mg/kg/d, maximal dose 2 g/d, intramuscularly or intravenously
Contacts (for 7 - 10 days)	
Erythromycin	40 mg/kg/d, maximal dose 1 g/d
Penicillin V	125-250 mg four times daily
Benzathine	600,000 IU for children < 6 years of age, 1 injection, intramuscularly
penicillin	1.2 million IU for persons > 6 years of age, 1 injection, intramuscularly

[1]azithromycin and clarithromycin are also recommended

The eradication of *C. diphtheriae* should be confirmed by at least two cultures taken at least one week after treatment. Both the throat and the nose and, in cutaneous diphtheria, the wound should be cultured to verify clearance [Lyman and Youngstrom 1956, Mofredj and Guerin 1993]. For treatment of patients and their close contacts, mainly erythromycin or other macrolids or various preparations of penicillin are recommended (Table 7).

5.4 Other treatment options

The prevention and treatment of myocarditis has gained much attention. Dextrose infusions and intravenous administration of co-carboxylase enzyme have had their proponents, but no proof of their efficacy has been reported [Naiditch and Bower 1954]. In one study carnitine supplementation was tried for severely sick children with diphtheria [Ramos et al. 1992]. In this open comparative trial with 625 diphtheria patients, carnitine decreased both the incidence of myocarditis (38.8% vs. 49.0%, $p<0.01$) and the risk of death (1.8% vs. 8.4%, $p<0.01$). This result has not been confirmed by further studies.

During the recent diphtheria epidemic in Russia, positive experience in the treatment of severely sick patients was reported in non-comparative trials on vitamin supplementation, plasmapheresis [Postnikov and Sonina 1995, Grinchuck 1996] and hyperbaric oxygen therapy [Ivanov et al. 1992]. The results of these studies are not

evaluated in a way to merit firm conclusions on the efficacy of these modes of therapy. There are no studies on the use of diphtheria toxoid vaccine in the treatment of diphtheria.

There are conflicting results on the effect of steroids in the treatment of severe diphtheria [Thisyakorn et al. 1984, Rub et al. 1989]. There is no convincing evidence of either their beneficial or deleterious effect. Corticosteroids might be considered for relieving oedema, if swelling of the airways is threatening the mainstay of open airways. Intubation can be life saving when the laryngeal oedema is severe. When the pseudomembranes obstruct the peripheral airways, artificial ventilation seems to be of little benefit. In such cases bronchial toilet is rarely helpful, because usually the membranes are firmly attached. For patients with severe problems in heart conduction and with severe cardiac failure, electrical pacing of the heart and counterpulsation in the aorta have been tried but, according to case reports, with little success. Even with the means of advanced intensive care the death rate in patients with total atrio-ventricular dissociation and circulatory failure remains around 80% [Salih et al. 1981, Araujo et al. 1990, Havaldar 1992, Stockins et al. 1994]. For patients with diphtheritic polyneuropathy, temporary use of artificial ventilation can be life saving when there is paralysis of the diaphragm and other respiratory muscles [Longina and Donaghy 1999].

Tonsillectomy is occasionally performed for patients with diphtheria, usually due to an erroneous diagnosis of peritonsillar abscess. There is old data suggesting that patients who have undergone tonsillectomy have a decreased risk of contracting diphtheria, as evidenced by an increased proportion of Schick skin-test negativity among them [Naiditch and Bower 1954]. Tonsillectomy has been practised, reportedly with success, for treating chronic carriers of *C. diphtheriae* [Weaver 1921, Grinchuck 1996]. There is no evidence that tonsillectomy would be deleterious when performed during acute diphtheria, but there is a theoretical concern that surgical procedures in the throat would liberate an excess of toxin into the circulation and thereby increase the risk of systemic complications.

6 Prevention

The cornerstones of diphtheria control are high standards of hygiene and national vaccination programmes. In addition to these, during outbreaks prompt recognition and management of diphtheria patients and rapid investigation and management of close contacts of cases are crucial [World Health Organization 1994]. Besides the increase in wealth and hygiene as such, the most dramatic change in the epidemiology of diphtheria was achieved by immunization programmes for children. Since they were started, there

has been a steady decline in the incidence of diphtheria and successful programmes have completely prevented population-wide epidemics for 40 years. Swabbing and treating close contacts with antibiotics can prevent some secondary cases of diphtheria.

6.1 History

Before the era of active immunizations, diphtheria caused dramatic epidemics at intervals of 20 to 25 years. The cyclic nature of the epidemics was probably created by the increasing level of herd immunity in the population during the epidemic years and the waning of this immunity during the years when the incidence of diphtheria was low.

Hygiene and other general measures

In the 19th century it was still the prevailing view that the origin of diphtheria was "miasma" and the disease was a manifestation of the Almighty's retribution for original sin. For believers of this doctrine, the prevention of diphtheria might have been in the hands of the clergy. But there were also those who believed that diphtheria was contagious. Therefore, quarantine was used for diphtheria patients and their families. The prominent New York physician Abraham Jacobi wrote in favour of quarantine in 1880: "In such cases it is not society that tyrannizes the individual, it is the individual that endangers society".

The germ theory and the isolation of *C. diphtheriae* in the 1880s provided a scientific basis for quarantining those with positive cultures, regardless of symptoms, as well as rational criteria for ending quarantine [Kleinman 1992]. In 1903 the Michigan State Board of Health declared that quarantine had reduced the average number of deaths per diphtheria outbreak by a factor of five [Kleinman 1992]. With hindsight, it is easy to see other possible explanations for the decreasing mortality of diphtheria, such as changes in living conditions and a little later also antitoxin therapy. The relation of diphtheria to poor hygiene or "filthiness" was discovered during the epidemics of the 18th century [Kleinman 1992]. At that time, it was difficult or impossible to separate as risk factors poverty as such from overcrowded tenements and poor hygiene. In respect to specific measures in hygiene, before the 20th century only good oral hygiene was stressed [Kleinman 1992].

The observation that diphtheria was transmitted from person to person led to prevention strategies that brought great inconvenience and suffering to the patients, who were mainly children, and to their families. If the patient was treated at home, he was

not allowed to leave the room, where he was to be alone [Pipping 1914]. All upholstered furniture and carpets were removed from the room. Nobody was to touch the patient, except a specially dressed nurse, if a nurse was available. The patient should lie on straw, which could be burned after the recovery or death of the patient. Burning or a 5% solution of Lysol was used to decontaminate excrements and bed linen. The family members were prohibited to have intercourse with other families. After the recovery or death of the patient, the house was fumigated. If the patient died, the funeral was private [Kleinman 1992].

For patients who could be taken to a hospital, strict but not always rational isolation precautions were taken. At least, an effort was made to take them. During diphtheria epidemics a quarter of all the patients in a hospital might be ill with diphtheria [Kass 1993]. In Boston in the 1880s, the diphtheria epidemic had become very severe. Overcrowding in the medical wards and the risk of contagion to other patients and hospital staff forced the city to appropriate two new buildings, one for scarlet fever and one for diphtheria [Kass 1993]. Patients, even children, were forbidden visitors and doctors were not supposed to visit the regular wards after attending the contagious wards. The entire medical and nursing staff was required to wear special gowns and to disinfect their hands and hair. Nurses, along with the medical staff and other hospital employees, had no protection from the major contagious diseases to which they were continuously exposed. Morbidity and mortality among the personnel was not inconsequential. From 1895 to 1912, in Boston City Hospital, there were 431 cases of diphtheria among the nurses, house staff and employees working in the "diphtheria building" with 260 beds. However, there were no fatalities among the staff, probably due to acquired immunity and early antitoxin therapy [Kass 1993].

Mass swabbing campaigns were carried out in the beginning of the 20th century. In these campaigns carriers of *C. diphtheriae* were the prime, almost exclusive consideration [Hooker and Bashford 2002]. Initially success was claimed for this approach. Later it became evident that attempts to eradicate the organism from a community by searching for and treating carriers are not reasonable, except in closed communities [Hewlett 1985].

Measuring immunity

The earliest methods for measuring immunity to diphtheria were developed in the late 19th century. The guinea pig was used as a sensitive detection system for titration of toxin neutralization by serum antitoxin. In clinical and epidemiological practice this laborious method was replaced by the Schick test, invented in 1913 by the Hungarian

paediatrician Béla Schick (1877–1967) [Kleinman 1992]. In the Schick test diphtheria toxin (one fiftieth of the minimal lethal dose for a guinea pig) is injected intradermally. If this leads to an immediately visible inflammatory reaction the test is regarded as positive and indicating a lack of protective immunity. There is a vast amount of experience to support this conclusion. Soon attempts were made to find a test that would measure the protective level of antitoxin *in vitro* [Karasawa and Schick 1910]. Observations have shown that a serum antitoxin level of 0.01 to 0.03 units per millilitre correlates to a negative Schick test [Gibbard et al. 1945, Ipsen 1946, Ipsen Jr. 1954] and probably to protection [Yokoi K, 1932, Ipsen Jr. 1954].

Vaccine

In 1888, Pierre Paul Emil Roux (1853–1933) isolated the diphtheria toxin [English 1985]. Two years later, Emil Adolpf von Behring (1854–1917) used bacterial toxin to immunize animals [Grundbacher 1992]. The door was open for the development of diphtheria toxoid vaccine. Active immunization was first used on humans in 1913, but it was not until 1919 to 1925 that it was widely used in the general population [Collins 1946, Kleinman 1992, Hooker and Bashford 2002]. This took place first in Canada and the United States and a few years later in Australia. The vaccine was given free of charge to those who could not afford it [Collins 1946, Kleinman 1992]. The Schick test was used to identify the children who required immunization. Vaccination of those who were already immune was not desirable because of the cost and the risk associated with the early vaccines.

It was difficult to assess the immunizing efficiency of the diphtheria plain formol toxoid vaccine used in the early days. There was considerable variation in the procedures used, such as the size and number of doses and the timing and method of administration. As a matter of fact, purified formol toxoid vaccines were shown later to be very poorly immunogenic [Medical Research Council Committee on Diphtheria Toxoid 1962]. The American experience did not convince Europeans. As late as 1949, two critical comments were published in Presse Médical, Paris, claiming that the reduced incidence of diphtheria in North America was a natural development and not the credit of vaccinations [reviewed in Anonymous 1949].

The decline in the incidence of diphtheria started in 1900 in the United States, before vaccination was introduced [Collins 1946]. In the late 1930s, the vaccination coverage among children in North America approached 50% [Collins 1946, Naiditch and Bower 1954]. In the late 1930s in Europe, only preliminary small-scale vaccinations were performed, especially in Germany and Hungary [Collins 1946]. The incidence of

diphtheria had decreased in the Unites States from 100 / 100,000 population in 1920 to 3 / 100,000 in 1938. On the contrary, in 1938 in Europe, the incidence of diphtheria remained high. It was 30 to 100 / 100,000 population in most Northern and Central European countries [Collins 1946]. In Europe vaccination programmes for children were started mainly in the 1940s to combat the devastating epidemics that started during World War II [Carter 1947].

6.2 Vaccination

The aim of vaccination is to achieve an amount of diphtheria antitoxin in the blood sufficient to neutralize all absorbed and circulating toxin in case of an infection. Vaccine does not induce the production of antibodies to components of *C. diphtheriae* other than the toxin. Therefore, not surprisingly, it has been concluded from retrospective data that vaccination against diphtheria offers no protection against the carrier state of *C. diphtheriae* [Miller et al. 1972, Munford et al. 1974], thus reducing its value in ending outbreaks.

Current vaccines

Full-strength diphtheria toxoid is used for primary immunization of children. The vaccines currently in use are diphtheria-tetanus-pertussis (DTP) or diphtheria-tetanus (DT) vaccines. Also DTP vaccines combined with *Haemophilus influenzae* type B, polio and hepatitis B virus antigens are available [Kansanterveyslaitos 2002]. One immunizing dose usually contains 7–25 flocculation units (Lf) of diphtheria toxoid [Galazka and Robertson 1996]. WHO requirements state that the potency of diphtheria toxoid shall not be less than 30 International Units (IU) per human dose when tested in animals with an appropriate reference material calibrated against the International Standard [Galazka and Robertson 1996]. For those 7 years old and older, only a tetanus-diphtheria vaccine (Td) containing fewer flocculation units (1.5–5 Lf) of diphtheria toxoid per dose should be used [Advisory Committee for Immunization Practices 1991]. An oral (liquid) diphtheria vaccine has been developed [Mirschamys et al. 1994]. It has been shown to be immunogenic in rabbits, monkeys and human volunteers. Because of the simplicity of the introduction procedure it could offer practical benefits especially in the developing counties, if proven efficacious and practical to store.

Immunogenecity and adverse events

Two doses of absorbed diphtheria toxoid elicit conversion to negative Schick test [Hewlett 1985] and a protective level of antitoxin [Cohen and Nagel 1984, Galazka and Robertson 1996] in at least 95% of children. A booster dose administered 12 to 18 months after completion of the primary series stimulates abundant production of diphtheria antibody, with mean levels above 1 IU/ml [Galazka and Robertson 1996]. The proportion of children who have a protective antibody level following a booster dose has been in the order of 95%, and a protective level remains for at least 6 years [Myers et al. 1982, Ramsay et al. 1993, Galazka and Robertson 1996].

Following primary immunization in childhood the immunity wanes relatively quickly, if not reinforced by constant natural exposure to *C. diphtheriae* [Scheibel et al. 1966, Galazka and Robertson 1996]. In surveys performed 10 years after vaccination, the proportion of unprotected persons (antibody titers < 0.01 IU/ml) has been 24% if the primary series contained only two doses of toxoid and 10% if it contained three doses [Volk et al. 1962]. Twenty-five to thirty years after three doses of toxoid without a booster dose, the proportion unprotected has been 26% [Kjeldsen et al. 1985]. A booster dose for adults (dT) has been shown to boost well the titers of those whose immunity before vaccination was low (< 0.1 IU/ml) [Mortimer et al. 1986, Simonsen et al. 1986a]. In adults with a non-protective level of antitoxin, a booster dose gives a protective level for 60-80% [Allerdist and Ehrengt-Lange 1982, Bayas et al. 2001, Vellinga et al. 2001]. In adults infected with human immunodeficiency virus the mean increase in antioxin levels induced by a booster dose is very low, even in persons with a fairly good $CD4^+$ cell count of 300 x 10^6/L [Kroon et al. 1995].

In early studies severe reactions were observed in adults immunized with a full dose of diphtheria toxoid (DT). A clear dose-response relationship in adverse events has been observed [Simonsen et al. 1986b]. In most reports low-dose booster toxoid vaccine (Td) for adults has shown to carry a low risk of adverse events [Mortimer et al. 1986, Galazka and Robertson 1996]. Mild tenderness or pain in the movement of the hand has occurred in 8–21% of those vaccinated. In some studies, however, a high prevalence of local reactions, up to 40%, has been reported [Palmer et al. 1983, Allerdist and Ehrengut-Lange 1992]. The authors of these reports have recommended that adults should not be vaccinated without previous screening for susceptibility to diphtheria. This would hardly be practicable. Systemic reactions and severe swelling of the entire limb have been reported after immunization with a current DTP vaccine [Rennels et al. 2000]. The reaction was probably connected to the diphtheria toxoid part of the vaccine. Observations in laboratory animals indicate that such reactions might be triggered also by endotoxin in the vaccine [Ansher et al. 1992].

6.3 Who is protected?

Individuals

Protection against diphtheria can be acquired from three different sources: immunity after infection, by maternally acquired antibodies and by immunization with vaccine. Naturally acquired immunity is extremely rare in countries with a low incidence of diphtheria, and it cannot reach the level needed for prevention of outbreaks even where diphtheria is endemic [Hewlett 1985]. For prevention of diphtheria epidemics, vaccination coverage of at least 95% of the population is regarded as necessary [World Health Organization 1995].

Since the 1970s, *in vitro* methodologies have been used to measure immunity. It is well documented that tissue culture neutralization tests are as reliable as *in vivo* neutralization for titrating the level of antitoxin antibodies in sera. The diphtheria neutralization test in *Vero* cells (green monkey renal epithelium) is currently the reference assay [von Hunolstein et al. 2000]. Different modifications of enzyme-linked immunosorbent assay (ELISA) are also in use. With these methods one can distinguish human antibodies from horse antibodies given as antitoxin therapy for diphtheria [Camargo et al. 1984, Efstratiou and George 1996, Bonin et al. 1999]. In general, ELISA tests have performed better than the earlier used passive haemagglutination tests, when the *Vero* cell test has been used as a reference test [Walory et al. 2000]. However, ELISA tests have been shown to have a poor correlation with tissue culture and *in vivo* neutralization tests for sera containing less than 0.1 IU/ml antitoxin with a significant risk of false-positive interpretations of immunity [Melville-Smith and Balfour 1988, von Hunolstein et al. 2000].

Diphtheria antitoxin antibody levels of 0.1 IU/ml are considered as minimum protection, especially when *in vitro* techniques are used. Levels between 0.01 and 0.09 IU/ml are thought to give uncertain protection, and patients with antibody levels lower than 0.01 IU/ml are considered unprotected [Ipsen 1946, Ipsen Jr. 1954, Edmunds et al. 2000]. An antibody level of > 1 IU/ml is considered to indicate good long-term protection [Farizio et al. 1993]. However, there is no sharply defined level of antitoxin that gives complete protection from diphtheria [Christensson and Böttiger 1986, Mofredf and Guerin 1993]. For epidemiological purposes the minimum protective level is considered to be 0.01 IU/ml of diphtheria antitoxin in a serum sample. A higher level of 0.1 IU/ml is desirable for individual protection [Efstratiou and George 1996].

Diphtheria toxoid vaccine gives about 90% protection against clinical disease, and 50-80% protection against the late cardiac and neurological complications and death [Carter 1943, Collins 1946, Ipsen Jr. 1954, Naiditch and Bower 1954, Taylor et al.

1962, Glinyenko et al. 2000, Khetsuriani et al. 2000, Quick et al. 2000a, Quick et al. 2000b]. From retrospective data it has been concluded that vaccination against diphtheria [Miller et al. 1972, Munford et al. 1974] or high antitoxin antibody levels [Yokoi 1932, Björkholm et al. 1986] offer no protection against a carrier state of *C. diphtheriae*.

Population

In industrialized countries, generally over 95% of children are immunized against diphtheria [Schill and Buisson 1990, Galazka and Robertson 1996]. Several recent surveys have shown that even in countries with a successful childhood vaccination programme, the proportion of protected persons is low in older age groups. It is generally 40–60% among those over 40 years of age [Working Group 1978, Kerttula et al. 1980, Nauman et al. 1983, Kjeldsen et al. 1988, Klouche et al. 1995, Maple et al. 1995, Edmunds et al. 2000, Egemen et al. 2000, de Melker et al. 2000, von Hunolstein et al. 2000, Hasselhorn 2001, Walory et al. 2001, Cristensson et al. 2001, McQuillan et al. 2002]. In many countries men are better protected than women, because men receive a toxoid booster during military service. The Swedish experience shows that a vaccine campaign targeted at increasing adult immunity to diphtheria can be successful [Christensson et al. 2001].

A serological survey from rural Africa shows that there are not enough natural challenges to *C. diphtheriae* to give protection at the population level [Kurtzhals et al. 1997]. In countries which have implemented diphtheria vaccination programmes only recently, the population at large is vulnerable to diphtheria. In developing countries, an infant vaccination coverage exceeding 80% was reached in 1995, as reported by the WHO Expanded Programme on Immunization [Galazka and Robertson 1996].

6.4 Vaccination recommendations

In most western countries the routine vaccination programme offered for the whole population is four doses of the toxoid vaccine by approximately 18 months of age. The doses are given at 2, 4, 6 and 15 to 18 months of age. A booster dose at the age of 4 to 6 years is recommended [Ullberg-Olsson 1983, Advisory Committee for Immunization Practices 1991, World Health Organization 1994, Galazka and Robertson 1996]. To preserve immunity, a booster vaccination should be received every 10 years. Until recently, the value of routine boosting at school entry or later has been questioned

[Editorial 1985, Mathias and Scheichter 1985]. Especially after the East European diphtheria epidemic in the 1990s, adults are advised to keep up their immunity by booster doses either routinely or when travelling to an endemic area.

Diphtheria is one of the target diseases of the WHO Expanded Programme of Immunization, launched in 1974 [Farizio et al. 1993, World Health Organization 1994, Efstratiou and George 1996]. To achieve the elimination of diphtheria in Europe, a minimum immunization coverage rate of 90% in children and 75% in adults is regarded as necessary. The targets proposed by WHO are that each district should achieve 95% coverage with the primary immunization series by two years of age, and every district should include a booster dose of a diphtheria toxoid-containing vaccine for children at school age and achieve a 95% coverage for this dose [Efstratiou and George 1996].

6.5 Other modes of prevention

Hygiene and other general measures

In hospital, the patient should be nursed in strict isolation [World Health Organization 1994] or barrier nursed [Bonnet and Begg 1999] until bacteriological clearance has been demonstrated by negative cultures of nasopharynx and throat swabs obtained 24 hours after completing treatment. All articles in direct contact with the patient and articles soiled by discharges from the patient should be disinfected while the patient is in isolation. If patients are treated at home, visitors are not allowed and the members of a household should be swabbed and treated with antibiotics.

Antibiotics

An attempt to eradicate the organism from the community during outbreaks by searching for carriers is not reasonable [Hewlett 1985]. Antibiotic treatment of carriers is practical only when they are recognized in relation to documented clinical diseases. Treating either all close contacts or those found to harbour *C. diphtheriae* is recommended and may be efficient in preventing secondary cases. However, this has not been proven. WHO has given a list of those regarded as close contacts [World Health Organization 1994]. It includes household members, friends, relatives and those caring for the patient who regularly visit the home, kissing/sexual contacts, school classroom contacts, those who share the same room at work and health care staff exposed to oropharyngeal secretions of the case. The communicable Disease Surveillance Centre in London would limit antibiotics for health care worker to those who have given mouth to

mouth resuscitation, and would limit routine antibiotics in school classes to those known to be improperly vaccinated or who have had especially long close contact with an index case [Bonnet and Begg 1999]. Any close contacts should be clinically assessed for symptoms and signs of diphtheria and kept under daily surveillance for seven days from the date of the last contact with the case. The surveillance should include daily inspection of the throat for the presence of a membrane and measurement of the temperature [World Health Organization 1994]. Close contacts should be given penicillin, preferably a single dose of intramuscular benzathine penicillin (Table 7).

In the search for cases and carriers, both nasal and pharyngeal swabs should be obtained from all contacts regardless of vaccination status [World Health Organization 1994]. In one study from the 1950s, for 22% of the carriers *C. diphtheriae* was recovered only from the nose [Lyman and Youngstrom 1956]. Contacts should also be examined for the presence of wounds or skin lesions and any such lesion should be swabbed. For those found to carry *C. diphtheriae*, a control culture is recommended two weeks after completion of antibiotics.

All close contacts who have received less than three doses of diphtheria toxoid in the past, or whose immunization status is unknown, should be given an immediate booster dose, then complete the full immunization series according to the nationally recommended schedule [World Health Organization 1994]. Contacts who have had three doses of vaccine in the past should also receive an immediate booster dose, unless the last dose was given in the previous twelve months.

III THE PRESENT STUDY

7 Aims of the study

The general aim of the study was to investigate various clinical and epidemiological aspects of diphtheria of the respiratory tract during the diphtheria epidemic in St. Petersburg that started in 1990.

The specific questions were:

1. How is diphtheria transmitted and what are the risk factors for acquiring the disease?

2. Will the epidemic spread to Finland, and how probable would such a development be?

3. What is the clinical spectrum of diphtheria like today in a population-wide epidemic?

4. How common is diphtheritic myocarditis in adults?

5. What are the individual risk factors for acquiring diphtheritic myocarditis?

8 Materials and methods

8.1 Subjects and data collection

Study I is a report on the first case of imported diphtheria in the West during the diphtheria epidemic of the 1990s in Eastern Europe. The disease was acquired by a western tourist on a short trip to Russia. The clinical and epidemiological features are presented.

Study II was conducted as a retrospective review of the records of unselected adult diphtheria patients treated in Botkin Hospital, St. Petersburg, in 1993 and 1994. The study comprised 1,860 patients. Botkin Hospital is a 1,400-bed hospital for adults with infectious diseases. It serves the whole of the city of St. Petersburg. Nearly all of the adult patients with diphtheria in St. Petersburg are treated in this hospital. Under Russian regulations also mild cases not needing hospital treatment are admitted to Botkin Hospital for isolation [Ministry of Health 1984].

The date of the patient's first contact with the health care services, date of hospital admission, clinical presentation, severity of the disease and treatment and outcome were collected from the hospital patient records on a form designed in advance. The results of laboratory tests, chest X-rays and ECG examinations were also recorded. Data on the vaccination history was collected by questioning and from written documents, where available. An autopsy with confirmation of the cause of death was performed on all deceased patients.

Study III presents clinical and microbiological data on patients with a clinical diagnosis of respiratory tract diphtheria treated in Botkin Hospital during the period 1991 to 1995. It presents the clinical and autopsy findings for all the 112 patients from whom only a non-toxigenic strain of *Corynebacterium diphtheriae* was isolated.

Study IV is a clinical and epidemiological report on all the Finnish citizens who acquired diphtheria during the Russian diphtheria epidemic from 1990 to 1998. The study comprises 10 patients. The cases were identified from the national infectious diseases register at the National Public Health Institute, Helsinki. To estimate the number of visits from Finland to Russia and *vice versa*, data from the Finnish Ministry for Foreign Affairs, the Finnish Frontier Guard and the National Agency for Promotion of Tourism were used.

Study V is a prospective study focusing on the incidence and risk factors for diphtheritic myocarditis in adults. The study comprises 122 patients. They were enrolled from the inpatient service of Botkin Hospital between January 1 and May 31, 1995. Patients were enrolled from all hospital wards including the intensive care ward. Criteria for enrolment included age $\geq$16 years and a clinical syndrome compatible with diphtheria of the respiratory tract. In principle the patients were enrolled at random, and

there is no obvious bias in the selection of patients. Clinical evaluation, phlebotomy and ECG recording were performed on admission, weekly while in hospital and at the time of discharge. A follow-up evaluation was conducted one to three months after discharge. All ECG recordings were interpreted by one of the investigators (Kaj Groundstroem) who was provided only with the age, sex and data on pre-existing cardiac disease for each subject.

Study VI is a case report on a Finnish unvaccinated infant that died in diphtheria. The disease was acquired from a visitor from Russia. The epidemiological, clinical and immunological aspects are described.

8.2 Microbiological methods

In the studies where the microbiological diagnosis was made in the Russian Federation (II, III, V), the diagnosis of diphtheria was based on a typical clinical presentation and it was confirmed or excluded by isolation of *C. diphtheriae.* All isolated strains were tested for toxin production *in vitro*. For bacteriological diagnosis, for each patient swabs were taken from both the pharynx and the nose. The microbiological identification methods used were in accordance with those recommended by WHO [Efstratiou and Maple 1994]. The diphtheria cultures were performed on tellurite blood agar. The identification of bacterial strains was made on the basis of production of cysteinase and fermentation of liquid tellurite media. Toxin production was demonstrated by immunoprecipitation on agar as described by Feldman [Feldman et al. 1987]. The cultures for identification and toxin production were repeated four times to confirm the results. To search for possible double infections, for each patient throat cultures were performed for *Streptococcus pyogenes*, *Staphylococcs aureus* and *Streptococcus pneumoniae*.

In studies where the microbiological diagnosis was made in Finland (I, IV, VI), the methods for identification of the *C. diphtheriae* strain and assay of toxin production were based on WHO guidelines [Feldman et al. 1987, Efstratiou and Maple 1994]. All isolates were also referred to the Diphtheria Laboratory at the National Public Health Institute, Helsinki, for further characterization. In Study VI, a direct diphtheria toxin gene determination was made of a throat swab by a method using polymerase chain reaction [Nakao and Popovich 1997]. The strain isolated in Study VI was later genotyped to determine the ribotype [De Zoysa et al. 1995].

Human antitoxin concentrations were assessed by a routine indirect alkaline phosphatase enzyme immunoassay method [Melville-Smith 1990] and with a functional *Vero* cell assay (I, IV, VI) [Miyamura et al. 1974] at the National Public Health Institute in Helsinki and the Microbiological Laboratory of Turku University. In Study VI, the level of free toxin in serum and the specificity of the toxin that killed the *Vero* cells was

verified by a neutralization assay with diphtheria antitoxin. The test system was specially developed for this study in the National Public Health Institute, Helsinki.

8.3 Definitions

In Studies I, II and IV to VI, diphtheria was defined as an exudative inflammation of the upper respiratory tract (with or without pseudomembranes) and a positive culture for a toxin-producing strain of *C. diphtheriae*. In Study III, the diagnosis was made by the same criteria for the clinical presentation and an infection by a non-toxin producing strain of *C. diphtheriae*.

In Study II and Study V, the clinical disease of respiratory tract diphtheria was divided according to the Russian guidelines into clinical forms and degrees of toxicity. The disease was classified as catarrhal if inflammation of the tonsils and the pharynx was mild with no haemorrhage or membranes. This condition is characterized by subfebrile body temperature, throat pain, hyperaemia and enlargement of the tonsils. The disease was described as local if inflammation and exudate or membranes were restricted to the tonsils or nostrils. In the spread or extensive form the signs of inflammations were observed also in the uvula, soft palate, posterior pharynx, larynx or the lower respiratory tract. In the combined forms, which were always also extended forms, membranes were found in both the upper and lower respiratory tract. The disease was classified as toxic if there was subcutaneous swelling. Three grades of toxicity were defined. In grade 1 the swelling did not extend beyond the first cervical fold, in grade 2 the swelling extended further but no further than the clavicle, and in grade 3 there was swelling beyond the clavicle. For the statistical analysis in Study V, the severity of the acute diphtheria was classified for three features: presence or absence of pseudomembranes, anatomical distribution of pseudomembranes (extended or not) and toxicity (toxic or not). Neuropathy was defined as the new-onset of sensory or motor defects in cranial or peripheral nerves detected on physical examination. The clinical classification of neurological disease was based on the distribution of physiological abnormalities as observed by one of the investigators (Oleg Melnick).

In Study V, the patient was regarded as evaluable for myocarditis if at least two ECG recordings were available, and at least one of them was taken between day 7 and day 21 from the onset of respiratory tract symptoms. The classification of ECG changes was based on previously published criteria for myocarditis [Boyer and Weinstein 1948, Morgan 1983, Morgera et al. 1992]. The following were defined as myocardial abnormalities: arrhythmias (supraventricular and ventricular extrasystoles), abnormal Q-waves and repolarization abnormalities. Specific criteria included ST-segment elevation >1mm in at least two chest leads or one limb lead, ST-segment

depression ≥1mm, T-wave inversion (except in leads V1 and AVR, isoelectric T-waves), right and left atrial and ventricular hypertrophy and QTc interval >0.39 seconds for males and >0.41 seconds for females. The following were defined as conduction abnormalities: atrio-ventricular block, bundle branch block, and hemiblock. The ECG abnormalities were regarded as diphtheria related if there was a change from abnormal to normal or the reverse, or if there was no other explanation (such as underlying cardiovascular disease or medication) for a constant abnormality.

There are no published classification systems for social class, income, education, mode of habitation or alcoholism in the Russian Federation. After testing with poor success several scoring systems for classifying patients according to these features, we had to accept the investigator's (Oleg Melnick) subjective assessment on a dichotomous scale. These data were used in the statistical analysis in Study V. In St. Petersburg there were no vaccination records for adults. Data on diphtheria immunization were based on patient histories. As protected was regarded a person with a basic vaccination series of at least three doses less than 10 years previously or a basic series given earlier than that with a booster dose in the 1990s.

8.4 Statistical methods

In Study V, one-way frequency tables and cross tabulations were generated using SPSS for Win 6.1. Chi-square and Fisher's exact tests were used to test association between myocarditis and risk factors. Relative risk was calculated by dividing the incidence in the myocarditis group by the incidence in the non-myocarditis group. Ninety-five percent confidence intervals were calculated using the CIA (Confidence Interval Analysis) program [Gardner and Altman 1989]. After preliminary frequency analysis, multivariate associations were evaluated in a multiple logistic regression model based on forward stepwise selection [Hosmer and Lomeshow 1989]. The procedure allows estimation of the strength of the association between each predictor and the dependent variable (myocarditis), taking into account the potential confounding effects of the other independent variables. The predictor is added to the model if the probability of the likelihood ratio statistic is <0.10. The method used is based on the maximum-likelihood estimate. Three models (demography, socio-economic status and severity of the acute disease) were constructed.

9 Results

Studies I, II, and VI describe the clinical manifestations and epidemiology of Finns who acquired diphtheria between 1990 and 2002 as a consequence of the Russian epidemic. Four of the 12 patients described had a severe disease (Table 8). In three of the patients, no membrane was observed at any time by direct inspection of the pharynx. In one patient, the infant, a pseudomebrane was later found around the epiglottis by laryngoscopy. In two of the patients, a 43-year-old male and a 45-year-old male, a local oedema in the pharynx was misdiagnosed as a peritonsillar abscess. After unsuccessful attempts to drain pus by needle and incision, a bilateral tonsillectomy was performed on both patients.

Three patients received antitoxin therapy. The time from onset of symptoms to administration of the first dose varied from 4 to 7 days (Table 9). Two patients acquired clinically significant myocarditis and one acquired peripheral polyneuropathy. Two patients died despite intensive care; the 45-year-old man from cardiac failure and the 3-month-old infant by suffocation due to pseudomembranes obstructing the peripheral bronchi. Only one patient, the 45-year-old man who died, was adequately vaccinated. The level of antitoxin antibodies was low in all four patients when first tested on or soon after admission to hospital (Table 8). In the infant, free diphtheria toxin was observed in the serum on the seventh day of the disease, before the administration of antitoxin.

Table 8. Characteristics of the four patients with a severe form of diphtheria acquired from the Russian diphtheria epidemic.

Age/sex	Locality of infection	Strain (biotype/ toxin production)	Vaccination status[1]	Acute disease	Complication	Antibody level day 1-7[2]
43y/M	St. Petersburg	*gravis*/toxin$^+$	not vaccinated	local, tonsillitis non-toxic	myocarditis polyneropathy	0.01
57y/M	Viborg	*gravis*/toxin$^+$	not known	extensive toxic[3]	bleeding tendency renal insufficiency	0.06
45y/M	Viborg	*gravis*/toxin$^+$	vaccinated	extensive toxic[3]	myocarditis bleeding tendency death (cardiac failure)	0.08
3mo/M	Finland	*mitis*/toxin$^+$	not vaccinated	extensive non-toxic	death (suffocation)	<0.003

[1] a patient was regarded as vaccinated if having a basic series of at least three doses and a booster dose less than 5 years before falling ill
[2] an EIA test for human antibodies expressed as IU/ml
[3] subcutaneous oedema

Table 9. Various delays expressed as days from the onset of diphtheria symptoms in four Finnish patients with a severe disease.

Age/sex	First health care contact	Admission to hospital	Antitoxin given	Microbiological diagnosis	Myocarditis	Neuropathy	Death
43y/M	0	1	No	6	9	30	No
57y/M	3	7	7	9	No	No	No
45y/M	0	1	7	7	4	No	11
3mo/M	0	4	4	7	No	No	7

All four patients with a severe form of diphtheria had had direct saliva contact with a person living in Russia. The three middle-aged men had kissed a Russian woman and two of them had had commercial sex during a visit to Russia. A visitor from Russia had kissed the infant during a visit to the home of the family. Altogether 227 close contacts of the four severe cases of diphtheria were screened for *C. diphteriae* with a throat and a nose swab. Only two contact persons were found to be positive. One was a male travel companion of the 43-year-old male, who had sex with a Russian woman during the trip to Russia. The other was the sister of the infant, who was, like the infant, kissed by the visitor from Russia. Fifty-five schoolmates of the sister were swabbed. All had negative cultures. None of the 91 health care workers caring for the patients were positive.

Study II reviews the clinical data on all 1,860 adult patients treated for diphtheria at Botkin hospital in St. Petersburg during a two-year period (Table 10). In about two thirds of the patients, no pseudomembrane was observed. Of all the patients with diphtheria, 16% had an extensive disease (membranes spreading outside tonsils) and 8% had a toxic disease with subcutaneous swelling. Due to the retrospective nature of the study, it was not possible to assess reliably the rate of late complications of diphtheria; myocarditis or neuropathy.

Table 10. The division of diphtheria patients at presentation by clinical form of disease.

Affected	Number	%
NON-TOXIC FORMS, total	1,709	91.9
Pharynx or tonsils without membranes	1,256	67.5
Tonsils with membranes	150	8.1
Uvula, soft palate, posterior pharynx	268	14.4
Larynx or lower respiratory tract	35	1.8
TOXIC FORMS[1], total	151	8.1
Grade 1	69	3.7
Grade 2	57	3.1
Grade 3	25	1.3
TOTAL	1,860	100

[1] clinical forms with pharyngeal or subcutaneous swelling

Forty-two patients died, giving a case fatality ratio of 2.3%. Asphyxia caused by pseudomembranes was contributory in 24 (57%) of the deaths, myocarditis in 15 (36%) and polyneuropathy in 6 (14%). Nearly half the deaths (43%) occurred on the first day in hospital. Eighty-six percent of the deaths occurred in patients 40 years old or older. Those vaccinated had a significantly lower risk for an extensive form of disease (7% vs. 18%) and for a toxic disease (6% vs. 14%).

Of the patients with a clinical diagnosis of diphtheria treated in Botkin Hospital, St. Petersburg, during the period 1991 to 1995, 112 had only a non-toxigenic strain of *C. diphtheriae* isolated (III). They represent 2.4% of all patients with a clinical diagnosis of diphtheria treated in the same time period. Of the patients with a non-toxigenic strain, 13% had a disease classified as moderate or severe, in contrast to 30% of patients with a toxigenic strain. Three (2.4%) patients with a non-toxigenic strain died. All had a membraneous infection. Two on them had clinically evident myocarditis verified by an ECG recording and the third had peripheral polyneuropathy. In all cases one or more pathologic changes typical of toxic diphtheria were found in the autopsy: extensive pseudomembrane, subcutaneous oedema, capillary microthrombi, haemorrhages in various organs, myocarditis and demyelinization of peripheral nerve fibres.

Study V is a prospective study designed to investigate the incidence and risk factors of diphtheritic myocarditis in 122 patients treated in hospital. Eighty-eight patients had a sufficient number of ECG recordings with adequate timing to be evaluated according to heart involvement. In the ECG analysis, 25 (28%) of the 88 evaluable patients had changes regarded as evidence of diphtheritic myocarditis. Eighteen patients had electrophysiological findings indicating myocardial involvement only, seven had findings of both myocardial involvement and abnormal conduction (Table 11).

Having a more severe acute form of diphtheria was the greatest risk factor for myocarditis. Patients who had fever on admission, membraneous disease, extensive form of diphtheria or toxic disease all had at least a two-fold increased risk for myocarditis (Table 12). Nevertheless, twelve (18%) of the 65 patients with non-membraneous diphtheria had myocarditis. Patients with diphtheritic neuropathy had a risk of cardiac involvement equal to that of patients with no neuropathy (36% vs. 26%, p=.48). The incidence of myocarditis for males was 36% (13/36), and for those ≥40 years old 46% (16/35) (Table 12).

Table 11. Electrocardiographic changes among patients classified as having diphtheritic myocarditis. A patient may appear in more than one category.

Change	Patients	
	N	% of those with myocarditis
MYOCARDIAL INVOLVEMENT, total	25	100
ST-T changes	20	81
T-wave inversion	19	78
Prolongation of the QTc interval	16	67
Ectopic atrial tachycardia	1	4
Supraventricular extrasystoles	1	4
Ventricular extrasystoles	1	4
ABNORMAL CONDUCTION, total	7	28
Atrio-ventricular block 1°	3	12
Atrio-ventricular block 3°	1	4
Left anterior hemiblock	1	4
Left posterior hemiblock	1	4
Right bundle branch block	1	4
MYOCARDIAL INVOLVEMENT AND ABNORMAL CONDUCTION	7	28

In a univariate analysis the odds ratio of higher age (≥40 years) was statistically significant. There was a trend for increased risk of myocarditis in those sharing a dwelling with non-family members (p=.12), in alcoholics (p=.11) and in persons having limited education (p=.12). In the logistic regression analysis, using demographic determinants as explanatory variables, age ≥40 years (OR 4.12, 95% CI 1.55-10.95) was related to myocarditis (Table 12). In a similar analysis, but using the determinants of the socio-economic variables, the risk of myocarditis was associated to sharing a dwelling with non-family members (OR 2.93, 95% CI 1.00-8.61). Of the determinants of the severity of the acute disease, having fever on admission (OR 4.17, 95% CI 1.05-16.57) and having both a spread and toxic form of diphtheria (OR 6.99, 95% CI 1.16-42.22) were statistically significant predictors of myocarditis. When all four significant variables were included in a model, the odds ratio for each was in the same range.

For testing the association of age with clinical manifestations of the respiratory tract disease, an age cut-off of 40 years was used. Age ≥40 was associated with extensive disease (p=<.001, Fisher's exact test), toxic disease (p=.010) and inflammation with pseudomembranes (p=.011). Gender was not associated with any of these clinical features. Among clinical characteristics, alcoholism was associated only with the risk of myocarditis and the risk of diphtheritic polyneuropathy (43% vs. 11%, p=.049).

Table 12. Univariate and multivariate analysis of the determinants of myocarditis among 88 patients with diphtheria.

Feature	N	Percent with myocarditis	Fisher's exact test p-value	Univariate analysis		Multivariate analysis[1]	
				OR	95%CI	OR	95%CI
Demography							
Female	52	23.1		1.00			
Male	36	36.1	.231	1.56	.81-3.03		
<40 years	53	17.0	1.00	1.00			
≥40 years	35	45.7	.007	2.69	1.34-5.40	4.12	1.55-10.95
Alcoholic							
No	79	25.3		1.00			
Yes	9	55.6	.111	2.20	1.09-4.40		
Acute disease							
Fever >37°C[2]							
No	71	19.7		1.00		1.00	
Yes	15	66.7	.001	3.38	1.87-6.10	4.17	1.05-16.57
Pseudomembranes							
No	65	18.5		1.00			
Yes	22	54.5	.002	2.95	1.56-5.59		
Toxic disease							
No	75	22.7		1.00			
Yes	12	58.3	.017	2.57	1.36-4.86		
Extensive disease							
No	70	21.4		1.00			
Yes	17	77.8	.005	2.75	1.51-5.00		
Toxic and extensive							
No	78	21.8		1.00		1.00	
Yes	8	77.8	.001	3.57	2.07-6.16	6.99	1.16-42.22
Other features							
Toxoid booster in the 1990s							
No	32	25.0		1.00			
Yes	44	25.0	1.00	1.00	.45-2.20		
Streptococcus pyogenes							
Present	25	24.0		1.00			
Not present	63	30.2	.612	1.26	.57-3.70		

[1] Multivariate logistic regression (forward stepwise selection). The predictor was added to the model if the probability of the likelihood ratio statistic based on the maximum-likelihood estimate was <.10. Three models (demographic, socio-economic and severity of the acute disease) were constructed.
[2] Fever measured on admission

10 Discussion

10.1 Diagnosis

Clinical diagnosis

There is no uniformly accepted clinical case definition of diphtheria. The World Health Organization [1994] has given one; presence of a pseudomembrane and a positive culture for *C. diphtheriae* is required. The definition excludes patients with no pseudomembrane. In the studies presented in this thesis all patients had microbiological diagnosis performed according to the WHO criteria [World Health Organization 1994]. Study II is the largest clinical series of patients with respiratory tract diphtheria ever published. The second largest clinical report is a 10-year material from the United States in the 1940s [Naiditch and Bower 1954]. In that study the patients had a "bacteriologically proven" diagnosis, but it is not stated whether toxin production by the isolated bacterium was determined. In several of the earlier studies, the proportion of diphtheria patients with pseudomembranes varied between 30% and 70% [Johnson 1947, Paley and Truelove 1948]. In the studies presented here, a quarter (V) to a third (II) of the patients with microbiological proof of *C. diphtheriae* had a pseudomembrane observed.

On the basis or the data in Studies II and V it seems that the definition requiring the presence of a pseudomembrane for the diagnosis of diphtheria is too narrow for clinical purposes and excludes true mild cases of the disease. Several observations support this view. The point prevalence of healthy or convalescent carriers of *C. diphtheriae* is only 0.1 to 2.4% in a general urban population during epidemics or high endemicity [Weaver 1921, Taylor et al. 1962], and the median period of bacterial carriage after acute diphtheria is only 2 to 3 weeks [Hartley and Martin 1920, Weaver 1921]. Therefore, a patient harbouring *C. diphtheriae* in the throat, but having an exudative but non-membraneous throat infection caused by some other microbe should be a rarity. The clinical faith of the patients in Study II with two-thirds of the patients with non-membraneous disease was not much different from that in a an old report from the United States, in which only 3% of patients presented without pseudomembranes (Table 13) [Naiditch and Bower 1954]. Moreover, in Study V, 12 out of the 65 patients (18%) with mild (non-membraneous) infection had myocardial involvement, supporting our definition of diphtheria. As in an earlier report [McCloskey et al. 1971], in Study V, too, almost a third of the patients with pharyngitis and a positive culture for *C. diphtheriae* had also *S. pyogenes*, group A in the throat making a false diagnosis of

Table 13. Demographic description and percentages of the clinical distribution and outcome of respiratory tract diphtheria in the 1940s in the United States compared with those in the 1990s in Russia [Naiditch and Bower 1954, II, V).

Feature	Patients in the US in the 1940s (N=1,372)	Patients in Study II (N=1,860)	Patients in Study V (N=88)
Demography			
Males (%)	53	46	42
Age range (years)	0- >50	16-72	17-71
Mean age (years)	10-20 (median)	36[1]	36
Alcoholic (%)	4	20[1]	10
Other features			
Immunized (%)	50[2]	50[2]	42
Delay in admission (median, days)	3	6	6
Clinical form			
Localized[3]	71	76	82
Extensive[4]	29	24	18
Membraneous	97	32	25
Toxic[5]	18	8	14
Polyneuropathy	6	ND[6]	12
Fatal	10	2	1

[1] Data obtained retrospectively from patient records for this study
[2] Estimated on the basis of other studies
[3] Catharral forms without membranes and tonsillar forms with pseudomembranes
[4] Faucial, pharyngeal, laryngeal and lower respiratory tract forms with pseudomembranes
[5] Subcutaneous swelling or marked lymphadenopathy ("bull neck")
[6] Not determined

streptococcal angina possible in several of diphtheria patients. In Study V, the risk of myocarditis was similar for the patients with and without streptococci.

Observations on the four Finnish patients with severe diphtheria (I, IV, VI) [Groundstroem et al. 1996] illustrate the problems with the diagnosis of diphtheria in a non-endemic country. Even if the Finnish doctors were well informed about the epidemic of diphtheria east of the Finnish border [Jahkola et al. 1993], the diagnosis of diphtheria was delayed by at least four days counted from the first health care contact. Antitoxin given later than the third day of disease is probably ineffective [Naiditch and Bower 1954]. Two of the patients had marked local swelling of the throat, which is typical for diphtheria. In a country where diphtheria is not endemic, an abscess of the peritonsillar tissue is a more common cause of this phenomenon than diphtheria. On two of the patients an unnecessary tonsillectomy was performed. These experiences stress the value of a careful patient history to discover any possible predisposition to diphtheria such as travel to an endemic area.

Microbiological diagnosis

Non-toxigenic strains of *C. diphtheriae* are considered emerging pathogenes. They have caused mild diphtheria-like upper respiratory tract infections or pyogenic infections, such as wound infections, endocarditis, septic arthritis and abscesses in both immunocompetent and immunocompromized persons [Jepchott and Gillespie 1975, Isaac-Renton et al. 1981, Afghani and Stutmen 1993, Farizio et al. 1993, Tiley et al. 1993, Poilane et al. 1995]. However, the patients in these reports did not have the typical pathological signs of toxic diphtheria, such as extensive membrane formation, myocarditis or peripheral neuropathy. In the United States the case fatality rate in infections caused by non-toxigenic isolates of *C. diphtheriae* was only 0.5% as opposed to 9.9% for diphtheria caused by a toxigenic strain [Munford et al. 1974].

Using isolation of a toxin-producing strain of *C. diphtheriae* as a prerequisite for the diagnosis of diphtheria brings problems. Study V describes the clinical characteristics of 112 patients with a disease clinically compatible with diphtheria but with isolation of only non-toxigenic *C. diphtheriae.* It is likely that the severe complications occurring in the three deceased patients described in detail in the report were caused by diphtheria toxin which was not detected in diagnostic tests, even though the methods used for identification of *C. diphtheriae* and detection of its ability to produce toxin *in vitro* are regarded as reliable for the diagnosis of diphtheria. Because of the properties of the culture media such as incomplete iron depletion, routine *in vitro* methods may fail to detect all *C. diphtheriae* strains producing exotoxin *in vivo*. It is known that phenotypic variation of toxin production exists *in vitro* [Pappenheimer and Murphy 1983]. A DNA sequence, referred to as a DNA insertion element, has been shown to be capable of interrupting the gene coding for *C. diphtheriae* virulence, converting the bacterium from toxigenic to non-toxigenic [Rappuoli et al. 1987]. This type of phenotypic change in the bacterium *in vitro* was probably the reason for failure to show toxin production in routine tests. The microbiological diagnosis or the evidence for toxin production by the isolated *C. diphtheriae* should not be prerequisites for antitoxin treatment in patients with a history and clinical picture supporting diphtheria. If a PCR test is available and its application validated in clinical patient samples, the results of PCR assays capable of detecting sequences of the diphtheria toxin gene may prove useful in determining the choice of therapy [Michailovich et al. 1995].

10.2 Clinical characteristics

Acute disease

There is no internationally accepted clinical classification for respiratory tract diphtheria. Such a division would be useful for epidemiological purposes, for the choice of treatment, and for predicting the outcome. Especially, clinical classification is a necessary tool in scientific work, e.g. in treatment trials. In Studies II and V, the Russian clinical classification system [Ministry of Health 1986] for diphtheria was used (Table 10). It divides the disease according to the presence or absence of a pseudomembrane, the extent of the pseudomembrane and toxicity of the disease. The Journal of Infectious Diseases in its special issue in 2000 entitled "Control of Epidemic Diphtheria in the Newly Independent States of the Former Soviet Union" (Volume 181, Supplement 1) later adopted the same classification as was used in Studies II and V. This classification system seems to divide patients adequately in relation to the risk of death (II) and the risk of acquiring diphtheritic myocarditis (V). It is, with fair accuracy, possible to reclassify the patient materials reported earlier according to this classification.

An example of the use of this clinical classification is its application for assessing whether diphtheria has changed as a clinical disease over the last decades. Such a change could be expected, as there have been marked changes in host factors like nutritional status and immunization histories. There may also have occurred variation in bacterial virulence factors like the prevalent biotypes of the bacterium or their capacity to produce toxin. In contrast to the Russian epidemic, the latest big epidemics 40 to 50 years ago hit children under 5 years of age hardest. In the early epidemics the proportion of the patients who had been immunized was fairly low or unknown. Also access to early diagnosis and treatment were probably different from the current situation. In spite of all this, the comparison of the clinical presentation of the population-wide epidemic in St. Petersburg in the 1990s (II) to the presentation of the endemic diphtheria in the United States 40 years earlier [Naiditch and Bower] shows that there are no marked differences in outcomes (Table 13). The biggest difference can be seen in the case fatality ratio; 9.6% in the United States in the 1940s vs. 2.3% in Russia in the 1990s. The likely explanation for this is that during low endemicity (the study in the United States) the diagnosis of diphtheria is frequently missed in mild non-membraneous cases, because *C. diphtheriae* is not detected by routine throat cultures. In the Russian epidemic, however, a special diphtheria culture was routinely performed for all patients with pharyngotonsillitis (II).

Myocarditis

Study V is the first and so far the only clinical study of diphtheria with a prospective design. It provides data on the incidence of and risk factors for cardiac involvement in respiratory tract diphtheria among a group of unselected adult patients. The study was conducted during a nation-wide epidemic. Our definition of diphtheria was more inclusive than in some other studies by considering also mild, non-membraneous cases of diphtheria.

Since the introduction of ECG in clinical practice in the early 1920s, more than 30 studies on cardiac involvement in respiratory tract diphtheria have been published. Only twelve studies have included more than 10 adult patients and none of them has been prospective and unbiased in regard to the patient population [Ball 1945, Carter 1947, Altshuler et al. 1948, Boyer 1948, Gore 1948, Brainerd and Bruyn 1951, Naiditch and Bower 1954, McCloskey et al. 1971, Dobie and Tobey 1979, Harnisch et al. 1989, Quick et al. 2000b, Kadirova et al. 2000] (Table 4). The reported risk of clinically evident cardiac involvement has ranged from 2% [Havaldar et al. 1989] to 33% [Nathanson 1928], typically from 10% to 20%, and the risk of ECG-verified myocarditis from 7% [Vichitbandha et al. 1969, Dobie et al. 1979] to 89% [Alstead 1932]. In the prospective study presented here (V), a 28% incidence of ECG-verified diphtheritic myocarditis was observed among 88 adults with respiratory tract diphtheria.

There are only three studies, none of them prospective, where any factors related to the risk of cardiac involvement have been reported. In a study where serial ECG recordings were used to identify cardiac involvement, the patients with myocarditis had a higher average age than patients without myocarditis (25 years vs. 12 years) [Boyer 1948]. A small study using only clinical criteria for complications showed that patients older than 60 years had the most complications [Harnisch et al. 1989]. In a large study with serial ECG recordings from each patient, no obvious correlation was found between the severity of acute diphtheria and the severity of ECG changes [Altshuler et al. 1948].

In the old studies where determinants of increased risk of death have been sought, associations have been found with male gender [Naiditch and Bower 1954, Munford et al. 1974, Jones et al. 1985], age below 5 or 6 years [Honey 1934, Burkhard et al. 1938, Quick et al.2000b], high age [Brainerd and Bruyn 1951, Harnisch et al. 1989, Kadirova et al. 2000], alcoholism [Naiditch and Bower 1954], having a severe inflammation in the respiratory tract [Honey 1934, Carter 1947, Naiditch and Bower 1954, Dobie and Tobey 1979], lack of prior immunization [Taylor et al. 1962, Miller et al. 1974, Jones et al. 1985, Quick et al.2000b, Kadirova et al. 2000, Khetsuriani et al. 2000] (II) and delay in admission [Kadirova et al. 2000, Quick et al. 2000b]. Because in diphtheria half the deaths have been [Brainerd and Bruyn 1951, Naiditch and Bower 1954] and still are (II)

[Quick et al.2000a] caused by cardiac complications, it is logical that the risk factors for myocarditis found in Study V are mainly the same as the risk factors for death found in other studies.

Although severe infection was found to be a risk factor for cardiac involvement in diphtheria (V), there is a considerable risk for those with a mild disease without pseudomembranes. Since even mild ECG changes have been associated with fatal outcome [Bethell et al.1995], patients with diphtheria should be followed with serial ECGs. Because of the observed delay in the onset of ECG abnormalities (V), recordings should be performed weekly for three weeks to identify most patients with myocarditis. If resources are limited, particular attention should be given to patients with the highest risk; those with severe respiratory tract inflammation, the elderly, persons with low socio-economic status and probably the alcoholics. If a cardiac ultrasound investigation is available, it may be the most sensitive way of diagnosing early myocarditis (I) [Groundstroem et al. 1986]. Although not proven, early recognition of disturbances in cardiac conduction may improve outcomes for diphtheritic myocarditis.

10.3 Transmission

Diphtheria is regarded a highly contagious disease. It was one of the first diseases for which legal quarantine against the will of the patients was used [Kleinamn 1992]. Even today, the WHO recommends strict isolation for patients with respiratory tract diphtheria, a practice usually recommended for diseases transmitted by an aerosol of respiratory tract secretions [World Health Organization 1994].

Finnish citizens made 6.2 million trips to epidemic areas in Russia during the 1990-1998 epidemic. This travel resulted in only ten observed cases of diphtheria (IV). Only one of those found infected was a secondary case; the child of a father who had travelled to Russia. During the years 1990 to 2001 Russian citizens made over five million trips to Finland. The visits of Russians to Finland led to only two certain and one possible transmission in Finland; all in one family. For all four Finnish patients with a severe form of diphtheria, kissing was the most probable mode of transmission. In none of the cases there was suspicion of acquisition of the infection from poorly washed dishes or contaminated food, or simply from joining crowds in theatres, public transport or the like. Transmission of diphtheria via food or by sharing contaminated dishes has, however, been proposed on the basis on two reports [Christenson et al. 1989, Quick et al. 2000b]. Neither of these studies considered the possible role of hands contaminated with excrement containing *C. diphtheriae*.

In the recent East European epidemic, clusters of multiple cases and carriers have been observed in families and schools but not in workplaces [Vitek et al. 1999, Magdei et al. 2000]. In a Finnish school, no spread was observed in connection with one child with a positive throat culture for *C. diphtheriae* (VI). There is anecdotal data on the risk of nosocomial diphtheria in health care personnel during the recent epidemic in Belarus [Filonov et al. 2000]. When treating the four severely sick patients with diphtheria in Finland, many health care personnel were heavily exposed to the patients' respiratory tract secretions. The patients were treated for several days in intensive care and operations were performed on the respiratory tract. Three of the patients were initially treated without suspicion of diphtheria and only the usual barrier precautions were used. Still, none of the 91 most heavily exposed health care workers who were screened with a throat and a nose swab was positive for *C. diphtheriae* (I, IV, VI).

The low incidence of imported cases of diphtheria in Finland despite the frequent travel of residents to an endemic area suggests that aerosol or droplets of respiratory tract secretions as well as food and poorly washed dishes are inefficient in transmitting diphtheria. Even using only usual barrier precautions in the treatment of patients, the risk of nosocomial spread is low.

10.4 Immune protection

From retrospective data, it has been concluded that vaccination against diphtheria offers no protection against a carrier state of *C. diphtheriae* [Miller et al. 1972, Munford et al. 1974]. Diphtheria toxoid vaccine gives about 90% protection against clinical disease, and 50% to 80% protection against severe disease forms, the late cardiac and neurological complications and death (II) [Naiditch and Bower 1954, Taylor et al. 1962, Glinyenko et al. 2000, Khetsuriani et al. 2000, Quick et al. 2000a , Quick et al. 2000b].

There are no studies correlating the pre-existing antibody level to the clinical course of diphtheria. Diphtheria antitoxin antibody levels of 0.1 IU/ml are considered as minimum protective, especially when *in vitro* techniques are used. Levels between 0.01 and 0.09 IU/ml are thought to give uncertain protection, and patients with antibody levels lower than 0.01 IU/ml are considered unprotected [Ipsen 1946, Ipsen Jr. 1954, Farizio et al. 1985]. In epidemiological studies, an antibody level of ≥1 IU/ml is considered to indicate good long-term protection [Golaz et al. 2000]. It can be assumed that high antitoxin levels prior to infection protect persons from myocardial and neural damage and death [Ipsen Jr 1954, Hadfield et al. 2000]. However, there is no sharply defined level of antitoxin that gives complete protection from diphtheria [Christenson and Böttiger 1986, Mofredj and Guerin 1993].

All four patients described in Studies I, IV and VI had a non-protective level of diphtheria antitoxin antibodies in their first serum sample (Table 8) and all had an equally severe acute respiratory tract infection with extensive pseudomembranes and a toxic disease. The pathogenesis of both the acute disease and its complications, such as myocarditis and neuropathy, is regarded as a consequence of the bacterial exotoxin [Pappenheimer Jr 1984]. Passively given antitoxin provides protection from complications of diphtheria and death if given within the first few days of the disease [Naiditch and Bower 1954]. Due to delay in diagnosis one of the patients did not receive antitoxin treatment and for the others the antitoxin was administered late, about one week after falling ill (Table 9). Two of the three middle-aged patients described in Study IV did not show a rise in antitoxin antibody titres during the first 2 weeks of infection to a level regarded as protective. Both of these patients had myocarditis and one developed a severe diphtheritic neuropathy. The third patient had a rapid rise of antibodies to the protective level and recovered without complications. This may indicate that the ability to produce an early antibody response to diphtheria toxin protects a patient with low antitoxin levels prior to the infection from late complications of diphtheria. A prospective study would be needed to confirm this.

Antitoxin treatment is used to neutralize diphtheria toxin in the blood. The doses recommended depend on the clinical severity but are not based on clinical trials. There are no reports in the western literature where free or complex bound diphtheria toxin has been measured from patient sera. In a report published in Russia, levels as high as 0.65 Lf/ml of free diphtheria toxin in the serum are described in the acute phase of the disease [Melnikova et al. 1996]. These toxin measurements were made by using monoclonal antibodies to COOH-terminal site of a toxin molecule in an enzyme immunoassay. The level of 0.03 Lf/ml found in the Finnish child (VI) with the *Vero* cell assay is relatively high. On the basis of toxin concentration and the volume of blood in a 3-month old infant, the estimated amount of toxin in the blood was approximately 6 Lf. Intravenous administration of 40,000 IU of equine antitoxin, like performed to this child, should result in a blood antitoxin concentration of approximately 100 IU/ml. All circulating toxin should have been neutralized after treatment.

Virtually all human cells have receptors for diphtheria toxin [Pappenheimer Jr 1984, Collier 2001]. It has been suggested that, after the toxin is bound to the receptor, the progression of the disease cannot be influenced by giving antitoxin [Pappenheimer Jr 1984, Collier 2001]. Clinical patient series indicate that antitoxin given later than three days after the onset of symptoms has only minor or no impact on the outcome of the disease [Brainerd and Bruyn 1951, Naiditch and Bower 1954]. The deceased infant received antitoxin late, seven days after the onset of respiratory tract symptoms (VI). Finding free antitoxin in the serum of a patient so late as on the seventh day after the

onset of disease may indicate that even a delayed administration of antitoxin could be of benefit at least in preventing the late manifestations, myocarditis and polyneuropathy, in diphtheria.

In most developed countries, the diphtheria immunization coverage of children is over 90%. However, booster vaccinations are not performed routinely for adults, and a third of the middle-aged population is without sufficient protection [Edmunds et al. 2000]. Despite this, in the light of the observations in Studies I, IV and VI it seems highly unlikely that major outbreaks of diphtheria would occur in countries with a high standard of living and high general level of hygiene, e.g. in Finland. However, doctors should be aware that even in a population with very high vaccination coverage there are persons vulnerable to diphtheria, such as infants born to mothers who have not received booster vaccinations (VI). Even properly immunized persons can have asymptomatic infections of *C. diphtheriae* and can transmit diphtheria.

11 Observations on the Finnish-Russian scientific partnership

The selection of studies presented here is a result of a collaboration between Finnish investigators and their Russian colleagues. The starting point of these two parties was quite different in respect to clinical and scientific traditions. The project required us to adopt common clinical definitions and study methods. In addition to this the limitations of human and material resources in Russia made it difficult to adhere to every component of the proposed study designs.

The main counterparts were Tampere University Hospital in Finland, Botkin Hospital of infectious diseases, St. Petersburg and St. Petersburg Medical Academy for Postgraduate Studies in the Russian Federation. In the early 1990s, communication between Finland and Russia was troublesome as fax and e-mail connections did not work. Mail took more than two weeks to arrive and language mismatch made telephone contacts difficult. The author of this thesis, alone, had to pay more than twenty visits to St. Petersburg before the two extensive retrospective reports (II, III) could be published and the first patients could be enrolled for the first prospective study (V) [Lumio 1995].

A few examples of the practical obstacles may be presented. The original intention was to conduct a case control study on risk factors for acquiring diphtheria and so obtain valuable data for prevention of the disease both locally in the endemic area and for those travelling to the endemic area. The lack of a validated classification of social groups in Russia and our failure, despite vigorous attempts, to validate such a classification together with some other cultural determinants made this kind of scientific

approach impossible. We were tempted to perform prospective randomised trials on different treatment schedules, but such studies could not be performed due to the strict guidelines of the Russian Ministry of Health on the treatment of diphtheria [Ministry of Health of the USSR 1986].

Although, it was possible to follow closely the progress of the study by regular visits, according to the regulations in Russian hospitals, no Finnish doctor could be placed on scientific patient work. The high standard of patient case records in Botkin Hospital was the prerequisite for the retrospective clinical studies (II, III) as the data had to be re-evaluated according to the definitions agreed upon for this project. In the routine of Botkin Hospital, a decursus with the findings of a clinical check-up and the changes made in treatment together with reasons had been entered in the records daily.

The Finnish-Russian scientific co-operation on the study of diphtheria has already yielded two Russian theses (Doctor Jelena Nosikova, Doctor Oleg Melnick) and numerous international lectures. The project has served as a framework for eight Finnish-Russian symposia for physicians representing various specialities (infectious disease, cardiology, oncology, urology, otorhinolaryngology, pulmonology) and general practitioners. We are confident that the project has provided unique and clinically valuable data on the epidemiology and clinical character of diphtheria and that it can serve as an example of scientific partnership across national and cultural barriers.

12 Summary and conclusions

The re-emergence of respiratory tract diphtheria in an industrialized country for the first time in nearly 50 years made this series of studies possible. The epidemic in the 1990s was nation-wide in the Russian Federation. The subjects of primary interests were the reasons for emergence of the epidemic, the mode of transmission of the infection, evaluation of the possibility that diphtheria would spread to neighbouring countries like to Finland and the risk factors and clinical characteristics of the disease. Within this framework the projects were designed *ad hoc,* largely depending on the resources available and other circumstances prevailing in Russian society at the time of the study. The clinical data were acquired by a retrospective review of patient records. Epidemiological observations were acquired from the national statistics of the Russian Federation and of Finland and by observations made in connection with the cases of diphtheria in Finland. Risk factors for cardiac complications of diphtheria were studied in a prospective trial.

The findings and conclusions based on the results of the studies are:

1. Diphtheria is not as easily transmissible as earlier considered. The principal mode of transmission of diphtheria is probably direct contact with a person carrying *Corynebacterium diphtheriae*. As cases of diphtheria have been rare in travellers to the epidemic area, aerosol spread and spread by inanimate objects such as poorly washed dishes seem to be inefficient in transmitting diphtheria. In hospitals, barrier precautions like hand disinfection may be more important for protection than treating patients in specially designed isolation rooms.

2. It seems improbable that diphtheria will spread in epidemic proportions to the wealthy countries which have a high standard of hygiene, functioning infrastructure and high coverage of childhood immunization for diphtheria.

3. In epidemics that affect society as a whole and are not confined to special risk groups like alcoholics and intravenous drug abusers, the clinical spectrum of diphtheria seems to be fairly similar to than what it was in United States and in Europe fifty years ago. In adults, mild, non-membraneous cases predominate, but up to a third of the cases are of a severe or complicated nature.

4. Approximately one in four cases of diphtheria in adults is complicated by myocarditis. Even among the cases with mild, non-membraneous disease the risk of myocarditis is considerable. To identify patients with myocarditis, serial ECG recordings during the first three weeks of disease are needed.

5. Patients with a severe acute inflammation of the respiratory tract, persons over 40 years of age, persons with low socio-economic status and alcoholics have the highest risk for myocarditis in diphtheria.

IV ACKNOWLEDGEMENTS

The study was carried out at the Department of Internal Medicine, Tampere University Hospital, and at Botkin Hospital, St. Peterburg. It proceeded in tight connection to the St. Petersburg Medical Academy for Postgraduate Studies and University of Tampere.

A study performed on two geographical areas and amidst two different cultures leaves you with a debt of gratitude in various quarters; both scientific and non-scientific.

First of all I want to address my cordial thanks to my Russian collaborators. They go to Professor Aza Gazanovna Rakhmanova, MD, for her intelligent mind and warm heart and to Professor and Chief of Botkin Hospital Alexei Yakovlev, MD, for the brilliant management of a scientific project with limited resources. Without Doctor Oleg Borislavovich Melnick, PhD, carrying the favorite name “the Snake”, not much would have happened in the clinical work. My gratitude concerns both the partnership and the friendship.

Two expansive Cief physicians of Pirkanmaa Hospital District deserve my dearest thanks. Professor Arto Uusitalo, MD was the key figure in the beginning of this study. He was the person who signed a cultural exchange agreement with Botkin Hospital on 4 July 1994. Both Professor Uusitalo and Professor Ossi Auvinen have given both their concrete and abstract support to the efforts during the whole of the study. I greatly appreciate their help and encouragement.

I am deeply grateful to my two supervisors, Professor Jukka Mustonen, and Docent Petri Ruutu, who’s impact in the late stages of the work was crucial. I also want to express my sincere thanks to the official referees, Professor Jukka Nikoskelainen, MD, and Professor Ville Valtonen, MD.

Many experienced scientists in Finland have contributed to the scientific yield. For this, I would especially like to thank Docent Kaj Groundstroem, MD, Docent Jaana Vuopio-Varkila, MD, MSc Rose-Marie Ölander, and MSc Heini Huhtala.

Several travel companions have not only contributed to the success of the scientific project but have helped in enduring the unavoidable frustrating vicissitudes. In the beginning of the project late Doctor Matti Jahkola, MD, and Doctor Jukka Saikku, MD, both slavofils I dear say, shared the pains in building relations in St. Peterburg. I reminisce with warmth the seemingly endless nights in Hotel Moscow when they shared their experience with me.

The impact of many persons outside the medical or scientific faculties have been indispensable in bringing the project this far. Especially I would like to thank warmly Consul Olli Kuukasjärvi for teaching me long-suffering and philanthropy and Consul Teela Pakkasvirta for giving with great all the kindness and practical help one can desire. I am grateful also to bibliophile and superior of Helsinki City Library Kimmo Haapala, for leading me to the relevant Russian fiction on diphtheria.

After sitting during this study more than 600 hours in train between Tampere and St. Petersburg I am deeply grateful for the personnel and the passengers I have met. The conductors who helped me to regain the confiscated microscope from the Viborg customs, the young Finnish activists on their way to help abandoned children on the street, the American rabbi on his way to restore the icons in Kazans Cathedral, the forest experts of the United Nations traveling to the East-Karelian forests. Thank you for your time with me, thank you for the discussions – they have been part of the reward for this project.

The study was financially supported by the Medical Research Fund of Tampere University Hospital, and Tampere Tuberculosis Foundation. Ministry of Social Affairs and Health of Finland, National Research and Development Center for Welfare and Health, and the Finnish Lung Health Association have supported the Russian party in some of the trips to international conferences where our results have been published.

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