The Pathology of Diphtheria

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Diphtheria is an acute, communicable disease caused by Corynebacterium diphtheriae. The disease is generally characterized by local growth of the bacterium in the pharynx with pseudomembrane formation or, less commonly, in the stomach or lungs; systemic dissemination of toxin then invokes lesions in distant organs. Acute disease of the upper respiratory tract usually involves one or more of the following: tonsillar zones, larynx, soft palate, uvula, and nasal cavities. A recent epidemic in Russia emphasized the role of vaccination in reducing disease in children and adults.

Clinical descriptions of diphtheria appeared in Hippocratic writings, but the illness was not clearly differentiated from other upper respiratory disease until clinician-pathologist Pierre Bretonneau first described its unique clinical characteristics [1, 2].

Diphtheria is an acute, communicable disease caused by exotoxin-producing Corynebacterium diphtheriae. Review of pathology in archived cases and the literature shows that C. diphtheriae usually localizes in the upper respiratory tract, ulcerates the mucosa, and induces the formation of an inflammatory pseudomembrane. The exceedingly potent toxin is absorbed into the circulation and damages remote organs, potentially resulting in death. Although primary infection can occur at sites other than the pharyngeal mucosa, lesions usually occur as local pseudemembranous inflammation on mucosal surfaces of the upper respiratory tract and systemic lesions of the heart and (to a lesser extent) nerves.

C. diphtheriae is usually transmitted by direct contact or by sneezing or coughing. No age group is completely immune, but nonimmune children are commonly affected before age 5 [3]. In populations with a high rate of immunization, improved coverage in children has shifted the age distribution of those afflicted to unimmunized or poorly immunized adults [4]. Furthermore, relatively increased isolation of nontoxigenic strains of C. diphtheriae versus toxigenic strains is noted when immunization rates are improved [5].

A widespread epidemic of diphtheria began in 1990 in the former Union of Soviet Socialist Republics (USSR). This epidemic was notable for the high incidence of infection in adults and the extent of disease.

Respiratory Tract Diphtheria

The upper respiratory tract mucosa is the most common site of infection in children but was a rare site for localization of diphtheria in adult patients in a recently described epidemic occurring in the former USSR. In adult patients with oral mucosal lesions, unusual sites of infection included buccal mucosa, upper and lower lips, hard and soft palate, and tongue [6].

Anterior nasal infection presents with serosanguinous or seropurulent nasal discharge, which is often associated with subtle whitish patches on the mucosal membrane of the septum. The discharge can incite an erosion of the external nares and upper lip, but symptoms are usually mild. Faucial diphtheria involves the posterior structures of the mouth and proximal pharynx. This is the area of infection most characteristic for clinical diphtheria. A membrane typically develops on one or both tonsils, with extension to the tonsillar pillars, uvula, soft palate, oropharynx, and nasopharynx. C. diphtheriae multiplies on the surface of the mucous membrane, resulting in the formation of the “pseudomembrane” [7]. Initially, the pseudomembrane is white, becoming a dirty gray color over time. Late in the course of infection, the membrane may have patches of green or black necrosis.

Our review of diphtheria shows that laryngeal and tracheobronchial diphtheria are uncommon. Both can be primary infection sites or extensions of a pharyngeal infection. Edema and pseudomembrane coating of the trachea and bronchi can reduce and eventually block air flow through the respiratory tree, resulting in cyanosis or suffocation of the infected person.

The organism establishes itself on the surface of mucous membranes, and primary foci of infection coalesce during the course of the disease. Satellite infections may occur in the esophagus, stomach, or lower airways. The growth of organisms remains localized, but exotoxin is absorbed into the blood and evokes severe systemic pathology. The exotoxin inhibits cellular protein synthesis by stimulating adenosine diphosphate ribosylation, thus inactivating protein synthesis elongation factor 2. In addition, Lessnick et al. [8] and Chang et al. [9] re-
ported an alternative cytotoxic pathway consisting of a nuclease activity stimulated by Ca" and Mg"+, which comigrates with the A subunit of the toxin. They propose that the toxin directly attacks the chromosomal DNA to initiate cell lysis.

In fatal diphtheria, gross findings often include disseminated ulcers of the mucosa covered by a “dirty” coating. Pharynx, larynx, trachea, and the main bronchi may be covered by pseudomembranes that may or may not be firmly attached to the tissues. Smaller bronchi are often reddened and coated with a similar thin membrane. Chest films reveal a bronchopneumonia [10]. The lungs are hemorrhagic with a moderately solid consistency [11]. Edema and hyperemia of the affected epithelial surface appear first. This is followed by necrosis of the epithelium, accompanied by outpouring of a fibrinopurulent exudate. The coagulation of this exudate on the ulcerated necrotic surface creates the characteristic tough, dirty gray to gray-white superficial pseudomembrane (figure 1). The pseudomembrane also contains necrotic sloughed epithelial cells and numerous colonies or organisms of C. diphtheriae (figure 2).

Caution should be exercised in the histologic diagnosis of diphtheria because nonviral diphtheroids are normal inhabitants of the oral cavity and may also be enmeshed within the pseudomembrane. However, in diphtheria, the membrane has patches of intense collections of C. diphtheriae as opposed to the small collections of nontoxigenic diphtheroids seen in figures 3 and 4. Mild neutrophilic infiltration of the underlying tissues becomes progressively more severe with the continuance of the bacterial invasion. Severe lesions have marked vascular congestion, interstitial edema, fibrin exudation, and intense neutrophil infiltration. When the pseudomembrane is torn off this highly vascularized bed, bleeding occurs. By centrifugal spread, the pseudomembrane reaches the larynx, trachea, and even the lower respiratory tract. Likewise, it may rise to cause nasal obstruction. Occasionally, intense suppurative inflammation and necrosis of the subjacent tissues permit spontaneous dislodgment and aspiration of the membrane.

Pseudomembranes may show an inner band of fibrin and a lumenal zone of polymorphonuclear leukocytes, but this zoning may be reversed or absent. Over the vocal cords, the membrane is almost entirely fibrin, and in the main bronchi, it is usually a uniform mixture of fibrin and neutrophils. In laryngeal lesions, there is a deep layer of lymphocytes. The acute inflammatory reaction often extends into the mucous glands of the respiratory tract. Tonsil and laryngeal diphtheria lesions penetrate adjacent skeletal muscle. There may be marked hemorrhage in and around the parabronchial and mediastinal lymph nodes, with numerous polymorphonuclear neutrophils in the peripheral sinuses. Pulmonary pathology includes extension of the fibrinopurulent exudate to line and partly fill small bronchi, bronchioles, and alveoli, leading to early bronchopneumonia [10].

Diphtheria lesions in the stomach have superficial mucosal erosion with slight hemorrhage and an attached thick fibrinopurulent exudate. These pseudomembranes are indistinguishable from lesions in the respiratory tract [10].

**Lymph Nodes**

Lymph nodes in advanced cases are enlarged and may appear blackish-red and be hemorrhagic. Regional nodes respond, resulting in a nonspecific acute lymphadenitis, particularly in the cervical area. Respiratory embarrassment, severe adenitis, and soft tissue edema result in a “bull neck” appearance in advancing cases.

**Pathology Associated with Toxigenicity**

Diphtheriae toxin, which is secreted by toxigenic strains of C. diphtheriae, is a single polypeptide of M_58,342. Toxigenic strains of C. diphtheriae carry the tox structural gene found in lysogenic corynebacteriophages β tox', γ tox', and ω tox'. Highly toxic strains have two or three tox' genes inserted into the genome. Expression of the gene is regulated by the bacterial host and is iron dependent. In the presence of low concentrations of iron, the gene regulator is inhibited, resulting in increased toxin production. Toxin is excreted from the bacterial cell and undergoes cleavage to form two chains, A and B, which are held together by an interchain disulfide bond between cysteine residues at positions 186 and 201. As toxin concentrations increase, the toxic effects extend beyond the local area due to distribution of the toxin by the circulation. Diphtheriae toxin does not have a specific target organ, but myocardium and peripheral nerves are most affected [12].

**Myocardium**

Human diphtheria infection may terminate with acute cardiac failure or may prove fatal weeks later during convalescence. Examination of heart tissues after death demonstrates cardiac damage in many cases, although the pathologic lesions are varied and inconsistent, perhaps representing a spectrum of changes related to cumulative exposure to toxin. When toxin concentrations are low, the heart chambers may be dilated with no effusion or malformation. The valves, coronary vessels, epicardium, and endocardium are normal. The myocardium appears pale brown and soft. The myocardium appears distorted by widely distributed areas of granular degeneration and loss of cross striations. Neutral fat droplets occur in ~50% of fatal cases of diphtheria. The fat droplets appear as beaded configurations in the sarcoplasm of muscle cells with oil Red O stains. Nuclei may form caterpillar chromatoid configurations. Some nuclei are pyknotic. “Caterpillar” cells are histiocytes [13]. Neutrophils in the sarcoplasm of degenerating myocardial cells are rare except in areas of maximal degeneration, where they are...
Figure 1. Pharyngeal pseudomembrane. Epithelium is absent; at one side, inflammatory exudate extends to underlying muscle. Hematoxylin-eosin staining. Original magnification ×2.5.

Figure 2. Higher magnification (original ×25) of pseudomembrane comprising fibrin, neutrophilic inflammation, and gram-positive colonies of *C. diphtheriae*. Brown-Hopps tissue gram stain.

Figure 3. Irregularly staining rods of *C. diphtheriae* comprising small colony. Brown-Hopps tissue gram stain. Original magnification ×330.

Figure 4. Colony of *C. diphtheriae* with admixed gram-positive coci. Note relatively small size of rods. Brown-Hopps tissue gram stain. Original magnification ×330.

Figure 5. Cardiac muscle. Note loss of myofibrils, with extensive fatty change. Few mononuclear cells (macrophages and lymphocytes) are present. Hematoxylin-eosin staining. Original magnification ×25.
associated with slight hemorrhage. No other inflammatory cells are observed. Conduction tissue lesions are not extensive. Abnormalities of coronary vessels, endocardium, or epicardium are not seen [14].

In a study of 102 patients who died of diphtheria intoxication, dystrophic-necrotic processes in cardiac conduction and intramural nervous systems in contractile myocardium were observed between days 1 and 8 [15]. (Myocarditis progression undergoes two stages: early exudative [about day 3 of disease] and late productive [beginning ~9 days into disease]). The end result for patients in the study was myocardial infarction [15].

Grossly, hearts are dilated, pale, and flabby, with a characteristic “streaky” appearance in the myocardium. Sections of myocardium show extensive areas of hyaline degeneration and necrosis associated with active inflammation in the interstitial spaces. Infiltrates of mononuclear cells with eosinophilic cytoplasm are present within these areas (figure 5). Fluorescent antibody staining of tissue sections demonstrate toxin in a patchy distribution within myocardial fibers. Toxin is not observed in areas of advanced necrosis. Electron micrographs show striking ultrastructural changes within involved myofibers. Mitochondria are swollen and show loss of matrix and disorganization of the cristae. Dense osmophilic material is often seen within the mitochondria. Damaged myofibrils are disrupted at scattered foci, leaving empty, structureless, pale spaces. Numerous lipid droplets are seen within myofibers. Sarcoplasmic reticulum tubules show no significant changes. Chromatin granules are clumped and are located near the nuclear membrane [16].

Neurologic Toxicity

About three-fourths of patients with severe disease develop neuropathy. The incidence of neurologic complications is related directly to the severity of respiratory symptoms. About 20% of all symptomatic respiratory infections are followed by polyneuritis, but 75% of patients with severe disease develop some form of neuropathy. The first indication of neuropathy is paralysis of the soft palate and posterior pharyngeal wall. This often results in regurgitation of swallowed fluids through the nose. Thereafter, cranial neuropathies causing oculomotor and ciliary paralysis are common; dysfunction of facial, pharyngeal, or laryngeal nerves contributes to the risk of aspiration. Peripheral neuritis develops later, from 10 days to 3 months after the onset of oropharyngeal disease [17].

Occasionally, motor nerves of the trunk, neck, and upper extremities are involved, as are sensory nerves, resulting in a glove-and-stocking neuropathy. Examination of affected nerves by microscopy shows degeneration of myelin sheaths and axon cylinders. The basis of diphtheritic polyneuropathy is toxic myelopathy with paranodal demyelination, especially in large myelinated fibers. Early changes affect the paranodal myelin so that the nodes of Ranvier appear widened and altered in configuration. At this stage, segmental demyelination is rare; later (45–62 days in an animal model), segmental demyelination is the characteristic lesion [18]. Axonal degeneration is observed in the most severe cases and appears secondary to the axon being squeezed by the folded myelin and voluminous Schwann cell cytoplasm invaginating into the axon [19].

Muscle biopsies removed during acute stages of diphtheria polyneuropathy reveal scattered small, angulated muscle fibers adjacent to more generalized slight atrophy, predominantly of type 2B fibers and target-like phenomena or cores of type 1 fibers. Intramuscular vessels may exhibit a vasculitis comprising lymphoid cells [20].

Other Pathologic Effects

The exotoxin also causes myocardial-like changes in inflammatory polyneuritis. Lesions show degeneration of myelin sheaths and, less commonly, fatty degenerative changes or focal necroses of parenchymal cells in the liver, kidneys, and adrenals. Occasionally, Zenker’s hyaline degeneration, or necrosis, occurs in the striated voluntary and cardiac muscle fibers in the more severe cases.

In addition to the toxigenic manifestations of *C. diphtheriae*, disease associated with nontoxigenic strains occurs. Guran et al. [21] described a septic arthritis of the hip, which was caused by nontoxigenic *C. diphtheriae* in a child. Dissemination presumably occurred from skin lesions on the toes. Nontoxigenic *C. diphtheriae* was isolated from excoriated skin lesions on the toes and from articular fluid of the hip.

Nontoxigenic *C. diphtheriae* was recently described as a cause of sepsis and has been associated with splenic and hepatic abscesses [22]. Well-differentiated, diffuse lymphocytic infiltrates effaced the architecture of lymph nodes, spleen, and bone marrow. Similar interstitial infiltrates were present throughout the lungs, cortex, and medulla of kidney and hepatic portal triads. The liver showed multifocal panlobular areas of necrosis with a thin rim of scattered degenerating neutrophils and rare lymphocytes. A few gram-positive, rod-shaped bacteria were observed in the lesions. Similar lesions were seen in the spleen. Small foci of interstitial hemorrhage were present in the skin, renal cortex, lung, and liver. *C. diphtheriae* sepsis can lead to endocarditis [23].

Cutaneous Diphtheria

Today, we often think of diphtheria of the skin in the context of wound diphtheria, umbilical diphtheria, or impetiginous diphtheria. Skin lesions can be extremely variable owing to the ability of *C. diphtheriae* to colonize any skin lesion of other origin (e.g., surgical wounds, pyoderma, eczema, impetigo, dermatitis, and insect bites). Often, an ulcerative lesion (eczema diphtheriticum) is the presenting lesion. It begins as a vesicle or pustule filled with straw-colored fluid, which breaks down...
quickly. The lesion progresses to form a punched-out ulcer, single or multiple, measuring several millimeters to a few centimeters, with slightly curved and elevated margins. In addition, the margins may be slightly undermined, or inverted. Common sites for diphtheric lesions are the lower legs, feet, and hands. The lesions are painful and may be covered with an adhering eschar (dark pseudomembrane) during the first 1–2 weeks. Then the lesion becomes anesthetic, and the pseudomembrane falls away, leaving a hemorrhagic base, sometimes with serous or serosanguinous exudate oozing from it. The surrounding tissue is edematous and pink, purple, or livid in color and may show blisters or bullae [24]. Skin lesions yielding *C. diphtheriae* on cultures are indistinguishable from those associated with other bacteria and can include dry, nearly healed, scaly lesions.

**Conclusion**

The scientific battle against diphtheria is a great success story encompassing efforts from many aspects of scientific investigation: pathology, molecular biology, immunology, public health, and preventive medicine. The culmination of these efforts has resulted in the virtual eradication of this disease in the developed world. The recent epidemic in Russia demonstrates how rapidly progress can be reversed when these efforts lag. Clinicians and pathologists alike must remain alert to the varied and unusual clinical manifestations of this disease. Ongoing efforts to further enhance our understanding of this scourge must continue.

**References**


