

BACTERIOLOGIC STUDIES OF THE UPPER RESPIRATORY PASSAGES

V. THE DIPHTHERIA BACILLI AND DIPHTHEROIDS OF THE ADENOIDS AND TONSILS

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The tonsils are the foci that usually harbor diphtheria bacilli in carriers, and their removal frequently terminates the carrier state.

Pegler¹ was perhaps the first to observe this effect, which has been described also by Friedberg² who reported 6 carriers that gave negative cultures for *B. diphtheriae* after extirpation of the tonsils and adenoids. Ruh, Miller and Perkins³ obtained similar results in 19 cases and Rabinoff⁴ had the same experience in 10 instances. In his study of diphtheria carriers Weaver⁵ reports that cultures became negative in 40 persistent carriers within 18 days after the removal of the tonsils and adenoids. Keefer, Friedberg and Aronson⁶ state that in 77.2% of the carriers they studied the organisms were in the tonsils, often in pure culture, and that 91.3% became negative within 2 weeks after tonsillectomy.

The diphtheria bacilli apparently are in the crypts of the tonsils as well as on the surface. Ruh, Miller and Perkins³ call attention to 5 carriers who gave positive cultures of the crypts while the surface cultures were negative. Dwyer and Gignoux⁷ obtained 5 positive reactions from direct cultures of the crypts of 72 persons. Brown⁸ demonstrated in microscopic sections gram-positive bacilli morphologically like the diphtheria bacillus in the crypts and in the tissues beneath the thin epithelium of the tonsils of 7 carriers. Ballantyne and Cornell⁹ found diphtheria bacilli in the tonsillar crypts of 6 carriers and once in the adenoids. Hartley and Martin¹⁰ found the bacilli in sections of the tonsils, in the crypts but not in the tissues.

In all of these observations it is noteworthy that cultures were taken from the tonsils only occasionally, from the adenoids practically never, and apparently none of the bacilli identified was tested for

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¹ Brit. Med. Jour., 1905, 2, p. 621.

² Jour. Am. Med. Assn., 1916, 66, p. 810.

³ Ibid., p. 941.

⁴ Ibid., 67, p. 1722.

⁵ Ibid., 1921, 76, p. 831.

⁶ Ibid., 1918, 71, p. 1206.

⁷ Laryngoscope, 1910, 20, p. 1042.

⁸ Jour. Infect. Dis., 1916, 19, p. 565.

⁹ Brit. Med. Jour., 1917, 2, p. 686.

¹⁰ Quoted by Weaver.⁵

virulence. To determine the frequency in the naso- and oro-pharynx of virulent and avirulent forms of diphtheria bacilli and of related organisms, a bacteriologic study with special reference to these points was made of the extirpated adenoids and tonsils of the same patients.

The material was obtained from 100 children who entered the Cook County Hospital to have their tonsils and adenoids removed chiefly because of hyperplasia. They were afebrile and had no evidences of acute inflammation of the throat or respiratory passages. They varied from 5 to 15 years of age, and were boys and girls living in scattered parts of Chicago. A reliable history of an attack of diphtheria was not to be obtained in any case. The adenoids and tonsils were removed during the months of October and November, 1920.

The material was selected and cultures were taken only from adenoids which were not macerated. Cultures were obtained from the tonsils of the same persons. Altogether 100 adenoids and 100 pairs of tonsils were studied. Cultures were made in from 1 to 3 hours after the operation.

The adenoids revealed no marked pathologic change other than hyperplasia. There were no gross evidences of necrosis or of a purulent exudate. They consisted of lymphoid tissue from 0.5 to 1.5 cm. in all dimensions, presenting 3 to 6 folds and often cryptlike depressions not unlike the crypts of the tonsils. The tonsils, like the adenoids, often showed marked hyperplasia, some however, being of normal size without any gross change. Occasionally fatty debris and cholesterol crystals, rarely pus, were observed in the crypts. In none was necrosis seen.

Cultures were taken from the surface of the epithelial lining of the adenoids and the crypts of the tonsils. From the adenoids cultures were obtained by passing a wire loop over the surface, and then after carefully separating the folds or orifices of the depressions another culture was taken of the sides and bottoms of these structures. The tonsils were incised transversely and cultures made with a loop from the bottoms of crypts. Slants of Loeffler serum medium with a broad surface were used and examinations made at the end of 18 to 24 hours.

The diphtheria bacilli and diphtheria-like organisms grew as small gray opaque colonies and were identified with the methylene blue and gram stains. Bacilli with typical polar granules corresponding to the C and D and occasionally A types of Westbrook were indicated as diphtheria bacilli. In several instances the barred types C' and D' were encountered, but granular types were also present. The remaining

bacilli, all gram-positive, occurring often in palisades, were termed diphtheroids. Pure cultures were obtained from subcultures on blood-agar plates.

Diphtheria bacilli were recovered in 12 instances, both from the adenoids and tonsils (table 1). In one case the bacilli were found on the surface of the adenoids and not in the depths. In 5 instances the bacilli occurred in one tonsil and not in the other. In all instances when the bacilli were present they appeared in the adenoids as well as in one or both tonsils. The organisms on Loeffler's medium appeared frequently in pure culture or in predominating numbers (table 2). In the crypts of the tonsils they generally occurred in larger numbers than in the adenoids. It is of interest to note that two strains which proved virulent occurred in practically pure culture in both the adenoids and tonsils.

TABLE 1

THE INCIDENCE OF THE *B. DIPHThERIAE* AND DIPHThEROIDS IN THE EXCISED ADENOIDS AND TONSILS OF 100 CHILDREN

	<i>B. diphtheriae</i> , Percentage Positive	Diphtheroids, Percentage Positive
Surface of the adenoids.....	12	25
Depths of the adenoids.....	11	24
Surface and depths of the adenoids.....	12	30
Right tonsil.....	12	14
Left tonsil.....	7	11
One or both tonsils.....	12	17

TABLE 2

RELATIVE NUMBERS OF *B. DIPHThERIAE* IN THE ADENOIDS AND TONSILS OF 100 CHILDREN

	Surface of Adenoids, Percentage	Depths of Adenoids, Percentage	Right Tonsil, Percentage	Left Tonsil, Percentage
Pure culture.....	3	2	5	2
Predominating numbers.....	3	2	2	2
Moderate numbers.....	1	3	3	2
Few numbers.....	5	4	2	1
None.....	88	89	88	93

On blood agar 4 strains caused hemolysis; only one proved virulent. In 1% carbohydrate broth with Andrade indicator all 12 strains produced acid in dextrose and dextrin, 10 in maltose and lactose and none in saccharose and mannite.

Two strains were repeatedly virulent in doses of 1, 2 and 3 c c of 48-hour cultures of infusion broth for 300 to 400 gm. guinea-pigs, causing death within 24 hours with typical local edema and hemorrhage.

fluid in pleural cavities and hemorrhagic adrenals. The antitoxin controls inoculated with 250 units survived in all instances. A third strain showed low virulence, killing in doses of 3 and 5 c c serum broth, while the antitoxin controls survived. Three other strains in large doses (5 c c) were pathogenic, producing local abscesses, hemorrhages in mucous membranes and muscles with congestion of the kidneys and adrenals, less marked, however, than in the lesions of the more virulent strains. Subsequent cultures were totally avirulent. The remaining 6 strains were avirulent. The 3 strains of low pathogenicity correspond closely to the bacilli described by Hamilton¹¹ in otitis media complicating scarlatina.

In this connection it is worthy to compare the incidence of virulent and avirulent diphtheria bacilli in swab cultures of the throats of normal persons determined by several investigators with the incidence in the adenoids and tonsils. It should be remembered that a surface swab culture has been repeatedly shown not to be a true index of the flora in the crypts of the tonsils in which the organisms are present in larger numbers free from contamination of the bacteria of the saliva and sputum. It is also important to note that most of the statistics available are based on a single swab culture and that the percentage undoubtedly would be higher if cultures of the nasopharynx, each tonsillar surface and the pharynx were taken separately. The report of the Massachusetts Association of Boards of Health¹² indicates a percentage of 1 to 2% of the urban population and 5 to 8% in institutions, and of the strains tried 17% were found virulent. Pennington¹³ found that 9.3% of 375 school children carried the bacilli, of which 14% were virulent and 30% of attenuated virulence. Von Sholly¹⁴ obtained in the cultures of 1,000 normal throats virulent bacilli in 1.8% and nonvirulent in 3.8%. Goldberger, Williams and Hachtel¹⁵ report *B. diphtheriae* in 0.928% of 4,093 healthy persons, of which of 19 strains tested 10.5% were virulent. Guthrie, Gelien and Moss¹⁶ encountered the organisms in 3.55% of 2,507 children and adults, of which 18.18% of 33 strains were virulent. In general, the bacilli recovered from normal persons not convalescent, or contacts from 10.5 to 18.18%, were virulent comparing closely to the strains of the adenoids and tonsils, of which 16.66% were virulent.

¹¹ Jour. Infect. Dis., 1907, 4, p. 316.

¹² Jour. Mass. Assn. of Boards of Health, 1902, p. 1202.

¹³ Jour. Infect. Dis., 1907, 4, p. 36.

¹⁴ Ibid., 1907, 4, p. 337.

¹⁵ Bull. 101, Hyg. Lab., 1915, p. 29.

¹⁶ Bull. Johns Hopkins Hosp., 1920, 357, p. 388.

The diphtheroid bacilli appeared in the smears as nongranular, shorter and stouter than the Klebs-Loeffler organism. In the adenoids they occurred in 30% and in the tonsils in 17% (table 1); 9 of the 30 and 4 of the 17 resembled the D2 type of Westbrook or the Hoffman bacillus. In the adenoids the diphtheroids were usually encountered in larger numbers on the surface than in the depths. In the crypts of the tonsils these organisms were decidedly less common and numerous (table 3). In the 13 instances in which the diphtheroids were absent in the crypts, although present in the adenoids, the predominating growth of the crypt cultures were coccus forms chiefly streptococci. The greater incidence of the diphtheroid group in the nasopharynx than in the tonsils is of interest for it has often been observed that these organisms are more common in the nose and nasal passages than in the oropharynx (Gorham¹⁷). Twenty strains isolated in pure culture did not ferment dextrose, lactose, maltose, saccharose or dextrin resembling the strains from the tonsils studied by Eberson.¹⁸ None of the strains were hemolytic.

TABLE 3
RELATIVE NUMBERS OF DIPHTHEROIDS IN THE ADENOIDS AND TONSILS OF 100 CHILDREN

	Surface of Adenoids, Percentage	Depths of Adenoids, Percentage	Right Tonsil, Percentage	Left Tonsil, Percentage
Pure culture.....	3	3	0	0
Predominating numbers.....	2	2	6	0
Few or moderate numbers.....	20	19	14	11
None.....	75	76	86	89

SUMMARY

Cultures made of the excised adenoids of 100 children revealed *B. diphtheriae* in 12.

The crypts of the extirpated faucial tonsils of the same persons harbored the bacilli in 12. When present in the tonsils the bacilli also occurred in the adenoids of the same person.

In the tonsillar crypts the diphtheria bacilli were usually more numerous than in the adenoids.

Two of the 12 strains were virulent; one showed attenuated virulence; three were pathogenic in large doses of the first culture while subsequent cultures were without virulence; the remainder were totally avirulent.

Diphtheroids occurred in 30 of the adenoids and in 17 of the tonsils; when present in both they were decidedly more numerous in the nasopharyngeal vegetations than in the tonsillar crypts.

¹⁷ J. Med. Res., 1901, 6, p. 201.

¹⁸ Jour. Infect. Dis., 1918, 23, p. 14.