THE RELATION OF UPPER RESPIRATORY INFECTIONS TO RHEUMATIC FEVER IN CHILDREN

III. The Seasonal Bacterial Flora of the Throat in Rheumatic and Non-rheumatic Children

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In the preceding articles of this series the significance of hemolytic streptococci in the pharyngeal flora of rheumatic subjects has been discussed. In this paper a more detailed analysis of the bacterial flora of the throat in rheumatic and non-rheumatic children is presented.

Many investigators have studied the bacterial flora of the throat under various conditions of health and disease. Bloomfield (1) concluded from his results with six healthy individuals and a few others that a true picture of the normal pharyngeal flora is obtained only by making repeated cultures from the same individual. This procedure has been generally followed by most of the other workers in this field. Shibley, Hanger, and Dochez (2) made frequent cultures from thirteen individuals over a period of nine months. McCartney (3) reported the results of numerous routine throat examinations of healthy children. Fox and Stone (4), Meleney (5), Helmholz (6), and Bourn, Carpenter and McComb (7) were primarily interested in the occurrence of hemolytic streptococci in the throat.

These researches and many others establish the fact that there is a basic flora of the throat which remains fairly constant for each individual and contains non-hemolytic streptococci, gram-negative cocci of the catarrhalis, *pharyngis siccus,* or chromogenic types, *Staphylococcus albus,* or diphtheroids in various combinations. Hemolytic streptococci, pneumococci, *Staphylococcus aureus,* hemoglobinophilic organisms of the influenza or "X" types, Friedlaender's bacillus and others are frequently present as transient invaders.

Coburn (8) included an extensive survey of the situation with respect to throat cultures in his studies on rheumatic fever. He believed that the frequency of transient upper respiratory infection with hemolytic streptococcus is the environmental factor of importance in the genesis of the rheumatic state.

Weinstein and Styron (9) studied throat cultures from 148 rheumatic patients and 173 persons who were normal or had diseases other than rheumatic fever. They reported approximately the same percentage of cultures positive for hemolytic streptococci in the two groups and found that "throat cultures of patients with rheumatic fever taken during an infection of the upper respiratory tract showed no greater incidence of hemolytic streptococci than those from other persons who were suffering from a cold or from a sore throat." The majority of their rheumatic patients who had exacerbations showed hemolytic streptococci in their throat cultures; they believe that this fact suggests a possible relationship between this organism and the reappearance of symptoms.

The growing tendency to regard the hemolytic streptococcus as an important, or even an essential, factor in the etiology of rheumatic fever led us to undertake the present investigation. The subjects were children and a few adolescents, rheumatic and non-rheumatic; no adults were included. The two principal objectives were: (a) to see if there is a significant difference between the incidence of hemolytic streptococcal invasion of the throat in rheumatic and in non-rheumatic children, and (b) to obtain information as to the relationship of such invasion to upper respiratory infection and to rheumatic activity. The frequency and time of appearance of other transient invaders were also noted.

The children under observation were seen in the pavilions and clinics of New York Hospital, at Convalescent Cottages, in school, or at their homes

by physicians and visiting nurses who took specimens for throat cultures at frequent, regular intervals and kept a careful record of the state of health of each individual. The average time between cultures was one week. If a child showed signs of upper respiratory infection or rheumatic fever, cultures were made more frequently; a few of the inactive and normal children had cultures every two to four weeks. If any kind of illness appeared, the child was kept under close observation either at home or in the hospital.

TECHNIQUE

Collection of specimens: Dry, sterile, cotton swabs, put up in culture tubes containing 1 cc. of nutrient infusion broth, were used. With the throat well exposed, the swab was rubbed over both tonsillar areas and the posterior pharyngeal wall; it was then replaced in the tube and immersed in the broth at the bottom to prevent drying. In a series of preliminary tests, two or three separate swabs were used for the different regions of the throat; this showed no appreciable advantage over the single swab method. Different methods for the subsequent handling of the specimens were also tested: (*a*) blood agar plates were taken to the bedside or home and inoculated immediately; (*b*) swabs were taken to the laboratory at once, and plates were inoculated within five minutes if the patient was in the hospital; (*c*) an interval of one-half to three hours at room temperature was allowed between the collection of the specimens and the inoculation of the plates. There was no instance in which hemolytic streptococci were obtained by the first method and not by the others; in a few cases they were found by the second and third methods but not by the first. The third procedure was adopted because it was convenient and satisfactory. The time elapsing between collecting the specimens and inoculating the plates was never more than three hours.

Culture mediums: The original cultures from the swabs were made on standard blood agar plates, an entire plate for each specimen. The basic medium was beef-heart infusion containing 1.0 per cent bacto-peptone, 0.5 per cent sodium chloride and 1.75 per cent bacto-agar, pH 7.4 when ready for use. This medium was prepared every two weeks and was put on in 200 cc. lots. In preparing plates, the medium was liquefied in an Arnold sterilizer, cooled to 45° C., 10 cc. of defibrinated horse blood[1] were added to each 200 cc. bottle, and the mixture was distributed in thirteen or fourteen standard Petri dishes. This gave a blood agar layer about 2 mm. deep, as recommended by Brown (10). Only freshly prepared plates were used.

Inoculation and incubation: As soon as the specimens were received at the laboratory, plates were inoculated by rolling and rubbing the moist swab over an area about 1 cm. in diameter, without breaking the surface of the medium, then spreading the material over the rest of the plate with a platinum spatula, making six parallel streaks. Plates were inverted and placed in incubator at 37° C.

Reading the cultures: At first, readings were made after twenty-four and forty-eight hours incubation. The forty-eight hour readings were discontinued after a few weeks because in many instances hemolytic organisms, other than streptococci, caused partial or complete hemolysis of the entire plate. Readings were made with naked eye and a 6X hand lens. A 60-watt daylight electric lamp was used as a source of uniform light. The following organisms were recorded:

Streptococci—Brown's classification (10)
  Beta—Hemolytic
  Alpha; alpha prime—Green producing, with or without partial hemolysis
  Gamma—Neither green nor hemolytic.
Staphylococci
  Aureus, hemolytic or non-hemolytic; albus; citreus.
Pneumococci
  Serologic types were not determined.
Gram-negative cocci
  Catarrhalis; pharyngis siccus; chromogenic.
Gram-negative bacilli
  Friedlaender; coli; proteus.
Hemoglobinophilic bacilli
  Pfeiffer; bacillus "X."
Diphtheroids

The relative number of each type of colony was estimated. The predominating organism was listed as 4, others, less numerous, as 3, 2, 1, or few (5 colonies or less). During the first few months many types of colonies which could not be accurately identified by inspection were studied in Gram stained films and in subcultures on suitable differential mediums. Streptococci were grown in blood broth and blood agar dilution plates, pneumococci were examined for capsules and bile solubility, Gram negative bacilli were tested for their fermentation reactions, and the hemoglobinophilic strains were transferred to plain and chocolate agar slants. As the work progressed, the need for these supplementary examinations diminished; most of the organisms could be recognized by a careful study of colony morphology.

The more common dissociation forms of some organisms were noted but not tabulated. Mucinous, smooth, and rough colonies of hemolytic streptococcus were encountered, the rough being the most frequent, but there was no apparent connection between the type of colony and the clinical findings.

RESULTS

This analysis is based on 4857 throat cultures from 123 rheumatic children and 1231 cultures

[1] Horse blood, defibrinated by shaking with glass beads, was obtained from Lederle Laboratories, Inc. A fresh supply was received every two weeks.

from 109 non-rheumatic subjects. The percentage incidences, by months, of various organisms, are presented in Charts 1 and 2. In most individuals the basic flora of the throat was remarkably constant; transient invaders appeared frequently, but when they departed there was a rapid reversion to the original flora. The percentage infected persons. Many of the positive cultures in both the rheumatic and control subjects came from children who presented no symptoms of hemolytic streptococcus infection and were apparently healthy. Throat cultures were recorded as positive when hemolytic streptococci were present, regardless of the number of colonies. It was

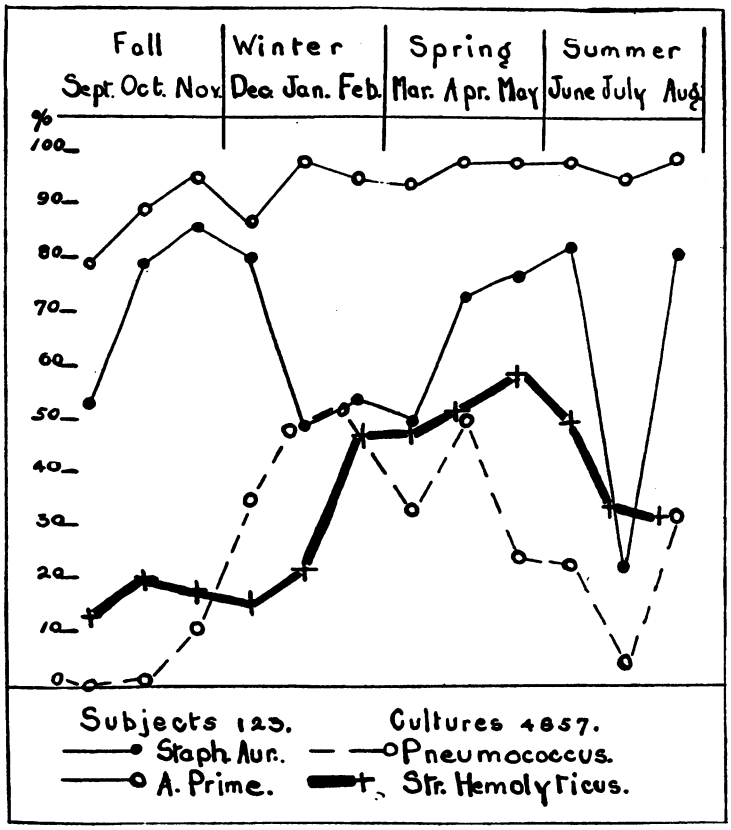

CHART 1. SEASONAL PHARYNGEAL FLORA IN RHEUMATIC SUBJECTS.*

of cultures positive for hemolytic streptococci shows a striking parallelism in the two groups. The increased prevalence of these organisms in the throat during the winter and spring months, in this locality, has been noted by many observers, but there has been too little emphasis on the fact that this increase shows in carriers as well as in found that the relative quantities of these organisms in repeated consecutive cultures varied from predominance to few or none. This was true for healthy carriers as well as for children with upper respiratory infections or with rheumatic activity. It seems evident, therefore, that etiologic significance can be attributed to an organism in the flora of the throat only when its occurrence in culture is associated with characteristic local signs and symptoms. Bacteriological evidence alone does not appear to be sufficient, since the findings in these cultures could not be correlated with the presence or severity of the local signs and symptoms. This point has been discussed in its rela-

* Alpha and gamma streptococci, gram-negative cocci and other organisms normally present in the pharyngeal flora showed no significant seasonal variations in their incidence. The curves for the hemoglobinophilic and Friedlaender bacilli remained below the 20 per cent line throughout the year.

tionship to rheumatic activity in the first paper of this series.

Other organisms, *Staphylococcus aureus* and pneumococcus in particular, show a seasonal variation similar to that of the hemolytic streptococcus. In the routine diagnostic work of this and other laboratories it has been found that in recent years *Staphylococcus aureus* infections, occurring primarily or as complications of upper respiratory disease, were unusually prevalent during November and December. Pneumococcus lobar pneumonia shows its greatest incidence during the winter and early spring months. The periods of maximum frequency of these organisms in the throat coincide with the seasons in which they show their greatest clinical activity, yet none of these children with positive cultures had a detectable staphylococcus infection, and there was not a case of pneumococcus pneumonia in either group.

A comparison of the flora of the throat of children with and without clinical evidence of upper respiratory infection is presented in Chart 3. Two representative months, March and November, have been selected. Here it is shown that there is no significant difference in the incidence of hemolytic streptococci in throat cultures from the two groups; other months showed similar percentages. There is no indication in these findings that upper respiratory infections are necessarily associated with any specific organism or group of organisms.

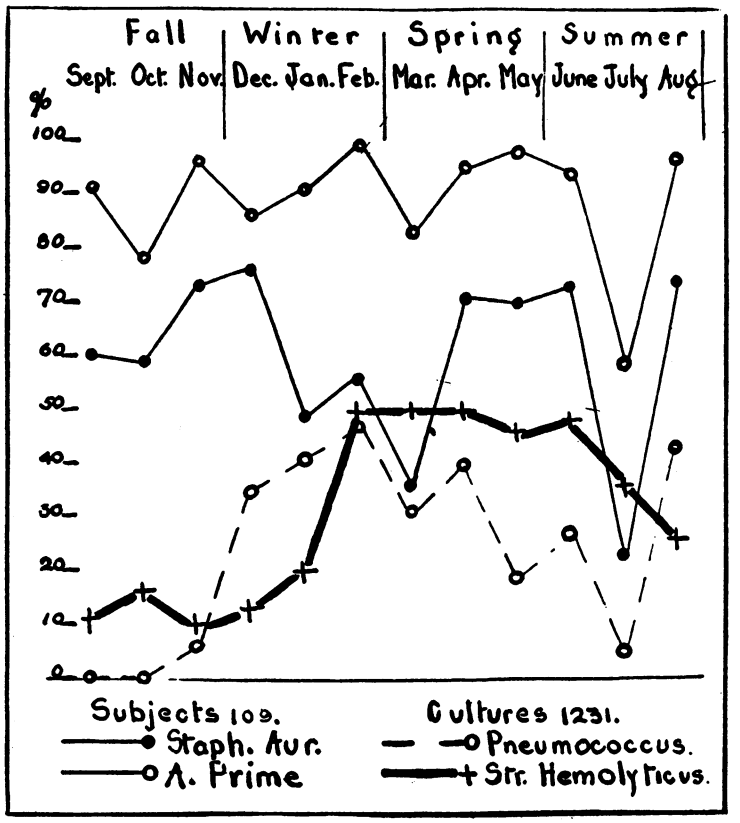

CHART 2. SEASONAL PHARYNGEAL FLORA IN CONTROL SUBJECTS.

SUMMARY AND CONCLUSIONS

1. The data presented are based on a twelve-month study of 4867 throat cultures from 123 rheumatic children and 1231 cultures from 109 non-rheumatic children.

2. In addition to the basic flora of the throat, which is relatively constant for each individual, transient invaders are frequently found and tend

to show their maximum incidence at well-defined seasons of the year.

3. The seasonal incidence of various organisms in the pharyngeal flora must be considered in evaluating their possible etiological significance.

4. A comparison of throat cultures from rheumatic and non-rheumatic children shows no significant difference in the frequency or time of appearance of hemolytic streptococci in the throat.

5. There was no noteworthy difference in the incidence of hemolytic streptococci in the throat during apparent health, upper respiratory infection or rheumatic activity.

6. These findings do not suggest any definite relationship between hemolytic streptococci in the throat and rheumatic fever.

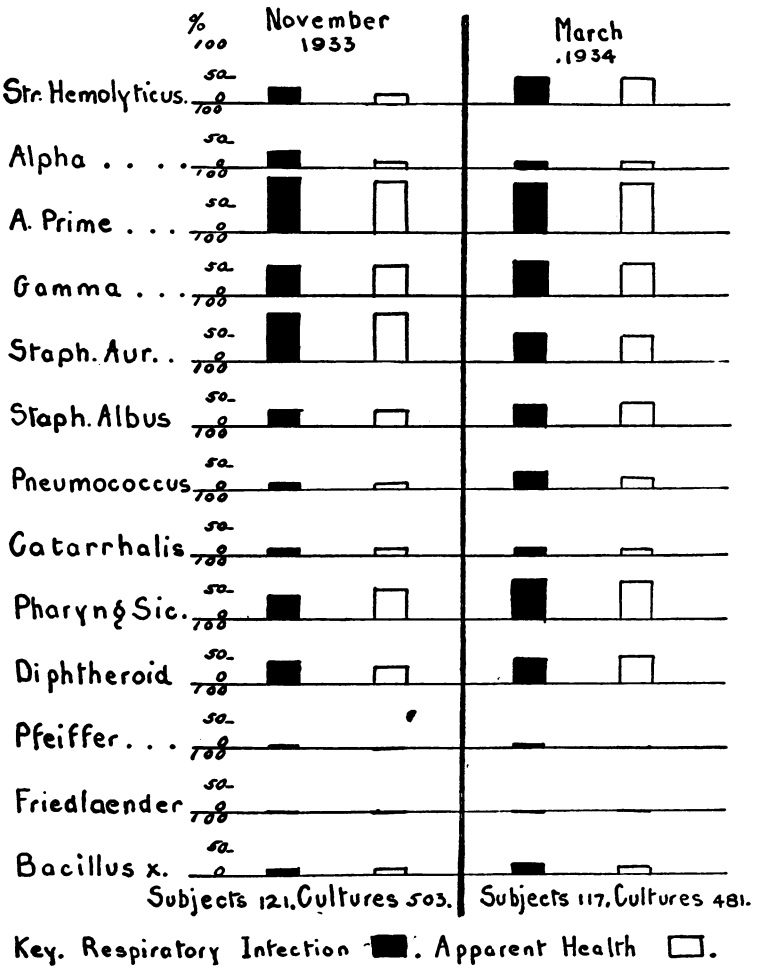

CHART 3. THE PHARYNGEAL FLORA IN RHEUMATIC SUBJECTS DURING RESPIRATORY INFECTIONS AND APPARENT HEALTH.

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