

STUDIES ON INDIFFERENT STREPTOCOCCI.

II. OBSERVATIONS ON THE DISTRIBUTION OF INDIFFERENT STREPTOCOCCI IN THE THROATS OF RHEUMATIC AND NON-RHEUMATIC INDIVIDUALS.

By C. H. HITCHCOCK, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 5, 1928.)

Within the past year the attention of investigators in the field of rheumatic fever has been sharply directed to the indifferent streptococci by the work of Small (1) and of Birkhaug (2). The former has cultivated organisms of this type from patients with the disease, and has prepared a therapeutic serum with which results are reported similar to those obtained with other varieties of antistreptococcus sera (3). He has assumed that these bacteria are to be regarded as the specific causative agents of the disease. Birkhaug has recovered streptococci of the same type from blood, throats, tonsils, and feces of patients suffering from the disease, and with culture filtrates of these organisms has obtained a high percentage of positive skin reactions in individuals "stigmatized by rheumatic fever or its syndromes." Neither of these investigators has adduced convincing evidence that indifferent streptococci are to be more seriously considered in connection with this disease than are streptococci of the *viridans* type.

It might be thought that, if the indifferent streptococci played a special rôle in the production of rheumatic fever, they should be particularly abundant in the throats of patients during the course of the disease or during early convalescence. If such were the case, they should be found with especial ease during the period of acute tonsillitis or pharyngitis which usually constitutes the initial stage of the illness and is frequently observable at the time of hospitalization. It seemed, therefore, of interest to study the flora of the throats of a group of patients suffering from one or another of the acute manifestations of rheumatic fever. As control groups there were studied (1)

patients who had recovered from the disease, symptom-free for at least several months and usually for a year or more; (2) patients suffering from other conditions; (3) normal individuals. The persons constituting the last two groups were free from any condition suggesting the rheumatic syndrome, and with one or two exceptions had never suffered from any of its manifestations.

Methods.

Cultures were obtained from throats by simple swabbing of the tonsils, or tonsillar fossæ, and pharynx. Initial seedings were made upon blood agar plates, spread radially with a platinum loop. At first plates of the inulin-bile-serum agar medium described by Birkhaug (2) were also employed, but these were found to present no unusual advantages and were soon abandoned for purposes of isolation. After an incubation period of from 24 to 36 hours the plates were studied, and a rough estimate was made of the percentage of indifferent colonies present. From six to eight colonies were then picked to blood agar plates, and after appropriate incubation smears were examined microscopically. All strains of indifferent streptococci were then transferred to plates of 1 per cent inulin agar enriched with chest fluid, and at the end of 48 hours the relative numbers of inulin-fermenting and non-fermenting strains were recorded. This method served roughly for initial differentiation, although with many strains it was found that inulin fermentation proceeded readily in tubes of Hiss serum water though it had failed to become evident when the plate method was employed. From the inulin agar plates strains were transferred to tubes of blood broth which were preserved in the ice box for further study. Usually two strains from each culture were thus preserved.

Final determinations of the capacity of the organisms to ferment inulin and salicin were made with tubes of Hiss serum water containing the appropriate carbohydrate. Inocula consisted of 0.2 cc. of a 24-hour culture in broth containing 0.05 per cent of dextrose. No result was recorded as negative until a 10-day period of incubation had elapsed. Two strains from each culture were examined serologically according to methods described previously (4). In most instances both agglutination and precipitation reactions were employed; after the close correspondence between the results of these two methods had been established, it was considered permissible in the case of a few strains to employ the reaction of agglutination alone.

RESULTS.

In Table I are presented data based upon the total number of cultures studied, analyzed with reference to the occurrence of indifferent streptococci.

Regardless of the presence or absence of rheumatic fever, there seems to be a tendency for these organisms to be found somewhat more frequently in the throats of individuals suffering from some condition of

TABLE I.
Incidence of Indifferent Streptococci in Throat Cultures.

	Total cultures	Occurrence of indifferent streptococci	
		Present	Absent
Active R.F. or early convalescence.....	34	29 (85%)	5 (15%)
R.F. recovered.....	26	24 (92%)	2 (8%)
Other conditions.....	36	32 (89%)	4 (11%)
Normal individuals.....	49	37 (76%)	12 (24%)
	145	122 (84%)	23 (16%)

TABLE II.
Incidence of Indifferent Streptococci in Throats of Individuals.

	Total persons	Occurrence of indifferent streptococci	
		Present	Absent
Active R.F. or early convalescence.....	28	26 (94%)	2 (6%)
R.F. recovered.....	23	21 (91%)	2 (9%)
Other conditions.....	36	32 (89%)	4 (11%)
Normal individuals.....	45	36 (80%)	9 (20%)
	132	115 (87%)	17 (13%)

disease than in those of normal persons. The differences are not, however, very striking. It is noteworthy that the bacteria under consideration were harbored quite as frequently by patients free from any taint of rheumatism as by those who were definitely suffering from

some manifestation of the disease. Certainly it would appear justifiable to conclude that the indifferent streptococci are widely distributed, carried more or less constantly by roughly 85 per cent of the population, and that there is a slight and entirely non-specific tendency for them to be found more frequently in the throats of hospital patients than in those of normal individuals. In view of the innate inexactness of the method, the results in the various groups are surprisingly concordant. Inasmuch as in a few cases more than one culture was made from a single individual, and as when indifferent streptococci are scarce they may be missed in the first culture and be found in the second, or *vice versa*, it has seemed desirable to present

TABLE III.
Percentage Incidence of Indifferent Streptococci in Throat Cultures.

	Total cultures	1-5%*	6-15%	16-30%	Above 30%	Present**
Active R.F. or early convalescence. . .	34	17 (50%)	5 (15%)	1 (3%)		6 (18%)
R.F. recovered.	26	16 (62%)	3 (11%)	3 (11%)	1 (4%)	1 (4%)
Other conditions.	36	22 (61%)	4 (11%)	3 (8%)	2 (6%)	1 (3%)
Normal individuals.	49	22 (45%)	9 (18%)	5 (10%)		1 (2%)

*Includes a few early cultures described as containing "few" or "very few."

**Includes a few of the first cultures in which estimates were not made.

Table II, in which the data are analyzed in terms of individuals rather than of total cultures.

Comparison with Table I shows that there is a tendency for the percentage of negative results to decrease when repeated cultures are taken from the same group of individuals. In all probability every person now recorded as free from these streptococci would eventually be found to harbor them, though in very small number, if the cultural procedure were repeated with sufficient frequency.

Although the indifferent streptococci were found no more frequently in the throats of one group of individuals than in those of another, it might be considered that in the case of patients suffering from some active manifestation of rheumatic fever these bacteria would consti-

tute a much greater percentage of the total flora than would be the case with other individuals. In Table III are presented data which bear upon this point.

The cultures from each group of individuals have been divided into subgroups, according as the indifferent streptococci constituted 1-5 per cent, 6-15 per cent, 16-30 per cent, or more than 30 per cent of the total flora, and the total number of cultures in each subgroup have been tabulated, together with the percentage of the main group formed by each subgroup. That the percentages under each group do not add up to 100 per cent results from the omission of a column for the negative cultures.

TABLE IV.

Incidence of Groups of Indifferent Streptococci in Throats of Individuals.

	Total strains	Distribution into groups	
		Type I	Group X
Active R.F. or early convalescence.....	43	21 (49%)	22 (51%)
R.F. recovered.....	32	13 (40%)	19 (60%)
Other conditions.....	43	20 (46%)	23 (54%)
Normal individuals.....	56	30 (53%)	26 (47%)

In slightly more than half of the individuals indifferent streptococci were found to constitute 1-5 per cent of the total throat flora, irrespective of the presence or absence of rheumatic fever or its stigmata. Thus, in 45 per cent of all the cultures from normal individuals these organisms were estimated as forming 1-5 per cent of the total flora. Reference to the remainder of the table shows that there was no tendency for these streptococci to occur in greater abundance in the throats of patients with rheumatic fever than those of other patients. In fact, some of the highest percentages recorded were found in persons free from any taint of rheumatism, while, conversely, it was several times observed that during very acute relapses of this disease there was a tendency for the plates to be crowded with *Streptococcus viridans*, largely to the exclusion of other types of organism.

In Table IV the total number of strains studied from each group of individuals is analyzed with respect to their serological characteristics (4).

In view of the fact that only one or two strains from each positive culture were studied, so that the element of chance played a large part, there is a remarkable agreement between the percentages for the various groups of persons. The probability that an indifferent streptococcus chosen at random from a throat culture will belong to Type I or will fall into the heterogeneous Group X is apparently independent of the condition of health of the individual from whom the culture is taken. In other words, there is no greater tendency for the organisms of Type I to be found more frequently in the throats of one group of individuals than in those of another, and in rheumatic fever there is no tendency for the incidence of this type to vary in either direction.

DISCUSSION.

The isolation of organisms of various types from lesions of rheumatic fever has been a more or less frequent occurrence. Many of the reports are open to grave suspicion of error in technique or of interpretation. The best accredited results are those in which streptococci of various types have been found. Never, however, has it been possible to secure experimental evidence that these organisms are directly concerned in the production of the disease. Their injection into laboratory animals has never been followed by the development of lesions closely resembling those of rheumatism. The pathological findings in the joints have been usually those of a proliferative and destructive arthritis (5, 6) while in the heart vegetative endocarditis has been present, together with the Bracht-Wächter bodies (7) in the myocardium. This has been the case regardless of whether the streptococci were of the indifferent or *viridans* type. Recent investigations (8, 9) have suggested that the failure of all these attempts may not mean that the bacterium used was unassociated with rheumatic fever, but that the error has been one of method. That is, the preliminary induction of the necessary tissue allergy in the experimental animal may be the *sine qua non* which hitherto has been neglected. Given the proper condition of tissue sensitization any streptococcus possessed

of the requisite allergizing capacity may be able to give rise to the peculiar condition called rheumatism.

The most recent organism to be ascribed a specific etiological rôle in rheumatic fever has been the indifferent streptococcus. The first object of this present investigation was to determine the extent of the distribution of these organisms among selected samples of the population and the percentage of the total flora of the throat which they formed. In spite of the fact that bacteriologists have been familiar with these organisms for decades, no such study has to our knowledge ever been carried out. With these fundamental facts established, it was then proposed to compare the flora of the throats of a number of groups of individuals, to determine whether in the case of patients suffering from rheumatic fever they occurred in greater incidence or in higher percentage. Such was not the case. There was found a slightly higher incidence of indifferent streptococci in the throats of hospital patients than in the throats of normal persons; but from the present study it is apparent that these bacteria are no more widely distributed among patients suffering from rheumatic fever than among other groups of patients. In the majority of throats they are present in approximately the same percentage relative to the total flora. Brown (10) has observed that in inflammatory conditions of the throat they tend to be overgrown by other organisms, notably the hemolytic streptococcus; and in a few cases of acute rheumatic fever included in the above tables there has been a tendency for *Streptococcus viridans* to displace them in similar fashion. Certainly such findings are not in harmony with the claim that these organisms are the sole cause of the disease.

Future investigation will probably determine the rôle which the indifferent streptococci actually play in the disease. Doubtless they must be considered along with the other types of streptococci in any comprehensive theory of its etiology. That they are not simple saprophytes is suggested by the facts that occasionally they may be the cause of bacterial endocarditis (11), that they are often efficient allergizing agents (12), and that they possess the capacity of yielding "toxic" filtrates (2, 13, 14). Therein they do not differ from many strains of *Streptococcus viridans*, and consequently are no more entitled to special consideration than are the latter.

It may be objected that the cultural and serological types here reported are not exclusively those used by Small (1) in the preparation of his SCA antiserum, and hence the validity of these conclusions may be questioned. In this connection attention should be directed to the fact that among the strains investigated in these two studies there were RF 1 recovered by Birkhaug from blood cultures of a rheumatic fever patient and Q 88 E and Q 97 EA similarly recovered by us from patients with the active disease. All of these fell into Type I. The R 1 strain and R 9 strain recovered by Small differ from each other and are also heterogeneous to Type I. While these strains are closely related as judged from their effect on blood agar, still their serological heterogeneity casts the same doubt on their specific etiologic rôle as was cast on *Streptococcus viridans* and for the same reason. We feel, therefore, that indifferent streptococci play a rôle in the production of this disease similar to that exerted by the green-forming streptococci.

SUMMARY AND CONCLUSIONS.

Indifferent streptococci occur in comparatively the same abundance in the throats of patients suffering from rheumatic fever or early in convalescence from the disease as they do in those who have recovered from the disease, or in those of patients suffering from other diseases.

There is a slightly increased incidence of these microorganisms in the throats of hospital patients as compared with those of normal individuals.

Type I occurs with comparatively equal frequency and abundance in the throats of all four classes of individuals studied.

BIBLIOGRAPHY.

1. Small, J. C., *Am. J. Med. Sc.*, 1927, clxxiii, 101; 1928, clxxv, 638, 650.
2. Birkhaug, K. E., *J. Infect. Dis.*, 1927, xl, 549.
3. Menzer, A., *Z. klin. Med.*, 1902, xlvii, 109.
4. Hitchcock, C. H., *J. Exp. Med.*, 1928, xlviii, 393.
5. Thalhimer, W., and Rothschild, M. A., *J. Exp. Med.*, 1914, xix, 444.
6. Cecil, R. L., *J. Exp. Med.*, 1916, xxiv, 739.
7. Bracht, E., and Wächter, *Deutsch. Arch. klin. Med.*, 1909, xcvi, 493.
8. Swift, H. F., Derick, C. L., and Hitchcock, C. H., *J. Am. Med. Assn.*, 1928, xc, 906.

9. Swift, H. F., Derick, C. L., and Hitchcock, C. H., *Tr. Assn. Am. Phys.*, 1928, xliii, in press.
10. Brown, J. H., The use of blood agar for the study of streptococci, Monograph of The Rockefeller Institute for Medical Research, No. 9, New York, 1919.
11. Kinsella, R. A., *Arch. Int. Med.*, 1917, xix, 367.
12. Hitchcock, C. H., and Swift, H. F., *J. Exp. Med.*, in press.
13. Kaiser, A. D., *J. Infect. Dis.*, 1928, xlii, 25.
14. Swift, H. F., Wilson, M. G., and Todd, E. W., personal communication.