THE RELATION OF CERTAIN CARRIER CONDITIONS TO
THE VIRULENCE OF HEMOLYTIC STREPTOCOCCI.*

By LEON C. HAVENS, M.D.,

*Associate in Immunology, School of Hygiene and Public Health, Johns
Hopkins University*

AND

MARGARET L. TAYLOR, M.A.,

*Assistant in Preventive Medicine and Hygiene, University of Iowa,
School of Medicine.*

(Received for publication October 25th, 1920.)

It has been demonstrated by a number of observers that the hemolytic streptococcus is a common inhabitant of the tonsils, especially of the crypts. Smillie (1917) found a large percentage of healthy carriers in a boys' school during an epidemic of sore throat. During the past few years numerous instances of a high incidence of healthy carriage of this organism in army camps have been reported. Nichols and Bryan (1918) report observations on the treatment of chronic carriers. They state that inasmuch as the hemolytic streptococcus appears to have a special predilection for the crypts of the tonsils rather than the surfaces of these and neighboring structures, it is especially difficult to treat carriers of this organism successfully. Their conclusion is that extirpation is the only satisfactory method. Davis (1920) states that the crypts are an almost constant source of hemolytic streptococci.

Studies of extirpated tonsils thus have added to our knowledge of carrier conditions. Since the crypts, in a large number of instances, contain hemolytic streptococci among their apparently normal flora, cultures of the tonsil surfaces and the pharynx, unless carefully made, will fail to reveal true carriers of this organism. Also it consequently appears that carriers of this organism are more common than formerly supposed.

* From the Department of Immunology, School of Hygiene and Public Health, Johns Hopkins University, and the Division of Preventive Medicine and Hygiene, State University of Iowa.

Altho reports of the incidence of carriers show these facts, studies of the relative virulence of the organisms carried and the relation of the acuteness or chronicity of the carrier state to the virulence of the strain harbored have not attracted much attention. It is a commonly observed fact that as the number of healthy carriers of any pathogenic organism increases in a group or community of individuals an epidemic is to be feared. This has been explained not only for the reason that there has been an increased opportunity for infection but that the virulence has been increased by more or less rapid passage from one individual to another.

The results of this report embody experiments dealing with the relative virulence for mice of strains of hemolytic streptococci obtained from different groups of healthy carriers, strains obtained from excised tonsils and strains from acute respiratory infections.

SOURCE OF CULTURES.

Cultures were obtained from the following groups: (1) chronic carriers, i.e., individuals who had yielded positive cultures of hemolytic streptococcus from their tonsils or pharynx at different intervals for a period of at least three months; (2) early carriers, individuals who had harbored the organisms for only a few days or weeks, and without history of prior infection; (3) cases of incipient acute bronchitis, laryngitis or tonsillitis; (4) cultures from tonsils removed several months following an attack of tonsillitis.

TECHNIC.

In obtaining the first three groups of cultures, the tonsils and pharynx were swabbed and the material streaked on blood agar plates, in the usual manner. Colonies were picked after 18-24 hours incubation and transferred to blood agar slants. In obtaining cultures from the extirpated tonsils cultures were made immediately after removal, both of the surfaces and of the crypts. To obtain the latter the tonsil was cut in half with sterile instruments and material obtained from several crypts. Both tonsils from each case were cultured in this way. It was usually found that the results were the same; occasionally, however, one tonsil yielded hemolytic streptococcus while the other would be negative.

For the determination of the virulence of a given strain the first blood agar slant culture, obtained from the streaked plate, was used.

One fifth of an 18-24-hour growth of such a slant, suspended in salt solution, was injected intraperitoneally into a mouse. Using an amount of culture of this size, the strain was not considered virulent unless the mouse died in 24 hours.

The number of positive cultures obtained from the extirpated tonsils, three to six months following the acute inflammation, is interesting. As is seen from Table I 96 per cent. of the 75 cases gave positive cultures, a finding in keeping with previous results (3). Furthermore, in over 93 per cent., in all but five of the seventy-five, hemolytic streptococci were present in large numbers, predominating over other organisms present. In two of the negative cases a hemolytic staphylococcus was present as the predominating organism.

TABLE I.

Hemolytic streptococci from tonsils removed three to six months following acute attack.

		Per Cent.
Exterior surface predominant	3	4.0
Crypts predominant	31	41.3
Approximately equal numbers—crypts and surface	36	48.0
Present in small numbers (a few colonies only)	2	2.6
Negative	3	4.0
Total	75	

VIRULENCE TESTS.

The following table shows the results of the virulence tests with the different strains obtained.

TABLE II.

Virulence tests.

	Virulent.		Non-virulent.		Total Strains.
	No.	Per cent.	No.	Per cent.	
Early carrier cultures	3	12.5	21	87.5	24
Chronic carrier cultures	4	10.0	37	90.0	41
Extirpated tonsils (3-6 months after acute attack)	7	14.5	41	85.5	48
Acute respiratory infections (tonsillitis, laryngitis, bronchitis)	19	86.4	3	13.6	22

DISCUSSION.

The difference in virulence for mice between healthy carrier cultures and strains obtained during an acute infection with hemolytic

streptococci, as shown in Table II, is marked. While no conclusions concerning virulence for one species can be drawn from evidence of virulence for another species of animal, yet the parallelism between these groups is sufficiently striking to be worthy of consideration. In view of the size of the inoculation it is interesting to find that only a small minority of the healthy carriers harbored strains that were at all virulent for mice, while in the group of cultures obtained from actual infections, the converse was true.

No differences in the virulence for mice of the strains obtained from early and from chronic carriers were observed. The majority of the early carriers in this particular group were associated with the carriers of longer duration and doubtless harbored much the same strains. Furthermore this group contains only individuals who gave no history of clinical infection. However, a comparison of the two groups seems to point to the fact that the duration of a strain in a healthy normal throat has little to do with its virulence as evidenced by mouse inoculation. This seems to be true for healthy throats.

A comparison of the group of strains obtained from acute respiratory infections with those from removed tonsils shows a general difference in the comparative virulence of each group for mice. The majority of the organisms isolated from the inflamed throats and tonsils, i.e., at the time when there is actual infection, were virulent for mice, usually in much smaller doses than the amount, one fifth of a culture, used in the tests. Yet after the infection has been overcome and the carrier state ensues, a marked decrease in the virulence of the strain apparently takes place. It would seem that during the acute infection, while the host is combatting the organisms, the latter, at least those successful in resisting for a time the defensive mechanism exerted by the host, develop an increased invasive power. Later, when the infection has been overcome, the organisms are living on the surface of the normal mucus membranes in a state of symbiosis, doing no harm, because, in the present state of the host's resistance, actual invasion is impossible. Under these conditions the invasive power—the virulence—of the organisms diminishes.

A further point of interest is brought out by Table I. This table seems to show that a high percentage of cases of tonsillitis become chronic carriers of hemolytic streptococci. Removal of the tonsils seems to be the only way to treat such cases, since the organisms occur deep in the crypts and usually in large numbers. While these strains apparently decrease rapidly in virulence for the individual

who harbors them and for mice, it is impossible to assume that these carriers are, therefore, harmless to others.

The decrease in virulence following an attack of tonsillitis may be a partial explanation of the small number of generalized infections following removal of the tonsils from three to six months after the acute attack, as compared with the higher incidence when they are removed soon after the actual infection. The organisms are still present but not only has the individual developed increased resistance; the bacteria, as well, have diminished in invasive power.

SUMMARY.

A low degree of virulence of hemolytic streptococci for mice is found to occur in cultures taken from healthy carriers, as compared with those from cases of actual infection.

No differences in virulence have been observed between strains from early and from late carrier states. The duration of a strain in a healthy throat seems to have little to do with its virulence.

A rather rapid decrease in virulence seems to occur after the acute infection has ended. Chronic carriers of hemolytic streptococci as a result of an attack of tonsillitis show a very small percentage of virulent strains.

A large percentage of cases of acute tonsillitis become chronic carriers of hemolytic streptococci, as evidenced by cultures of tonsils removed three to six months following the acute attack.

BIBLIOGRAPHY.

1. DAVIS, D. J.
1920. The tonsil in relation to infectious processes. Journ. A. M. A., LXXIV, 317.
2. NICHOLS, H. J. AND BRYAN, J. H.
1918. The tonsils as foci of infection in *Streptococcus hemolyticus* carriers. Journ. A. M. A., LXXI, 1813.
3. SMILLIE, W. G.
1917. Studies of the beta hemolytic streptococcus. Journ. Inf. Dis., XX, 45.