SYNTHESIS OF A POLYSACCHARIDE FROM SUCROSE BY STREPTOCOCCUS S.B.E.

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The identity of 113 cultures of streptococci recovered from cases of subacute bacterial endocarditis has been reported recently (Niven and White, 1946). Of this number, 42 seemed to represent a hitherto unrecognized variety, or species, which has been tentatively labeled *Streptococcus* s.b.e. In contrast with other streptococci associated with endocarditis those cases caused by *Streptococcus* s.b.e. respond sluggishly, or not at all, to Loewe's penicillin-anticoagulant therapy (Loewe, 1945*a*, 1945*b*; Loewe, Plummer, Niven, and Sherman, 1946).

Since prompt identification of *Streptococcus* s.b.e. may be a lifesaving measure, adequate methods for accomplishing this would be desirable. A detailed description of this streptococcus is to be given in a forthcoming publication. In the present report a unique physiological characteristic of *Streptococcus* s.b.e. will be described, namely, the ability to synthesize a polysaccharide from sucrose in broth culture.

METHODS AND RESULTS

In determining the fermentation characteristics of our collection of streptococci from endocarditis (White, 1944) it was noticed that some of the cultures when grown in a 1 per cent sucrose broth appeared to become slightly viscous. Practically all strains having this property were identified as *Streptococcus* s.b.e. In line with this suggestion, a large part of the collection has been restudied with respect to polysaccharide synthesis from sucrose.

The ability to form large, mucoid colonies on a 5 per cent sucrose agar (Niven, Smiley, and Sherman, 1941*a*) has become a convenient and accurate presumptive test for the identification of *Streptococcus salivarius*. As opposed to the picture presented by *Streptococcus salivarius*, none of the *Streptococcus* s.b.e. showed any evidence of mucoid colonies when streak cultures were observed on this medium after 24 hours' incubation at 37 C. However, after the incubation was continued for 3 additional days, 24 cultures showed slight evidence of polysaccharide synthesis. The individual colonies appeared very much like minute, clear-glass beads scattered over the surface of the plate. On the thickly seeded portion of the plate the mucoid appearance was somewhat more evident. The mucoid appearance upon the plate culture, however, could be easily missed by a casual observer. Eight of the *Streptococcus* s.b.e. cultures showed no evidence of polysaccharide synthesis upon continued incubation.

The cultures were then tested in a sucrose broth having the following composition: 1 per cent tryptone, 0.5 per cent yeast extract, 0.5 per cent K_2HPO_4, and 5.0 per cent sucrose; pH 7.4. The results obtained in this medium were striking.

After an incubation period of 3 days at 37 C, 31 of the 34 *Streptococcus* s.b.e. cultures tested showed a marked increase in the viscosity of the broth (table 1). Nine of the cultures actually solidified the medium, these cultures having the consistency of about a 1 per cent agar. The viscous nature of those cultures which did not solidify the medium could be easily determined by agitating the contents of the tubes so as to incorporate a few small air bubbles. When the agitation ceased, there was an immediate arrest in the motion of the bubbles and the suspension of bacterial cells. Upon continued incubation, there was little or no tendency for the cells to settle out of the sucrose medium, whereas the same strains grown in a 5 per cent glucose broth would tend to settle out leaving a clear supernatant.

The three strains which failed to synthesize the polysaccharide grew poorly in the sucrose medium. It is assumed that the reason for failure with these cultures was an insufficient medium for optimum growth. These strains, two of which had been isolated from one patient, had always grown poorly in laboratory media. When tested again in a medium containing only 3 per cent sucrose and fortified

TABLE 1

Polysaccharide synthesis from sucrose by endocarditis cultures

SPECIES OR VARIETY	CULTURES TESTED	CULTURES SHOWING CARBOHYDRATE SYNTHESIS	
		Sucrose agar streaks	Sucrose broth
Streptococcus s.b.e.	34	24 (slight)	32
Streptococcus mitis	29	0	2
Streptococcus bovis	7	7	7
Miscellaneous	18	0	0

with beef infusion, one of the three strains showed marked evidence of polysaccharide synthesis.

Among the collection of streptococci tested in the sucrose broth were seven cultures which had been previously identified as *Streptococcus bovis*. All these strains produced mucoid colonies on sucrose agar similar to certain members of this species from bovine sources (Niven, Smiley, and Sherman, 1941*b*). In the 5 per cent sucrose broth these cultures also synthesized a polysaccharide as evidenced by a marked increase in the turbidity of the culture medium, with a slight increase in viscosity. Only one strain, however, increased the viscosity to such an extent that it could not be distinguished from *Streptococcus* s.b.e. The *Streptococcus bovis* cultures from endocarditis were of the "indifferent" type on blood agar, in addition to having several other physiological characteristics which would afford easy differentiation from *Streptococcus* s.b.e.

Of the 29 strains of *Streptococcus mitis* cultures from endocarditis tested, none showed any evidence of mucoid colony production on sucrose agar, but two strains were found which appeared to be identical to *Streptococcus* s.b.e. in sucrose broth. In contrast to *Streptococcus* s.b.e., these strains fermented raffinose, but

not inulin, and failed to produce ammonia from arginine. They could also be differentiated from *Streptococcus* s.b.e. by serological methods.

Of the remaining 18 streptococcus cultures from endocarditis tested, none was found to show any evidence of polysaccharide synthesis from sucrose, either on the agar plate or in broth culture. In this group were cultures which had been identified as *Streptococcus agalactiae*, *Streptococcus faecalis*, Lancefield group G (non-minute variety), and four strains which could not be identified by either physiological or serological methods.

POLYSACCHARIDE SYNTHESIS FROM OTHER SUGARS

Three strains of *Streptococcus* s.b.e., one *Streptococcus bovis*, and the two polysaccharide-synthesizing cultures of *Streptococcus mitis* were inoculated into broth media containing 5 per cent each of all the various sugars, polysaccharides, and higher alcohols commonly used in fermentation tests. Also included was a broth containing both glucose and fructose at a level of 2.5 per cent each. There was no evidence of polysaccharide synthesis by any of the strains on any of the test substances except sucrose.

Of interest is the fact that none of the strains synthesized visual quantities of polysaccharide from raffinose. Although *Streptococcus* s.b.e. does not characteristically ferment this sugar, one strain was included which possessed this property. The three *Streptococcus bovis* and the two *Streptococcus mitis* cultures included were able to ferment raffinose. In contrast to these findings, all *Streptococcus salivarius* strains from the human throat are able to synthesize a levan from both sucrose and raffinose (Niven, Smiley, and Sherman, 1941*a*).

One strain of *Streptococcus* s.b.e. was tested for its ability to synthesize a polysaccharide from sucrose in cell suspension. The cells were grown for 18 hours in 0.3 per cent sucrose broth, then centrifuged out, washed once, and concentrated tenfold in a phosphate-buffered, 5 per cent sucrose solution, pH 7.4. The suspension was incubated at 37 C and neutralized occasionally with strong NaOH. Within 8 hours the contents of the flask had solidified.

PHYSICAL AND CHEMICAL PROPERTIES OF THE POLYSACCHARIDE

Two liters of the 5 per cent sucrose medium were inoculated with a strain of *Streptococcus* s.b.e. and incubated at 37 C. At occasional intervals the acid produced was neutralized with strong NaOH. When acid production ceased (in 48 hours), the flask was steamed for 30 minutes to kill the cells. Ethyl alcohol was added to the semisolid medium, with vigorous agitation, to approximately 50 per cent concentration. At this stage a large quantity of gelatinous precipitate settled out of the medium. The precipitate was washed several times in 50 per cent alcohol and then resuspended in water. The precipitation procedure was repeated three times, after which the precipitate was suspended in water and dialyzed for 24 hours against running tap water.

The suspension was then precipitated with excess alcohol, washed twice with 95 per cent alcohol, and then spread out to dry for 24 hours at 110 C. Even though no attempts were made to recover the polysaccharide quantitatively,

21 grams of the dried substance were obtained from the 100 grams of added sucrose in the original medium.

This dried substance appeared to be a mixture of two polysaccharides, one fraction (estimated to be about 10 per cent) being soluble in water. The water-soluble component was removed by suspending the dried material in water with mechanical stirring, followed by centrifuging. The operation was repeated until no alcohol-precipitable material was found in the supernatant. No attempts were made to identify the water-soluble fraction.

Even though it is insoluble in water, the larger fraction is characterized by its marked ability to imbibe water, resulting in a viscous suspension at a 1 per cent concentration. It can be centrifuged out easily to a thick, gelatinous mass, or can be precipitated out with 50 per cent alcohol. In normal HCl it goes into a turbid colloidal suspension with loss in viscosity. It is soluble in normal NaOH. There was found to be 0.1 per cent nitrogen and 0.5 per cent ash in the purified and dried material.

The polysaccharide was difficult to hydrolyze. A 2 per cent suspension in normal HCl heated for 4 hours at 100 C did not result in complete hydrolysis, as judged by the rate of increase in reducing the sugar content of aliquots taken at 30-minute intervals. At the end of the heating period 83 per cent of the carbohydrate could be accounted for as reducing sugars (calculated as glucose). The optical activity of the clarified hydrolyzate indicated 84 per cent glucose. Therefore, it was tentatively concluded that the insoluble polysaccharide produced by the *Streptococcus* s.b.e. strain was a dextran.

DISCUSSION

As this report was being prepared, our attention was called to a recent article by Hehre and Neill (1946) concerning the production of a dextran from sucrose by 22 of their 45 cultures of streptococci isolated from cases of subacute bacterial endocarditis. From the description given by the authors it would appear highly probable that most, if not all, of their dextran-producing cultures are identical with *Streptococcus* s.b.e. Our tentative identification of the polysaccharide as a dextran is in entire conformity with their results employing serological methods.

An interesting observation reported by Hehre and Neill is that, although very little or no dextran is synthesized by their cultures on sucrose agar incubated aerobically, all strains produced mucoid colonies on the same agar under anaerobic conditions. We have not tested our cultures in this manner.

Assuming that the cultures of Hehre and Neill are identical with *Streptococcus* s.b.e., it is interesting to note that this organism has been found to occur in a large proportion of the cases of endocarditis, as determined independently in two different laboratories. As will be pointed out in a future publication, this streptococcus (with one exception) has not been encountered from sources other than cases of endocarditis.

Because of the relatively large proportion of fatal cases due to this organism, it might at times be highly desirable to recognize this organism during the early

stages of the disease. The production of a dextran in sucrose broth (or perhaps on sucrose agar plates incubated anaerobically) might prove to be a valuable presumptive test.

Although *Streptococcus salivarius* was not encountered in our collection of endocarditis cultures (nor were there any in the collection of Hehre and Neill), it may at times be necessary to differentiate between these two groups of streptococci. Although there are many other physiological differences which could be used, this can be accomplished easily by merely observing the types of colonies on sucrose agar and the changes taking place in sucrose broth. *Streptococcus salivarius* produces large mucoid colonies on sucrose agar within 24 hours (aerobically), and in sucrose broth a bluish opalescence is developed in the supernatant with no apparent increase in viscosity. Some cultures of *Streptococcus salivarius* also synthesize an insoluble dextran from sucrose (Niven, Smiley, and Sherman, 1941*b*), but this material settles to the bottom of the tube in a flocculent mass along with the bulk of the bacterial cells.

Since a few cultures of *Streptococcus bovis* and *Streptococcus mitis* which produce a viscous dextran in sucrose broth may also be encountered in endocarditis cases, this test cannot be considered a perfect one. Other physiological or serological procedures would also have to be used in order to identify *Streptococcus* s.b.e. positively.

Since some strains of *Streptococcus* s.b.e. solidify the sucrose broth, whereas others merely increase the viscosity of the medium, it might be concluded that these organisms are synthesizing different types of polysaccharides. It is felt, however, that this is merely a quantitative effect; those strains which solidify the broth may be synthesizing larger quantities of the same polysaccharide.

SUMMARY

Thirty-two of the 34 strains of *Streptococcus* s.b.e. recovered from cases of subacute bacterial endocarditis synthesized large quantities of a polysaccharide from sucrose in broth culture, as determined by an increase in viscosity or by actual solidification of the medium. These cultures synthesized little or no polysaccharide when streaked on sucrose agar.

This unique property may be helpful in the identification of *Streptococcus* s.b.e., such cultures having been recovered from approximately one-third of the cases studied.

The polysaccharide has been tentatively identified as a dextran.

REFERENCES

HEHRE, EDWARD J., AND NEILL, JAMES M. 1946 Formation of serologically reactive dextrans by streptococci from subacute bacterial endocarditis. J. Exptl. Med., **83**, 147–162.

LOEWE, LEO. 1945*a* The combined use of penicillin and heparin in the treatment of subacute bacterial endocarditis. Can. Med. Assoc. J., **52**, 1–14.

LOEWE, LEO, 1945*b* The combined use of anti-infectives and anticoagulants in the treatment of subacute bacterial endocarditis. Bull. N. Y. Acad. Med., **21**, 59–86.

LOEWE, LEO, PLUMMER, NORMAN, NIVEN, C. F., JR., AND SHERMAN, J. M. 1946 *Streptococcus*, s.b.e.: A hitherto undescribed variety of non-hemolytic streptococcus recovered from patients with subacute bacterial endocarditis. J. Am. Med. Assoc., **130**, 257.

NIVEN, C. F., JR., SMILEY, K. L., AND SHERMAN, J. M. 1941*a* The production of large amounts of a polysaccharide by *Streptococcus salivarius*. J. Bact., **41**, 479–484.

NIVEN, C. F., JR., SMILEY, K. L., AND SHERMAN, J. M. 1941*b* The polysaccharides synthesized by *Streptococcus salivarius* and *Streptococcus bovis*. J. Biol. Chem., **140**, 105–109.

NIVEN, C. F., JR., AND WHITE, J. C. 1946 A study of streptococci associated with subacute bacterial endocarditis. J. Bact., **51**, 790.

WHITE, J. C. 1944 Streptococci from subacute bacterial endocarditis. **Thesis.** Cornell University.