fectious agent prompted a chemical examination of the blood. Analyses for calcium, inorganic phosphorus, and ketone bodies revealed values within the normal range for these constituents. However, abnormal amounts of blood sugar have been consistently encountered.† The sugar level ranged from approximately 3 to 61 mg per 100 cc. In one series of 20 affected pigs from 7 different litters, an average of 26 mg of sugar per 100 cc of blood was found. The blood sugar level in normal pigs of the same age ranged from 99 to 131 mg per 100 cc with an average of 113 mg.

A possible prenatal influence on this pathologic condition in newly-born pigs is obviously suggested; but until the underlying cause or causes are established, the cryptogenic nature of the acute hypoglycemia is recognized. Contributory evidence in support of the possible primary significance of acute hypoglycemia has been observed in the therapeutic response in naturally affected pigs following repeated injections of glucose solution. Pigs in the early stage of the disease show improvement in 2 to 3 hours following dextrose therapy, while repeated injections of dextrose together with forced feeding of milk have demonstrated that the life of naturally affected pigs may be prolonged and that in some cases the treated pigs may recover. However, glucose therapy even if repeated appears ineffective in the terminal stages of the disease.

13132

Influence of Sucrose upon Production of Serologically Reactive Material by Certain Streptococci.

JAMES M. NEILL, JOHN Y. SUGG, EDWARD J. HEHRE AND EVELYN JAFFE.

*From the Department of Bacteriology and Immunology, Cornell University Medical College, New York.**

This paper deals with the potential capacity of certain streptococci from man to produce material reactive with types 2 and 20 antipneumococcus and with antileuconostoc serums. The point of major interest is the influence of sucrose upon the production of the reactive material.

† The blood sugar was determined by the Shaffer-Hartmann-Somogyi method according to the technic of Koch. (Koch, F. C., *Practical Methods in Biochemistry*, Williams and Wilkins, Baltimore, 1934.)

* Aid was received by a grant from the Ruth B. Ettinger Fund.

It has long been known that some bacteria, especially *Leuconostoc mesenteroides,* produce abundant amounts of polysaccharide when grown in media containing sucrose but produce little or none when other common carbohydrates are substituted for sucrose. We[1] have found that the leuconostoc polysaccharides (dextrans) are reactive not only with antiserums of leuconostoc but also with antiserums of types 2 and 20 (and sometimes also of type 12) pneumococci. This newer evidence showed that the influence of sucrose upon the formation of the leuconostoc dextrans actually represented an example of the influence of a particular carbohydrate constituent of the culture medium upon the capacity of a microörganism to elaborate a serologically reactive polysaccharide. A study was then made to determine if the same or a similar phenomenon occurred among other sucrose-fermenting bacteria particularly among streptococci.

That some streptococci from man produce large amounts of "slime" or gum-like material when grown on sucrose media is an old observation. Hlava[2] isolated streptococci from patients with scarlet fever and other infections which were so similar to the common leuconostoc from plants in respect to mucoid colonies on sucrose and non-mucoid colonies on glucose media that he called them *Leuconostoc hominis.* Later Oerskov and associates[3] found that normal throats also had streptococci that produced large amounts of gum from sucrose or raffinose but not from other common carbohydrates. Recently Sherman and his associates[4] have reported that some members of the *Streptococcus salvarius* group possess the property of forming mucoid colonies on sucrose but not on glucose agar; their data on the serological properties of the polysaccharide formed by those streptococci have not yet been published. In addition to these reports there probably have been a number of unreported observations of the influence of sucrose upon the mucoid appearance of streptococcus cultures.

For the present study a collection of streptococci principally of human origin and a number of other sucrose-fermenting bacteria were tested. The streptococcus strains consisted of 30 group *A,* 5 *B,* 3 *C,* 8 *D,* 2 *E,* 5 *F,* 10 *G,* 8 *H,* 7 *K,* 2 "indifferent" strains which would be classified as *Strep. salivarius,* and 3 strains of *Strep. lactis* which were obtained through the courtesy of Dr. Sherman; most of

[1] Sugg, J. Y., and Hehre, E. J., in press.

[2] Hlava, J., *C. Bakt.,* 1902, **32**, 263.

[3] Oerskov, J., and Poulsen, K. A., *Z. Bakt. I. Orig.,* 1931, **120**, 125; Koch, F. E., *Z. Bakt. I. Orig.,* 1934, **130**, 381.

[4] Niven, C. F., Smiley, K. L., and Sherman, J. M., *J. Bact.,* 1941, **41**, 479.

the other streptococci were kindly supplied by Dr. Lancefield; all except 4 of group *D* fermented sucrose with acid production. The other bacteria tested were 5 strains of lactobacilli from plants, 5 of Friedlander *A, B,* and *C;* and one each of aerogenes (*A* 11), coli communior, proteus, and a spore-forming bacillus. For comparison with the streptococci the data on the serological properties of a collection of 28 strains of leuconostoc are included. These strains had been isolated from various plants obtained from diverse geographical sources; their mutual properties of fermenting sucrose and pentoses might justify the term *Leuconostoc mesenteroides*[5] for all of them. The chemical and serological properties of the leuconostoc dextrans have been described in another paper.[1] (In view of the serological relationship which will be shown to exist between them it should be noted that the leuconostoc strains can be clearly differentiated from the group *H* streptococci on the basis of other properties, among which are the failure of any of the group *H* to ferment arabinose or xylose and the higher optimum temperature for growth of the streptococci.)

Equal amounts of each of the strains were inoculated into 2 tubes of medium consisting of 2% Neopeptone, 0.5% sodium chloride, and 0.2% anhydrous disodium phosphate plus 1% dextrose or 1% sucrose. After 5 days of incubation the cultures were centrifuged and the supernatant fluids after being neutralized and heated at 70°C for 30 minutes, were used as antigens. Each antigen was tested in 1:10 and 1:100 dilutions against 1:10 dilutions of types 2, 20, 12 and 1 antipneumococcus serums and against the antiserums of two strains of *Leuconostoc mesenteroides* which are designated *A* and *B* in the protocol; the fluids which reacted in the 1:100 dilution were tested in 1:1,000 and 1:10,000 dilution. The pneumococcus serums had been produced by immunization of rabbits with bacteria grown in meat infusion peptone broth without added carbohydrate; the leuconostoc serums with bacteria grown in media containing sucrose. The results are summarized in Table I.

It is evident (Table I) that when grown in sucrose broth all of the leuconostoc and 7 of the 8 group *H* streptococci produced large amounts of material which was reactive with the antiserums of types 2 and 20 pneumococci and of the two strains of leuconostoc; the material produced by 4 of the leuconostoc strains had the additional property of reacting with type 12. In contrast in the culture mediums in which dextrose was substituted for sucrose, none of the

[5] Hucker, G. J., and Pederson, C. S., in *Bergey's Manual of Determinative Bacteriology,* Baltimore, 1939, p. 359.

leuconostoc or group *H* streptococci produced detectable amounts of the reactive material. That the capacity of elaborating it from sucrose is not as frequent among all groups of streptococci as among the group *H* variety is suggested by the negative results obtained with the strains of other groups which were tested. A more comprehensive collection will be studied in order to get better information on its occurrence among streptococci.

The group *H* strains which reacted had been isolated from throats; six were originally from Dr. Hare's[6] collection; one had been isolated by Dr. Julia M. Coffey in the laboratories of the New York State Department of Health. All of these strains were as non-reactive when grown in maltose or lactose broth as when grown in dextrose broth.

In order to distinguish between the antigen involved in the usual Lancefield grouping test and the antigens involved in the described reactions of the sucrose cultures of group *H* streptococci the following experiment was made. Two of the strains of group *H* streptococci which had reacted with the pneumococcus and leuconostoc antiserums were grown in meat infusion peptone broth containing 1% dextrose and in the same medium containing 1% sucrose. The 18-hour-old cultures were centrifuged at high speed and the supernatant fluids removed as completely as possible from the sedimented bacteria. The bacterial cells were then washed with physiological salt solution and recentrifuged. An HCl-extract of the washed cells was then prepared by the usual Lancefield[7] procedure. The supernatant fluids and the HCl-extract of the washed cells obtained from the dextrose and the sucrose cultures of the two strains were tested in a series of dilutions against 1:10 dilutions of the following rabbit antiserums: Group *H* streptococcus (which was kindly supplied by Dr. Lancefield), leuconostoc *(A* and *B)* and types 2, 20, 12 and 1 pneumococcus; the streptococci used for the immunization had been grown in broth containing dextrose, the leuconostoc in broth or agar containing sucrose and the pneumococci in broth containing no added carbohydrate. The results are in Table II.

The chief point in the results (Table II) was that when tested against the streptococcus *H* antiserum both the supernatant fluids and the HCl-extracts obtained from the dextrose both cultures reacted in as high dilution as did the corresponding materials obtained from the sucrose broth cultures. This point indicates that the antigens reactive with the group *H* antiserum are different from

[6] Hare, R., *J. Path. Bact.*, 1935, **41**, 499.

[7] Lancefield, R. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 473.

TABLE I.

Serological Reactions of the Supernatant Fluids of Sucrose and of Dextrose Broth Cultures.

Kind of bacteria	No. of strains	Sucrose cultures *vs.* the antiserums: Antipneumococcus 2	Antipneumococcus 20	Antipneumococcus 12	Antipneumococcus 1	Antileuconostoc A	Antileuconostoc B	Dextrose cultures *vs.* the same antiserums
Leuconostoc mesenteroides (28)	4	+++	+++	+++	0	+++	+++	0
	24	+++	+++	0	0	+++	+++	0
Streptococcus Lancefield *H* (8)	7	+++	+++	0	0	+++	+++	0
	1	0	0	0	0	0	0	0
Streptococcus A(30), *B*(5), *C*(3), *D*(8), *E*(2), *F*(5), *G*(10), *K*(7)	70	0	0	0	0	0	0	0
Strep. salivarius (2), *lactis* (3)	5	0	0	0	0	0	0	0
Other bacteria	14	0	0	0	0	0	0	0

0, no precipitation with 1:10 or higher dilutions of the antigens.
+++, precipitation with 1:1,000 and in some instances 1:10,000 dilutions of the antigens.

TABLE II.

Comparison of Reactions of Streptococcus *H* "grouping" Serum with the Reactions of Types 2 and 20 Antipneumococcus and of Antileuconostoc Serums.

Antiserums	Supernatant fluids of cultures of group *H* streptococci: Sucrose cultures Strain 1	Sucrose cultures Strain 2	Dextrose cultures Strain 1	Dextrose cultures Strain 2	HCl-extract of group *H* streptococcus cells: Sucrose cultures Strain 1	Sucrose cultures Strain 2	Dextrose cultures Strain 1	Dextrose cultures Strain 2
Streptococcus *H* (strain 1)	++	+	++	+	+++	++±	+++	++±
Leuconostoc (strain A or B)	++++	++++	0	0	+++	+++	0	0
Pneumococcus type 2 or 20								
" " 1 or 12	0	0	0	0	0	0	0	0

0 = no precipitation with undiluted or diluted antigen.
+, ++, +++, ++++ = precipitation with undiluted, 1:10, 1:100 and 1:1,000 dilutions of the antigens.

those involved in the reactions of the pneumococcus and leuconostoc antiserums.

Another item of interest is the comparative amounts of the material reactive with the different serums which are contained in the supernatant fluids of the sucrose broth cultures. For example, the supernatant fluids of the 7 strains of the group *H* streptococci grown in that medium invariably reacted in 1:1,000 and often in 1:10,000 dilution against the pneumococcus and leuconostoc serums whereas in experiments made against group *H* antiserums none of the same fluids reacted in 1:100 dilution and the majority failed to react in 1:10 dilution.

A polysaccharide has been prepared from sucrose broth culture of one of the group *H* streptococci and although its study is not completed it has been proved to be a dextro-rotatory substance. Further investigation of the relationships of this polysaccharide to the leuconostoc dextrans[1] has been delayed until we obtain potent antiserums by immunization with group *H* streptococci grown in sucrose media.

Summary. When grown in sucrose broth some strains of group *H* streptococci produced large amounts of material reactive with types 2 and 20 antipneumococcus and with antileuconostoc serums; little or none was produced by the same streptococci when grown in dextrose broth. This reactive material was different from the streptococcus antigen involved in the usual Lancefield grouping test; as much of the latter was produced in dextrose as in sucrose broth culture.

In addition to indicating an interrelationship of the different Gram-positive cocci the data furnished an example of the influence of a particular carbohydrate upon the capacity of some microörganisms to elaborate a serologically reactive polysaccharide.

13133

A Selective Medium for the Isolation of *Streptococcus salivarius.*

KENNETH D. ROSE AND CARL E. GEORGI. (Introduced by H. G. O. Holck.)

From the Department of Bacteriology, University of Nebraska, Lincoln.

Restaurant eating utensils have long been recognized as potential vectors in the transmission of respiratory and related diseases. Extensive investigations have been reported by many workers con-