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.

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

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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-

cerning technics applicable to sanitary surveys of restaurants. Their results have consistently demonstrated the appearance of Gram-negative bacilli and Gram-positive rods and micrococci on washed glasses. Recent trends have been directed toward the use of an organism, normal to the human mouth, as an index for the study of oral pollution. For the detection of a specific bacterium in a mixed population occurring on washed glasses, an inhibitory medium is desirable if not essential. These investigations concern the development of a selective medium for the isolation of a specific oral streptococcus, the presence of which on drinking glasses may be used as an index of salivary contamination.

Fleming and Young[1] and Bornstein,[2] utilizing the inhibitory powers of potassium tellurite on Gram-negative bacteria, employed this salt in media used for the isolation of fecal streptococci. Dick and Hucker[3] demonstrated that a 1:400,000 concentration of crystal violet inhibited Gram-positive rods and micrococci in enrichment broths intended for primary isolations of streptococci from washed glasses. Niven, *et al.*,[4] reported mucoid colony formation by certain "typical" strains of *Streptococcus salivarius,* characterized by Safford, *et al.*,[5] when cultured on a substrate containing sucrose. Combining these 3 factors, a medium was developed which would effectively eliminate Gram-negative bacteria and Gram-positive rods and micrococci from mixed cultures, but permitting "typical" *Streptococcus salivarius* to grow with a characteristic gummy or mucoid colony.

Methods and Results. Twenty species of Gram-negative and Gram-positive organisms and 14 strains of *Streptococcus salivarius* were employed in these experiments. Plates of the basal substrate containing varying amounts of the inhibitory substances were streaked with 24-hour-old broth cultures and incubated for 48 hours at 37°C prior to observation. The concentrations of the inhibitory agents arrived at and yielding the more promising results were determined as 0.03% potassium tellurite and 1:500,000 crystal violet. These concentrations agree favorably with those used by Garrod.[6] The basic substrate used was a modification of that suggested by Safford, *et al.*,[5] and is as follows:

[1] Fleming, A., and Young, M. Y., *J. Path. and Bact.*, 1940, **51**, 29.

[2] Bornstein, S., *J. Bact.*, 1940, **39**, 383.

[3] Dick, L. A., and Hucker, G. J., *J. Milk Tech.*, 1940, **3**, 307.

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

[5] Safford, C. E., Sherman, J. M., and Hodge, H. M., *J. Bact.*, 1937, **33**, 263.

[6] Garrod, P., *St. Barth. Hosp. Rep.*, 1933, **66**, 203.

Proteose peptone (Difco)	5.0 g
Beef extract (Bacto)	3.0 "
Yeast extract (Bacto)	5.0 "
Glucose	1.0 "
Sucrose	10.0 "
K_2TeO_3 (1%)	30.0 ml
Crystal violet (1%)	0.2 "
Agar (Bacto)	15.0 g
Distilled water to make	1000.0 ml
Adjust to pH 7.4	

Aqueous solutions of potassium tellurite and crystal violet were added aseptically after the basal medium had been autoclaved at 15 lb for 15 minutes. The effect of potassium tellurite, crystal violet and sucrose upon the growth of test organisms and on gum formation by *Streptococcus salivarius* is shown in Table I.

The colony formation is somewhat different from that described

TABLE I.
Effect of 0.03% Potassium Tellurite and 1:500,000 Crystal Violet on 21 Bacterial Species.

Bacterial species	No. of strains	Medium A	Medium B	Medium C
Streptococcus salivarius (typical strains)*	11	++†	++†	++†
Streptococcus salivarius (atypical strains)*	3	++	++	++
Streptococcus species (from chicken feces)	3	++	++	—
Streptococcus lactis	1	++	++	++
Streptococcus liquifaciens	1	++	++	++
Streptococcus pyogenes	1	++	++	++
Streptococcus equi	1	++	++	++
Streptococcus agalactiæ	2	++	±	—
Bacillus subtilis	1	++	++	—
Bacillus mycoides	1	—	—	—
Bacillus megatherium	1	—	—	—
Staphylococcus aureus	1	++	+	—
Staphylococcus albus	1	++	—	—
Gaffkya tetragens	1	++	—	—
Lactobacillus delbrückii	1	—	—	—
Mycobacterium lacticola	1	+	+	+
Escherichia coli	1	—	—	—
Aerobacter aerogenes	1	—	—	—
Eberthella typhosa (Hopkins)	1	—	—	—
Shigella dysenteriæ	1	—	—	—
Salmonella schottmuellerii	1	—	—	—
Neisseria catarrhalis	1	—	—	—

*One atypical and 2 typical strains obtained from Dr. J. M. Sherman, Cornell University.

— No growth.
+ Growth.
++ Good growth.
† Gum formation.
Medium A = Basal substrate without inhibitory agents.
" B = " " + 0.03% potassium tellurite.
" C = " " + " " " and 1:500,000 crystal violet.

by Niven, *et al.*[4] *Streptococcus salivarius* produces surface colonies 4 to 5 mm in diameter and hemispherical in shape. Potassium tellurite is generally precipitated as metallic tellurium at the periphery of the base of the colony while the crystal violet appears as a bluish halo in the medium directly surrounding the colony.

Before any practical application of this medium could be made, the distribution of the gum-forming "typical" *Streptococcus salivarius* had to be determined. Swabbings from human lips, washed and unwashed glasses, floor dust, storage trays and drain boards were obtained using the technic of Winslow and Sanjiyan,[7] modified by employing rolled cotton swabs instead of flannel-covered discs. Inocula from enrichment cultures of tap, wash and rinse waters were streaked on the selective medium. Restaurant air samples were obtained by exposing plates of the selective medium for 15 minutes. All plates were incubated at 37°C for 48 hours and then examined for the characteristic gummy colony of *Streptococcus salivarius*. The results of these distribution studies appear in Table II. Six of 50 strains of gum-forming streptococci isolated were studied critically. Their biochemical and cultural characteristics were found to be similar to the "typical" *Streptococcus salivarius* described by Safford, *et al.*[5]

Conclusions. A medium has been developed on which a specific oral streptococcus can be selectively isolated from mixed bacterial populations. The value of this selective medium as a detector of oral contamination of restaurant eating utensils is indicated by the distribution studies, which show that this bacterium is found normally in the human mouth and on articles which have come in direct contact with human lips.

TABLE II.
Recovery of "Typical" *Streptococcus salivarius* from Various Sources.

Source	Samples No.	*S. salivarius* No.	*S. salivarius* %
Human lips	45	42	93.4
Unwashed glasses	40	18	45.0
Washed glasses	40	4	10.0
Drain boards	6	1	16.6
Storage trays	9	0	0.0
Dust (Washroom)	12	2	16.6
Dust (Service area)	12	1	8.3
Tap water	5	0	0.0
Wash water	5	0	0.0
Rinse water	5	0	0.0
Air (Washroom)	3	0	0.0
Air (Service area)	6	0	0.0

[7] Winslow, C.-E. A., and Sanjiyan, D. H., *J. Bact.*, 1924, **9**, 559.