usually was elevated. Experiments repeated on patients who previously had shown anxiety or fear and whose blood had had the effects described, showed the blood to be without this pharmacologic activity after the emotional state had subsided (Fig. 2).

The nature of the substance in the blood that induces these changes is not yet known but the substance appears to be labile and disappears almost completely when the blood is allowed to stand for periods of from 15 to 20 minutes. However, the effects produced on the intestinal loop may persist for periods as long as 30 minutes when the blood has been used within 2 to 3 minutes after withdrawal. When the Ringer-Tyrode's solution containing the blood is replaced by fresh solution, normal muscular contractions return within from 5 to 8 minutes. This suggests that the substance can easily be washed out of the intestinal loop.

These observations indicate that the blood of patients showing fear and anxiety contains a substance having definite pharmacologic effects on the surviving intestinal muscle of the rabbit, and offer a possible explanation of the mechanism by which somatic changes in certain emotional states are mediated.

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Improved Technic for Demonstrating the Presence of *Streptococcus salivarius* on Eating Utensils.

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In a previous communication,[1] the use of a substrate containing sucrose, potassium tellurite and crystal violet for the selective isolation of "typical" strains of *Streptococcus salivarius* from washed restaurant utensils was reported. This medium demonstrated that common contaminants of drinking glasses, such as the spore-forming bacilli, coliform bacteria and staphylococci, were effectively inhibited, while streptococci apparently grew unhindered. The "typical" strains of *Streptococcus salivarius*, previously described by Niven *et al.*[2] grew by forming distinctive mucoid colonies (Plate I) and could be easily distinguished from other cocci which might occur. The number of isolations obtained during routine use of the medium were less than anticipated, indicating that the combination of potassium tellurite and crystal violet might be somewhat inhibitory to the desired organisms.[3] Such results might be expected in view of the relatively small inocula derived from glasses which have undergone washing.

Additional experiments have been undertaken to determine the selective inhibitory activity of 5 solid substrates. The media of Niven *et al.*[4] and of Stiles and Chapman[5] (minus crystal violet but with added sucrose, 1%) were compared with our previously reported basal substrate containing 3 combinations of inhibitors, *viz.*, potassium tellurite (0.03%), potassium tellurite (0.03%) + crystal violet (1:500,000), and potassium tellurite (0.02%) + sodium azide (0.02%). Twenty-four-hour broth cultures of 14 bacterial species, representative of those likely to occur on eating utensils, were streaked upon each of these media and incubated for 72 hours at 37°C. Adequate controls on non-inhibitory media were inoculated simultaneously. Results were recorded on a comparative basis using growth on the control plates as the standard of comparison. The data in

1 Rose, K. D., and Georgi, C. E., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 344.

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

3 Unpublished data.

4 Niven, C. F., Smiley, K. L., and Sherman, J. M., *J. Bact.*, 1942, **43**, 113.

5 Stiles, M. H., and Chapman, G. H., *J. Bact.*, 1942, **43**, 64.

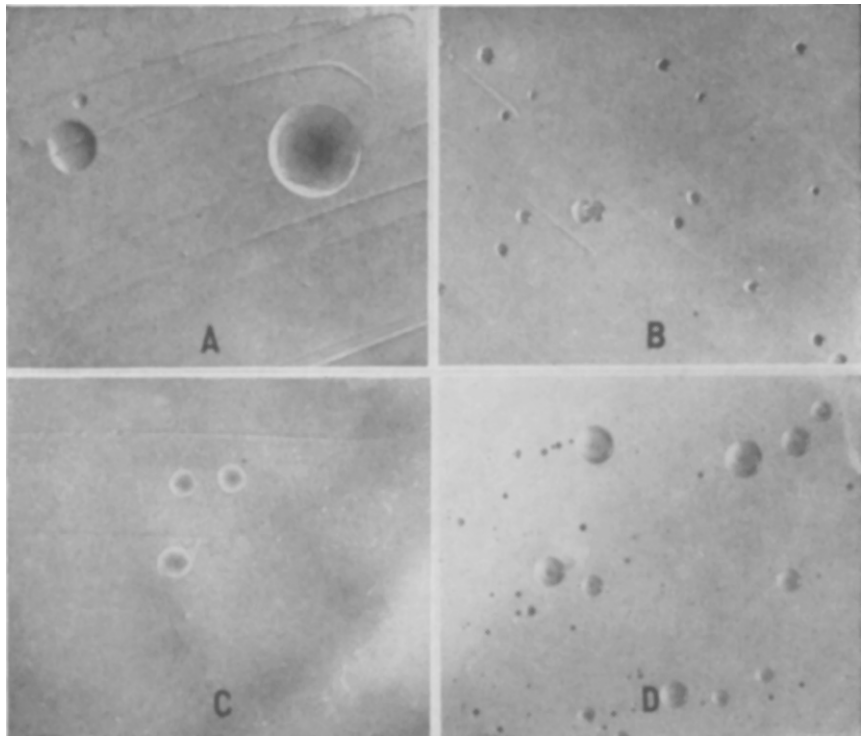

PLATE 1.
A. "Typical" *S. salivarius;* large mucoid colonies. B. Type "B" strain; hard gritty colonies. C. "Atypical" *S. salivarius;* flat. All are pure cultures, 72 hours. D. Direct smear from saliva showing typical and atypical forms. Age, 72 hours. Magnification, 2½ diameters.

Table I show that none of the media employed, with the exception of Medium IV, adequately inhibited the undesirable organisms while simultaneously permitting "typical" *S. salivarius* to grow unhindered.

Experiments to determine the effect of Media I, II and III on small inocula (10 to 200 "cell groups") of *S. salivarius* demonstrated that Media I and II were completely inhibitory. Medium III was non-inhibitory to inocula of *S. salivarius* as small as 25 cells, but its lack of inhibition of other organisms (Table I) eliminated its use for routine analyses. In fact, Walter[6] employed III in a restaurant survey, reporting no recovery of oral streptococci because of surface overgrowth by yeasts and coliform bacteria. Medium IV was also found unsatisfactory since a heavy inoculum of streptococci was required to initiate growth upon its surface. It became apparent that the use of a solid medium in the isolation of streptococci from washed glasses was attended by difficulties, not the least of which was the inability of small inocula of *S. salivarius* to grow well on a medium sufficiently inhibitory to eliminate undesirable organisms. An enrichment medium of some sort was deemed necessary.

A variety of enrichment media were tested and one containing sodium azide was found most satisfactory. Its composition follows:*

Proteose peptone (Difco)	5 g
Yeast extract (Bacto)	5 "
Beef extract (Bacto)	3 "
Glucose (C.P.)	10 "
Sodium azide (1% aq.)	20 ml
Distilled water	1000 "
Adjust to pH 7.0-7.2	

[6] Walter, W. G., *J. Bact.*, 1942, **43**, 114.

* Sodium azide is relatively stable to heat and can be added to the medium prior to sterilization. Care must be exercised to maintain the pH near 7.0 to 7.2. It was found that any marked divergence rendered the medium inhibitory to strains of *S. salivarius* employed in these studies.

TABLE I.
Growth of Microörganisms on Media Designed for Selective Isolation of "Typical" *Streptococcus salivarius*.

Bacterial species	Growth on selective media* I†	II	III	IV	V
Escherichia coli	—	—	++	—	±
Aerobacter aerogenes	±	±	±	—	±
Salmonella typhimurium	—	—	+±	—	+
Slow lactose fermenter No. 1	—	—	+	—	+±
Proteus morganii	+	+	+++	—	++
Proteus vulgaris	+++	+++	+++	—	++
Proteus species	++++	++++	++	+	++
Staphylococcus aureus	—	+++	+++	++±	++
Bacillus subtilis	±	+++	+++	—	++
Saccharomyces cerevisiæ	+	++	+++	+±	++
Torula lactosa	+	+	+	+	—
Streptococcus liquefaciens	++	++++	++++	+++	++++
Streptococcus salivarius No. 6	++	++	+++	++	++++
Streptococcus salivarius No. S25D	++	++++	++++	++++	++++
Streptococcus salivarius No. 83	++	++++	++++	++++	++++

* Results recorded on comparative basis with growth on the control = ++++.
† I = RG basal medium plus K_2TeO_3 (0.03%) and crystal violet (1:500,000).
II = RG basal medium plus K_2TeO_3 (0.03%).
III = NSS medium (0.02% NaN_3).
IV = RG basal medium plus K_2TeO_3 (0.02%) and NaN_3 (0.02%).
V = Modified Chapman's medium (0.02% NaN_3).

Experiments to determine the size of inoculum needed to initiate growth in this broth indicated that dilutions as high as 1:1 billion, or an inoculum of approximately 1 to 10 cells of "typical" *S. salivarius*, could initiate growth (Table II). *Staphylococcus aureus, Streptococcus liquefaciens* and an "atypical" (non-gum-forming) *S. salivarius* were also able to grow from minute inocula, whereas heavier inocula of *E. coli* and *Proteus* species were required. Organisms of the type *Proteus* species were particularly troublesome in routine isolations. They were encountered frequently in samples obtained from restaurant glasses and formed voluminous quantities of a mucoid material on sucrose media, readily distinguishable, however, from *S. salivarius*. These results suggest that preliminary cultivation in azide broth would tend to eliminate this group of organisms. In fact, *Proteus* species occurred frequently when direct streaks on solid media were employed, but they were not encountered in 398 field samples using an enrichment technic. Edwards[7] has demonstrated that inocula of *S. mastitis* consisting of 31 to 135 cells eliminated large inocula of *E. coli* when the 2 were grown in association in an azide-containing enrichment broth. Two experiments were conducted in which growth curves were obtained for *S. salivarius* and *E. coli* cultivated simultaneously in the enrichment broth. In both instances a heavy inoculum of *E. coli* was progressively reduced in numbers over a period of 16 hours by a smaller inoculum of *S. salivarius*. *S. salivarius* exhibited a normal growth curve and was unaltered by the association with *E. coli*.

TABLE II.
Effect of Inoculum Size on Initiation of Growth in Enrichment Medium Containing Sodium Azide.

Bacterial species	Greatest dilution of inoculum from which growth was initiated. (Avg of 3 trials) Basal medium	Basal medium + NaN_3
S. salivarius S25D*	1 x 10–9	1 x 10–9
S. salivarius S20B*	1 x 10–9	1 x 10–9
S. salivarius No. 6†	1 x 10–9	1 x 10–8
S. salivarius No. 208‡	1 x 10–9	1 x 10–8
Staphylococcus aureus	1 x 10–9	1 x 10–8
Escherichia coli	1 x 10–9	1 x 10–4
Proteus species	1 x 10–9	1 x 10–5

* "Typical" strain.
† "Atypical" strain.
‡ Strain "B."

For field experiments, a simple routine

[7] Edwards, S. J., *J. Comp. Path. and Therap.*, 1938, **51**, 250.

TABLE III.
Incidence of Oral Streptococci on Washed and Unwashed Glasses and in Wash and Rinse Waters as Determined by a Technic Involving Primary Enrichment in an Azide Broth.

Source of sample	No. of samples	Incidence of *S. salivarius*					
		"Typical" strain		Strain "B"		"Atypical" strain	
		No.	%	No.	%	No.	%
Unwashed glasses	44	23	52.3	13	29.5	25	57.0
Washed glasses	238	13	5.5	0	0	93	39.0
Wash water	20	5	25.0	0	0	10	50.0
Rinse water	9	0	0	0	0	4	44.4

technic was developed.† Cotton swabs on 5" applicators were suspended, through cotton plugs, in 5 ml of phosphate buffer at pH 7.0. For sampling purposes, the swab was slightly moistened in the buffer solution, the rim of a glass streaked vigorously and the swab returned to the buffer solution. In the laboratory, after vigorous agitation, the swab plus 1 ml of the phosphate solution was transferred to 9 ml of the enrichment medium and incubated at 37°C until growth appeared, but not longer than 48 hours. Large loopfuls (4.0 mm) of the cultures were then streaked on tellurite-crystal violet-sucrose agar plates and incubated 2 to 4 days at 37°C. The remainder of the phosphate buffer was used for the inoculation of other media. At the end of the incubation period, the plates were examined for colonies of "typical" *S. salivarius*.

Table III shows results obtained from 44 samples of unwashed glasses. "Typical" *S. salivarius* was recovered from 52% of the glasses tested, the results agreeing with the 45% recovery reported previously.[1] The "B" and "atypical" strains are 2 of *S. salivarius* which do not form mucoid colonies. The exact identity of strain "B" is not as yet established although it possesses several of the characteristics of the Lancefield Group H. The "atypical" strain resembles the "atypical" *S. salivarius* of Sherman.‡

Two hundred thirty-eight washed glasses in 24 different eating establishments were examined by the above procedure. These results are also presented in Table III and show that 5.5% of the glasses tested carried "typical" *S. salivarius*. The presence of "typical" *S. salivarius* on washed glasses demonstrates that the washing procedure employed was ineffective and that a potential chain of infection from the mouth of one individual to that of the next user exists. Although this paper is primarily a report on the development of a procedure, it is worthwhile to note that glassware in 37.5%[3] of the 24 restaurants examined showed "typical" oral streptococci still present on supposedly clean drinking glasses. The sanitary significance of these findings is obvious.

Summary. An improved technic for demonstrating the presence of oral streptococci on washed glasses is presented. The procedure involves primary enrichment of swab cultures in azide broth followed by inoculation onto sucrose-crystal violet-postassium tellurite agar. The simplicity of the technic, the ease of preparation of the media involved and the readily distinguishable colonies formed by *S. salivarius* on the selective agar substrate makes this procedure applicable to routine examination of restaurant ware. The data obtained by such routine examinations has public health significance which cannot be too strongly emphasized.

† Details will be reported more fully in another publication.

‡ Strain No. S44A received from Dr. James M. Sherman, Cornell, Ithaca, New York.