kya tetragena, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, and *Corynebacterium xerosis*.

A strain of *Lactobacillus acidophilus* showed poor recovery when dried directly from the broth culture, but when the cells were centrifuged and resuspended in a small amount of fresh broth and these washed cells dried on beads, the growth from the dried bead was vigorous and plentiful. However, when a strain of *Serratia marcescens* selected for intense pigment formation was dried on beads and recultured in broth from a bead, there developed many nonpigmented colonies with only a few pigmented colonies among them.

Some staphylococcal and enterococcal strains were preserved by this method. These strains were resistant to various antibiotics. After storage for 10 months on beads, they were reconstituted and rechecked for their resistance to one of the antibiotics. Table 1 shows that the resistance to this antibiotic did not change appreciably during the storage.

Although staphylococci are frequently unstable in broth or on agar medium, we have been able to maintain these and other strains for more than a year with subcultures from the beads showing the original characteristic colony form, mouse virulence, hemolysin, coagulase, pigment production, fermentation reactions, and antibiotic resistance.

MEDIUM FOR THE DIFFERENTIATION OF ACID PRODUCING COLONIES OF STAPHYLOCOCCI[1]

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In the course of studies on staphylococci of bovine origin the following medium was designed to aid in differentiation of acid producing colonies on agar plates: proteose peptone no. 3 (Difco), 2.5 g; yeast extract, 1.5 g; carbohydrate[2], 10 g; agar[3], 12 g; brom-thymol-blue[4], 0.05 g; $CaCO_3$[5], 10 g; distilled water, 1000 ml.

Various workers have recommended the use of $CaCO_3$ as an additive to culture media containing sugars for the differentiation of acid-producing colonies from non-acid-producing colonies. The value of its unique character of being opaque under alkaline conditions and yielding soluble calcium salts under acid conditions, which then results in a transparent culture medium, has long been recognized. Beijerinck (Centr. Bakteriol., **9**, 781–786, 1891) recommended the use of precipitated chalk ("geschlemmte Kreide") and clearly demonstrated that acid-producing colonies were surrounded by a transparent halo whereas non-acid-producing colonies had no such halo around them. Shank and Silliker (Bacteriol. Proc., **1956**, 34, 1956) recommended a procedure for the preparation of colloidal calcium carbonate for addition to a medium containing neutral red dye as a means of differentiating acid-producing colonies from non-acid-producing colonies of acid-producing spore formers.

Staphylococci do not produce as large quantities of acids from fermentable carbohydrates as some other groups of bacteria. Relatively long periods of incubation (48 to 72 hr) are therefore required to permit detection of transparent halos around acid-producing colonies. Various indicators were used in varying concentrations in conjunction with commercially available precipitated chalk. The contrast obtained between acid-producing colonies and non-acid-producing colonies was striking when brom-thymol-blue indicator was used. Non-acid-producing staphylococcal colonies remained essentially colorless with a bluish cast; acid-producing colonies be-

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[2] Mannitol, lactose, galactose, glucose, glycerol, and sucrose have been used.

[3] Davis New Zealand agar was used. Difco agar gave a comparable gel in a concentration of 15 g/L.

[4] Dibromothymolsulfophthalein; water soluble or in alcohol solution.

[5] Precipitated chalk, Baker and Adamson Code 1541, recommended by Dr. C. B. van Niel.

came bright yellow within 18 to 24 hr after inoculation. The surrounding medium remained opaque and blue. The $CaCO_3$ prevented changes in the color of the indicator in the medium surrounding the colonies, so that acid-producing sectors could be distinguished in a background of non-acid-producing colonial growth. After 48 hr incubation, colonies capable of reducing the dye were surrounded by a colorless zone. The concentrations of peptone and yeast extract should be minimal. Staphylococci are able to utilize amino acids as the energy source and if sufficient ammonia is liberated from the nitrogen source, greenish colonies result, thereby blurring the clear-cut difference between acid-producing colonies and non-acid-producing colonies.

IMPROVED MEDIUM FOR SELECTIVE ISOLATION OF VEILLONELLA

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A selective medium for the isolation and enumeration of the Veillonella from the mouth has been described previously by Rogosa (J. Bacteriol., **72,** 533, 1956). Considerable experience has now shown that in addition to the Veillonella, often a large background of small extraneous colonies, principally streptococci and diphtheroids, appears. Thus, it may be difficult to obtain pure isolates on first trial and also recognition of Veillonella colonies is sometimes uncertain. Lackey and Fitzgerald (J. Dental Research, **34,** 705, 1955 and Proc. Internat. Assoc. for Dental Research, 43, 1956) found that this genus was relatively insensitive to streptomycin and the antibiotic was therefore incorporated into the original medium at 5 μg/ml.

Since then, we have studied a newer antibiotic, vancomycin (obtained through the generosity of Eli Lilly and Company). Titration experiments with pure cultures of Veillonella showed that these organisms were resistant to 50 to 500 μg/ml of vancomycin. A wide spectrum of 44 strains of the genus *Veillonella*, comprising what is believed to be probably all the serological groups and subtypes,[1] has been tested for growth in the presence of vancomycin (20 μg/ml), or streptomycin (5 μg/ml). The level of streptomycin is that employed originally (Rogosa, J. Bacteriol., **72,** 533, 1956). Growth in the presence of vancomycin compared favorably with that on either streptomycin or the basal medium. Indeed, streptomycin may often be more inhibitory than vancomycin for certain strains of Veillonella, particularly in relatively early growth (24 hr or less). A concentration of 1 μg/ml or less of vancomycin has been found to be inhibitory for diphtheroids. With rare exceptions, the oral streptococci and micrococci are inhibited by 3 μg/ml or less.

[1] This serological study is to be described elsewhere.

TABLE 1

Comparative efficiency of isolation media containing streptomycin and vancomycin

Additive to Basal Medium	Amt	No. of Samples	Avg Plate Counts	
			Veillonella	Others
	μg/ml		*no.* × 10^6	*no.* × 10^6
Streptomycin	5	15	41	144
Vancomycin	5	15	66	7
Vancomycin	7.5	15	67	7
Vancomycin	10	15	67	7
Vancomycin	20	25	69	8

Salivary samples, collected by paraffin stimulation, were plated with basal medium containing either streptomycin (5 μg/ml) or vancomycin at 5, 7.5, 10, and 20 μg/ml. The results in table 1 clearly demonstrate that Veillonella are recoverable from saliva more readily in media containing vancomycin than streptomycin, and that extraneous organisms, principally streptococci and diphtheroids, are significantly reduced, to such an extent that they no longer interfere