line dipotassium salt), 2 per cent glucose, or 2 per cent sucrose; the base was 1 per cent tryptose, 0.3 per cent yeast extract, 0.5 per cent NaCl, and 0.05 per cent glucose; for the meningococci and gonococci, 0.2 per cent agar was added. After incubation at 37° C. for 7 days, the cultures which had good growth in all the mediums were centrifuged, and the supernatant fluids and the bacterial sediments were tested separately for starch-like material by observation for dark coloration upon addition of a solution containing 0.02 per cent I_2 and 0.2 per cent KI.

The chief point (Table 1) is that all the strains of *C. diphtheriae* produced material which gave a dark-blue to purple color with iodine when grown in the medium containing glucose-1-phosphate; the iodine-coloring material was readily demonstrable in the supernatant fluids as well as in the bacterial sediments. The same capacity was shown by some of the streptococci, but it did not occur regularly among the varieties which we tested; for example, only one of 5 group A, 2 of 3 group C, and 2 of the 9 dextran-forming streptococci gave a positive reaction. Except for the *N. perflava*, none of the other

TABLE 1

TESTS ON CULTURES GROWN IN BROTHS CONTAINING GLUCOSE-1-PHOSPHATE, GLUCOSE, OR SUCROSE

Bacteria	No. of strains tested	Dark color with iodine*		
		GP	G	S
C. diphtheriae	17	17	0	0
Streptococcus, groups A–F	12	4	0	0
" , others†	9	2	0	0
Other bacteria‡	40	0	0	0
N. perflava	3	1	0	3

* GP = glucose-1-phosphate; G = glucose; S = sucrose.

† Strains from subacute bacterial endocarditis which form dextran from sucrose (*2*).

‡ Included 3 diphtheroids, 3 staphylococci, 7 gonococci, 2 meningococci, 3 *E. typhosa*, 2 *Salmonella*, 3 *Shigella*, 1 *Bact. coli*, 2 *Bact. aerogenes*, 2 *Bact. Friedlanderi*, 2 *Proteus*, 1 *Ps. pyocyanea*, 1 *Cl. tetani*, 1 *Cl. histolyticum*, 2 *B. anthracis*, and 5 *B. subtilis*.

bacteria tested produced any detectable amount of iodine-coloring material, but the number of strains included are too small to warrant any conclusions on the entire lack of the capacity in any of those species. That all the 17 strains of *C. diphtheriae* and none of the 3 diphtheroids which we tested had the capacity raises the question of the possible use of tests for the production of starch-like material from glucose-1-phosphate as a descriptive, and perhaps as a differential, feature for *C. diphtheriae*.

The formation of starch-like material by *C. diphtheriae* apparently belongs to the same general class of reactions as those brought about by the starch-phosphorylases of tissue origin. As shown in Table 1, glucose-1-phosphate serves as substrate, and sucrose and glucose do not. Furthermore, in experiments which we have made with mixtures of resting cells plus glucose-1-phosphate, formation of polysaccharide occurred and was accompanied by an increase in inorganic phosphate and a corresponding loss in organic bound phosphate; also, the polysaccharide production was inhibited by high concentrations of inorganic phosphate. Although its chemical study has not been completed, the bacterial product has been found to be a mixture of amylose-like and amylopectin-like polysaccharides, and in that respect it can be regarded as a material similar to the starches formed by many plants.

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Vitamin Requirements of *Microbacterium lacticum* Orla-Jensen

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Investigations in progress on the metabolism and taxonomic position of bacteria in the genus *Microbacterium* Orla-Jensen (*1*) have shown that d-calcium pantothenate constitutes an essential nutritional requirement for some strains of *Mbm. lacticum*. In addition, thiamine was identified as one of the vitamins exerting a stimulatory effect upon growth.

TABLE 1

COMPOSITION OF BASAL MEDIUM

Glucose, c.p.	1.0 gram
Casein hydrolysate, vitamin-free	0.5 "
K_2HPO_4, c.p.	0.2 "
KH_2PO_4, c.p.	0.2 "
d-Calcium pantothenate	100.0 γ
Thiamine hydrochloride	100.0 γ
Distilled water to make	100.0 ml.
pH 6.6 ± 0.1	

Preliminary experiments indicated that several strains of *Mbm. lacticum* would grow in a mediun consisting of inorganic salts, glucose, vitamin-free hydrolyzed casein,[1] and a combination of most of the known bacterial vitamins. By a process of elimination it was found that a medium of the composition listed in Table 1 and designated as the basal medium would support growth of most strains tested.

TABLE 2

EFFECT OF D-CALCIUM PANTOTHENATE AND THIAMINE ON THE GROWTH OF *Mbm. lacticum*

Composition of medium	Strain designation						
	0J3	1PM1	1PM3	3RM2	3RM3	8180	S2
Basal minus d-Ca pantothenate and thiamine	0*	0	0	0	0	0	0
Basal minus d-Ca pantothenate	0	0	0	0	0	0	0
Basal minus thiamine	16	30	29	22	25	19	6
Basal medium	29	40	44	41	46	38	40

* Percentage of light absorbed through 10 ml. of medium measured with a Fisher Electrophotometer AC model, using filter 425 (blue). Readings after 4 days at 30° C.

A solution of the vitamin-free casein hydrolysate, neutralized to pH 6.6 ± 0.1, and solutions of glucose and phosphate salts were prepared and sterilized individually by autoclaving 15 minutes at 121° C. The d-calcium pantothenate and thia-

[1] A vitamin-free hydrolyzed casein designated as Lot #6 and obtained through the courtesy of M. L. Speck, National Dairy Research Laboratories, Baltimore, Maryland.

mine hydrochloride were sterilized individually by filtration through a Jena 1G5 auf 3 glass filter. Appropriate volumes of these ingredients were mixed together and then added to sterile test tubes in 10-ml. amounts, so that the final concentrations were as indicated in Table 1.

The inoculum for the basal medium was prepared in the following manner: Five ml. of a 4-day culture grown in yeast-extract-proteose-peptone-glucose broth (30° C.) was centrifuged and the sedimented cells washed with 5 ml. of 0.85 per cent saline. This was again centrifuged and the cells resuspended in 5 ml. of 0.85 per cent saline. One loopful of this suspension served as the inoculum for 10 ml. of basal medium. Ten of 14 strains of *Mbm. lacticum* were capable of growing on repeated subculture in the medium described in Table 1. The 4 strains which did not grow in this medium also failed to grow when most of the known bacterial vitamins were supplied.

Further observations indicated that d-calcium pantothenate was absolutely essential for the initiation of growth. Thiamine hydrochloride, however, was not. The 10 strains which grew in the basal medium also grew, though rather poorly, when thiamine hydrochloride was omitted. The effect of elimination of d-calcium pantothenate and thiamine hydrochloride separately and together on several representative strains is shown in Table 2.

The typical growth response of 4 strains of *Mbm. lacticum* to graded amounts of d-calcium pantothenate after 4 days at 30° C. is presented in Fig. 1. The minimal amount of this compound necessary for initiation of growth for most strains studied was approximately 0.02γ/ml., and maximum effect was usually obtained at a concentration of 0.1γ/ml. The response to thiamine hydrochloride was not linear in concentrations of 0.01–1.0γ/ml.

The results presented indicate that under the described conditions d-calcium pantothenate and thiamine hydrochloride are nutritional requirements for most strains of *Mbm. lacticum* used in this study.

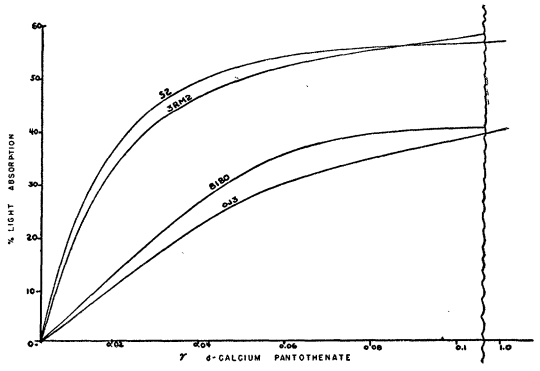

FIG. 1

Total growth in the basal medium as determined in these experiments was less than that in the natural medium, which indicates that an additional factor or factors are required for optimum growth.

Reference

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IN THE LABORATORY

A Rapid Method for Estimation of Use-Dilution Concentrations of Quaternary Ammonium Germicides

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The increased use of quaternary ammonium germicides as sanitizing agents for eating and drinking utensils, dairy and food processing equipment, etc., has accentuated the need for a satisfactory, rapid "use-dilution" test for determining the concentration of active quaternary ammonium agents in rinse waters. Dubois (*1*) has recently prepared a comprehensive review and evaluation of the methods that have been proposed for the chemical analysis of this group of compounds. While most of these give reliable and accurate results, under carefully controlled titration procedures, there still remains a demand for a simple test which is rapid and requires no elaborate apparatus. Furthermore, of particular importance to the sanitary engineer and public health officer is a test not only involving the latter requirements but also providing a reasonably accurate estimation of the bactericidally active concentration of quaternary ammonium germicide in the presence of inorganic and, in particular, organic matter.

It is generally recognized that many compounds of anionic nature, such as detergents and soaps, as well as proteinaceous substances, *i.e.* egg albumin, peptones, milk, serum, etc., are not compatible with the cationic quaternary ammonium germicides. This incompatibility is often evidenced by the appearance of a turbidity which varies in intensity with volumes and concentrations when the compounds are mixed. It was postulated, therefore, that it would be possible to arrive at a suitable volume and concentration of one of these incompatible substances, which when combined with a quaternary ammonium germicide, would give by the degree of turbidity a reasonably accurate indication of the concentration of the bactericidally active quaternary present in a solution.

For purposes of sanitation, most quaternary ammonium germicides are recommended for use in concentrations ranging from 166 ppm (1:6,000) to 250 ppm (1:4,000) (*3*). Thus, the objective of a rapid use-dilution test should be the demonstration of the presence or absence of such concentrations in the unknown solution.