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.

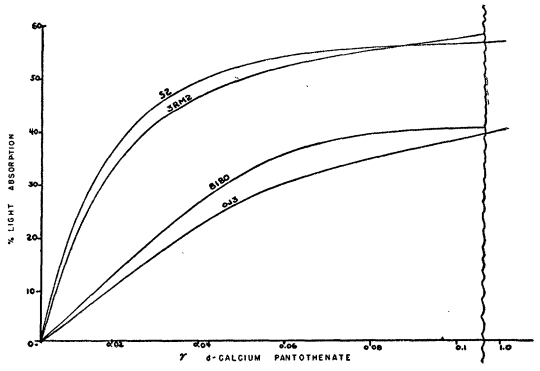

FIG. 1

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

1. ORLA-JENSEN, S. *The lactic acid bacteria.* Copenhagen: Andr. Tred. Host, 1919.

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.

In exploratory tests concentrations of 125, 166, 250, and 500 ppm of benzalkonium chloride (U.S.P. XIII) were prepared in water. To varying quantities of each of these concentrations were added varying amounts and different concentrations of several anionic detergents. The results of this study revealed, however, that the anionic substances failed to show any progressive differences in turbidity which could be correlated, within a reasonable degree of accuracy, with the concentration of quaternary ammonium germicide with which they were mixed. For this reason, further studies on anionic reagents did not appear warranted.

Encouraging results were obtained in studies using normal horse serum in combination with germicidal concentrations of quaternary ammonium compounds. As an example, when 1 drop of undiluted serum was added to 1-ml. quantities of varying concentrations of benzalkonium chloride, the following degrees of turbidity resulted:

1,000 ppm	(1:1,000)	++++	Marked turbidity
500 "	(1:2,000)	++++	" "
250 "	(1:4,000)	++	Moderate turbidity
166 "	(1:6,000)	+	Trace
125 "	(1:8,000)	−−	No turbidity

With the evidence obtained in the turbidimetric analysis of benzalkonium chloride, the investigation was extended to include the several other quaternary ammonium compounds of different structural configurations. By using a constant volume of 1 drop of horse serum, as before, results comparable to those noted in the benzalkonium tests were obtained with n(acylcolaminoformylmethyl)-pyridinium chloride, p-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, cetylpyridinium chloride and 9-octadecenyldimethylethylammonium bromide. The test for turbidity in all instances was read at a 15- to 30-second time interval following the addition and shaking of the horse serum in the unknown quaternary ammonium solutions. A moderate to heavy turbidity developing within the time indicated is taken as evidence that the sanitizing solution contains at least 250 ppm (1:4,000) of quaternary ammonium germicide. The addition of 10 per cent aqueous safranine (prepared from a saturated alcoholic solution), to give a final concentration of 4 per cent in the horse serum, will facilitate the ease in reading the degrees of turbidity, and chloroform will serve as a preservative for the serum.

The specificity of the test method in the presence of substances known to completely or partially neutralize the bactericidal action of quaternary ammonium germicides was carried out in the following manner: To an equal volume of 1,000 ppm (1:1,000) benzalkonium chloride was added a neutralizing agent. In the event a precipitate resulted in this combination, the mixture was clarified by filtration through paper. One drop of horse serum reagent was added to 1 ml. of the clear solution and the presence or absence of turbidity noted. For purposes of comparison, the mixtures were also tested by the Dubois modification of the Hartley-Runnicles colorimetric procedure (*2*). The results of this study are presented in Table 1.

Examination of the data reveals several pertinent points, among which are the following: (a) The colorimetric method on the unfiltered, turbid mixtures gives values which are consistently higher than the same solutions which have been filtered. While this may not directly indicate inactivation of the quaternary ammonium germicide, it is evidence of adsorption of the compound on the inactivating agent. Since in all instances filtration of turbid mixtures is necessary in carrying out the turbidimetric method, this obviates testing adsorbed quaternary ammonium compounds. (b) Certain quaternary-inactivator combinations will react with the indicator in the colorimetric test to give color complexes ("off color") not associated with the assay. In no instance was there any evidence of a similar interfering action in the horse serum reagent test.

TABLE 1

COMPARISON OF HARTLEY-RUNNICLES (DUBOIS) COLORIMETRIC AND HORSE SERUM TURBIDIMETRIC METHODS FOR DETERMINATION OF QUATERNARY AMMONIUMS

Quaternary ammonium (1:1,000 + inactivating agents)	Colorimetric (ml. Duponol "C")	Turbidimetric (1 drop serum)
Soap 1:1,000 (unfiltered)	1.5	−−−
" 1:1,000 (filtered)	0.3	0
" 1:100 (unfiltered)	No reaction	−−−
" 1:100 (filtered)	No reaction	0
Santomerse 1:1,000 (unfiltered)	1.0	−−−
" 1:1,000 (filtered)	0.3	0
" 1:100 (clear)	No reaction	0
Duponol "C" 1:1,000 (unfiltered)	Off color	−−−
" " 1:1,000 (filtered)	0.3	0
" " 1:100 (clear)	No reaction	0
Egg albumin 1:1,000 (clear)	2.4	+++
" " 1:10 (unfiltered)	Off color	−−−
" " 1:10 (filtered)	2.0	++
Evaporated milk 1:100 (unfiltered)	2.3	−−−
" " 1:100 (filtered)	1.6	++
" " 1:10 (unfiltered)	1.8	−−−
" " 1:10 (filtered)	0.6	Trace
Orange concentrate-undiluted (unfiltered)	Off color	−−−
" " " (filtered)	Off color	Trace
Ice cream 1:10 (unfiltered)	1.5	−−−
" " 1:10 (filtered)	0.7	Trace
Horse serum 1:10 (unfiltered)	2.5	−−−
" " 1:10 (filtered)	1.5	Trace
" " 1:5 (unfiltered)	Off color	−−−
" " 1:5 (filtered)	3.2	0
" " 1:2 (unfiltered)	Off color	−−−
" " 1:2 (filtered)	Off color	0
Control		
Quaternary 1:1,000	4.2	++++
" 1:2,000	2.3	++++
" 1:2,000 (filtered)	2.0	++++
" 1:4,000	1.2	++
" 1:6,000	0.7	0

"No reaction"—4.2 ml. or more of Duponol reagent results in no change in color.
"Off Color"—dye used in test reacts with color in agent.
−−−−solution turbid; impossible to test.
0—no quaternary demonstrable.
++ to ++++—moderate to marked turbidity.

Colorimetric and turbidimetric control tests on the several inactivating agents, in the absence of quaternary, in no instance showed evidence of a positive reaction indicative for quaternary ammonium compound. These data, therefore, were deleted from the table. Evidence will be presented elsewhere on the sensitivity of the present method for several homolo-

gous series of bacteriologically active and inactive quaternary ammonium germicides.

References

1. DUBOIS, A. S. *Soap San. Chem.*, 1946, **22**, 125.
2. DUBOIS, A. S., and DIBBLEE, D. D. *J. Milk Tech.*, 1946, **9**, 260.
3. JAMIESON, M. C., and CHAN, M. K. *Canad. Dairy Ice Cream J.*, 1944, **23**; KROG, A. J., and MARSHALL, C. G. *J. Milk Tech.*, 1942, **5**, 343; *Amer. J. Publ. Hlth*, 1940, **30**, 341; WALTER, W. G., and HUCKER, G. J. *Sanitarian*, June-July, 1942.

A Simple Device for Macroscopic Sectioning of the Brain

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The principle involved in the macrotome to be described is not original but was seen and used by one of us (R. F. B.) in a much more elaborate and expensive apparatus. We have been unable to discover any American firm which manufactures such a macrotome and have concluded that the one observed was of European make and, therefore, no longer obtainable. For this reason we designed and built an instrument along similar lines, but much simpler and less expensive, which has proved entirely satisfactory.

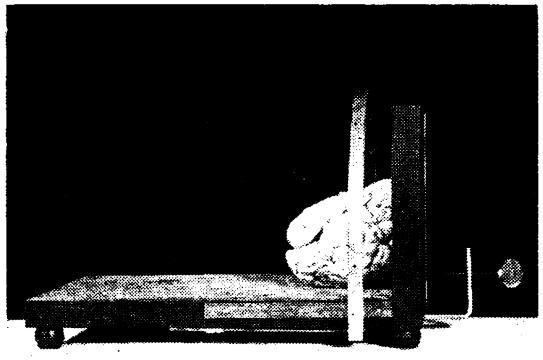

FIG. 1

For the study of the brain and other large organs, such as the liver, it is often desired to have macroscopic sections of uniform and known thickness. This is accomplished readily and easily with the macrotome to be described. The instrument consists of a base and an end board of hard wood with a metal arch, properly supported, which is used as a guide for the knife. The thickness of the section is determined by the distance between the arch and the end board as measured by a millimeter scale attached to the side of the base and adjusted by means of a thumb screw (Figs. 1 and 2). For the base, a piece of hard wood 12 x 9 x $\frac{7}{8}$ inches is used; for the end board one 9 x $6\frac{7}{8}$ x $\frac{7}{8}$ inches. Two countersunk screws are used to fasten the end board at right angles to the base. One piece of bar steel $30\frac{1}{2}$ x $\frac{3}{4}$ x $\frac{3}{16}$ inches is bent to form the arch, the ends being welded and the joint buffed smooth. Two pieces of bar steel $6\frac{1}{2}$ x $\frac{3}{4}$ x $\frac{3}{16}$ inches are used for the side guides, which prevent side sway and twisting of the arch. One piece of bar steel $12\frac{1}{2}$ x $\frac{3}{4}$ x $\frac{3}{16}$ inches forms the center guide and pressure bar to regulate the thumb screw. This piece must be drilled and threaded to accommodate a $\frac{3}{8}$ inch thumb screw having at least 2 inches of thread. A small piece of light metal should be fastened to the back of the end board for the thumb screw to work against and to prevent it from cutting into the wood. Four keepers of metal are used in which the guides move. To complete the apparatus four rubber feet are attached for support. Details of construction are shown in Fig. 2.

The blade used to cut the sections is 18 x $\frac{1}{2}$ inches and is drawn taut in a hacksaw handle.

To cut the sections, the thumb screw is turned until the arch reaches the desired distance from the end board, as measured by the millimeter scale. The specimen is placed on the base so that it touches the end board in the desired plane. The knife is then placed against the arch and drawn through the specimen with one long stroke, taking care that it touches both sides of the arch throughout the entire stroke. In holding the specimen on the base and against the end board only a moderate amount of pressure should be exerted, *i.e.* sufficient to keep the specimen from moving.

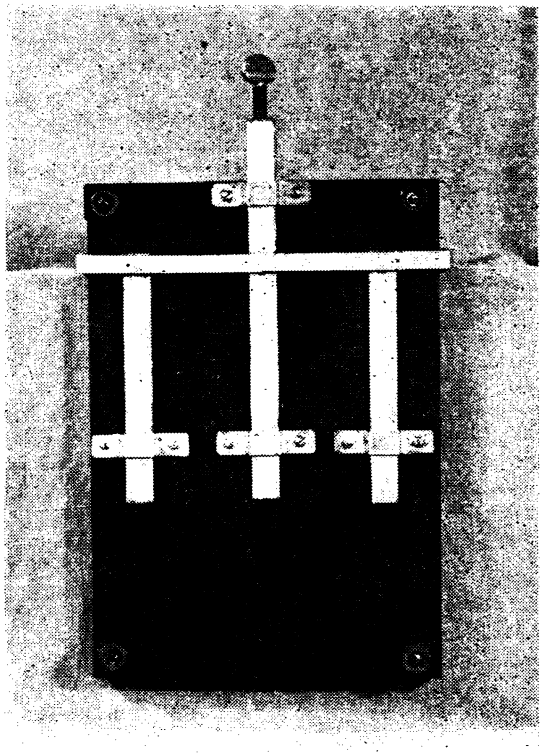

FIG. 2

Formalin-fixed specimens have been sectioned at 3 mm. with little difficulty and at 5 mm. with no difficulty. Thicker cuts have also been made. Removal of the pia mater facilitated sectioning of the brain. It was found, however, that brains embedded in agar and chilled did not section evenly because of the resistance offered to the knife by too firm a block.

[1] We wish to express our appreciation to Otto F. Kampmeier, head of the Department of Anatomy, for affording us the facilities to do this work.