just as the rise in pH became evident), were washed twice in 0.1 M potassium phosphate buffer (pH 6.4), and were suspended in various media at a concentration equivalent to that of the original culture. The more pertinent results are summarized in Table 2.

Results comparable to the G medium control were obtained in a buffered medium containing L-glutamic acid, L-methionine, lactic acid, and a mixture of inorganic salts (CDS medium). Favorable results were also observed in a medium containing acetic acid together with smaller amounts of lactic acid. These acids, added in the concentration at which they are normally produced from glucose during growth, were shown to be rapidly utilized once sporulation begins (Nakata, J. Bacteriol. 86:577, 1963). The replacement of lactate with equimolar concentration of acetic acid, however, was not consistently successful. These observations, coupled with those demonstrating a rapid conversion of lactate to acetate and carbon dioxide during the prespore stage (unpublished data), suggest a requirement for a reducing potential, together with acetate, for sporogenesis. In this CDS medium, lactic acid is added in an amount nearly equivalent to the combined molar concentration of acetate and lactic acid normally produced from 0.2% glucose.

The spores produced in CDGS or CDS media are microscopically indistinguishable from those produced in the G medium, and survive 20 min at 80 C without loss in viability. Only slight differences were noted in their dipicolinic acid (DPA) content, as determined by the method of Janssen et al. (Science 127:26, 1958). At pH 6.2, the CDGS- and CDS-grown spores contained 7.41 and 7.40% DPA, respectively, as compared with 6.44% for spores produced in G medium buffered at the same pH. At pH 7.0, the DPA contents were 6.30, 6.67, and 6.33%, respectively, for CDGS, CDS, and G medium spores.

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SELECTIVE MEDIUM FOR ISOLATION OF MIMA AND HERELLEA ORGANISMS

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Members of the provisionally so-called Mima and Herellea group, sometimes called Morazella by European investigators, were implicated in a number of human infections (Daley, Postic, and Kass, Arch. Internal Med. 110:580, 1962), including gonorrhea (DeBord, J. Lab. Clin. Med. 28:710, 1943). When they are the only organisms present in an infection, their isolation and identification do not present a problem; however, when present in an infection involving a variety of bacterial species, their isolation often presents difficulties because their colonial characteristics are not distinguishable. Our study of the role of Mima and Herellea organisms in gonorrhea, especially penicillin-resistant gonorrhea, has been hindered because of the uncertainty of recognizing Mima and Herellea colonies in the presence of large numbers of gram-positive cocci and gram-negative rods (usually members of the family Enterobacteriaceae) frequently encountered in urethral and vaginal discharges.

The medium reported here takes advantage of the inhibitory action of bile salts on gram-positive bacteria, the fermentative activity of the Enterobacteriaceae, and the biochemical inactivity of the Mima and Herellea group. The medium is prepared by adding to distilled water the following ingredients (in grams per liter): pancreatic digest of casein (Difco), 15; soy peptone (Difco), 5; sodium chloride, 5; lactose, 10; maltose, 10; bile salts, 1.25; bromocresol purple, 0.02; and agar, 16.

After 24 hr at 37 C, colonies of Mima and Herellea organisms were pale lavender, the color of the medium. All acid-producing colonies were yellow, surrounded by a yellow zone. The growth of gram-positive organisms was inhibited. In addition to color differentiation, Mima and
Herellea colonies were much more mucoid on the selected medium than on nutrient agar, blood-agar, and other media with a low carbohydrate content. Species of *Pseudomonas* and *Proteus* were not inhibited, nor did they produce acid from the carbohydrates present in the medium. However, *Pseudomonas* colonies were easily recognized by their gray-green color and diffusible pigment, when present. If desired, *Proteus* colonies can be differentiated by the addition of 5.0 g of phenylalanine and 0.5 g of ferric ammonium citrate to the medium, in which case *Proteus* colonies are brown, surrounded by a light-brown zone.

Table 1 lists the growth and fermentation characteristics of a number of bacterial species on this medium. The *Mima* and *Herellea* cultures were received from the Walter Reed Army Institute of Research, Washington, D.C.

This medium supported the growth of 30 laboratory strains of *Mima* and *Herellea* organisms. By the use of this medium, these organisms were easily isolated from clinical material containing large numbers of contaminating organisms.

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### RELATIONSHIP OF A $\beta$-ALANINE-PYRUVIC AMINOTRANSFERASE TO REVERSAL OF $\alpha$-SERINE INHIBITION OF GROWTH

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$d$-Serine inhibits biosynthesis of $\beta$-alanine in a species of *Flavobacterium*; the resulting inhibition of growth can be reversed by $\beta$-alanine, L-$\alpha$-alanine, L-aspartic acid, or pantothenic acid (Durham and Milligan, Biochem. Biophys. Res. Commun. 7:342, 1962). Kudlac et al. (Can. J. Microbiol. 10:307, 1964) reported that this species of *Flavobacterium* possesses an L-aspartic-4-carboxylase (IUB 4.1.1.12) which catalyzes the formation of L-$\alpha$-alanine and carbon dioxide from L-aspartic acid. This communication describes a $\beta$-alanine-pyruvic aminotransferase isolated from the *Flavobacterium* species, and establishes that the following pathway is prominent in the metabolic reversal of $d$-serine inhibition of growth in this organism.

$$\text{L-aspartic acid} \rightarrow \text{L-$\alpha$-alanine} \rightarrow \text{d-serine}$$

$$\beta-\text{alanine} \leftrightarrow \text{d-serine}$$

Cells were grown on nutrient agar for 12 to 15 hr at 37°C, harvested, washed twice, and suspended in 0.01 M tris(hydroxymethyl)aminomethane (tris) buffer (pH 7). The cells were ruptured in a French pressure cell at 20,000 psi at a delivery rate of approximately two drops per second, and were centrifuged at 25,000 × g for 60 min at 4°C. The supernatant solution (35 ml) was treated with 3 ml of protamine sulfate (saturated solution at 4°C), and was then centrifuged; ammonium sulfate (recrystallized) fractionations were made at 0.20, 0.45, and 0.70 saturation. Centrifugation was performed after each ammonium sulfate treatment. The ammonium sulfate precipitate was dissolved in 0.01 M tris buffer, and was dialyzed against 1 liter of tris buffer (changed after 12 hr) for a total of 36 hr. All additives were dissolved in 0.01 M tris buffer, adjusted to pH 7, and sterilized by filtration just prior to use. Protein was determined by