Aerobacter aerogenes. Consequently it seemed possible that incorporation of such compounds into an appropriate substrate might permit growth in air of those strains of *B. abortus* that normally require an increased atmosphere of carbon dioxide. The practical importance of this possibility prompted the following experiments.

The experimental methods were essentially those employed in previous work with brucellae (Gerhardt and Wilson: J. Bact., **56**, 17, 1948). Two CO_2-sensitive strains of *B. abortus* were selected that failed to grow in an air atmosphere even on prolonged incubation. Strain 19 of *B. abortus*, which grows in air, was used as the control. An inoculum of approximately 1×10^8 viable cells per ml (final concentration) was employed. The media used were tryptose broth and the chemically defined medium (minus asparagine) of Gerhardt and Wilson (1948). The compounds tested were added before autoclaving. Tubes containing 7 ml of medium were autoclaved, inoculated, and incubated statically at 35 to 37 C both in air and in an atmosphere containing 10 per cent CO_2. The latter served as the control and supported good growth of all strains with the various media used. Growth was measured turbidimetrically.

Although glutamic acid was reported by Ajl and Werkman (1948) to exhibit a maximum CO_2-replacement effect, and although it is readily utilized by brucellae, it did not obviate the need for an increased CO_2 tension by the CO_2-sensitive strains of *B. abortus* when added in amounts of 0.1 per cent to either tryptose broth or agar. When added to the chemically defined basal medium as the nitrogen source, L-glutamic acid again was ineffective, either as a liquid or as a solid substrate.

To the chemically defined basal medium containing 0.1 per cent L-glutamic acid as the nitrogen source was added singly 1.0 mM per 100 ml of the following compounds, which, according to Ajl and Werkman (1948), may substitute for CO_2: L-aspartic acid, L-arginine, L-proline, L-malic acid, fumaric acid, succinic acid, and *alpha*-ketoglutaric acid. Of the compounds tested, none replaced the effect of an atmosphere containing 10 per cent CO_2, although light growth was obtained after prolonged incubation (17 days) with arginine and with *alpha*-ketoglutaric acid. Growth comparable to that in the controls occurred in the basal medium when the culture tubes were stoppered tightly. Apparently this was the result of the CO_2 produced by the organism.

GROWTH OF THE YEASTLIKE PHASE OF HISTOPLASMA CAPSULATUM IN A FLUID MEDIUM

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The yeastlike (pathogenic) phase of *Histoplasma capsulatum* has been grown primarily on blood agar slants at 37 C. Recently, extensive growth has also been

reported in semifluid media with a small percentage of agar, silica gel, or a similarly acting substance (Salvin: J. Bact., **54**, 655, 1947; J. Infectious Diseases, **84**, 275, 1949). No liquid medium of simple composition has been described that can support extensive growth of the yeastlike phase.

The medium used in the experiments here reported has the following composition, per liter: "casamino acids" (Difco), 10 g; glucose, 3 g; sodium chloride, 2.5 g; potassium chloride, 2.5 g; disodium phosphate, 2.5 g; and biotin, 20 μg.

After adjustment to pH 7.0, the medium was dispensed in 1-liter Blake bottles, 50 ml per bottle. Inoculation with a suspension of yeastlike cells in the logarithmic phase of growth (about a 72-hour culture) and subsequent incubation at 37 C usually produced visible growth in 24 hours and extensive growth in 72 hours. The inoculum must contain a certain minimum number of cells, depending on the volume, surface, and composition of the medium, before growth can be initiated. The yeastlike cells grew throughout the medium, although as the culture aged they tended to clump and settle to the bottom. This type of growth was characteristic of all six strains tested.

Rapid and extensive growth of the yeastlike phase (to the exclusion of the mycelium) occurred when the surface area of the culture fluid was large and its depth small. Although the consistently best growth (4 to 5 $\times$ 10^8 cells per ml) was obtained with a mixture of amino acids, excellent growth also appeared in media with a peptone as the nitrogen source, such as Difco peptone, proteose-peptone, or tryptone. Yeast extract (3 g per liter) may be substituted for the biotin. Continued subculture in the amino acid medium did not result in a reduced growth of the yeastlike cells or in an appearance of mycelium.

When the culture fluid was kept in constant rotation, flasks with less surface area could be used to produce abundant growth. With an initial inoculum of 1 $\times$ 10^6 cells per ml, counts of 2 to 3 $\times$ 10^9 cells per ml were obtained in 500-ml Erlenmyer flasks containing as much as 200 ml of culture medium. This process of rotating the fluid may be viewed as another method of producing an extensive surface area. The growth of the organism under the conditions described above is striking, especially since it also has been grown extensively (3 $\times$ 10^8 cells per ml) under vacuum and in an atmosphere of nitrogen.

The foregoing technique has also been used for obtaining large numbers of the yeastlike cells of *Blastomyces dermatitidis*.

THE ISOLATION OF LISTERIA MONOCYTOGENES FROM FERRETS

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Listeriosis is a mildly infectious but highly fatal disease of several species of animals, including man. The disease is caused by *Listeria monocytogenes*, which has heretofore been isolated only from man and animals showing symptoms or