(guinea pigs and rabbits). Antibacterial properties of the drug have been discussed also by Büchi (Schweiz. Apoth. Z., **83**, 198, 1945) and by Junod (Med. et Hyg., **4**, 1, 1946).

We became interested in comparing the morphological and cytochemical effects of cepharantine[1] on *Escherichia coli* with those of penicillin and streptomycin. The standard cylinder-plate technique was used.

After a suitable incubation period, each cylinder from which cepharantine (0.25 μg per ml) was diffusing was surrounded by a small bacteriostatic zone, which in turn was circumscribed by a sharply defined ring of enhanced growth. Cells of *E. coli* in the "static" area appeared isodiametric, in the shape of vesicles approximately 5 to 7 microns in diameter. Often they were associated two by two or were serially arranged in rows. This is in contrast to the effects of penicillin and of streptomycin, which cause pronounced elongation of *E. coli* into myceliumlike filaments.

The vesicular cells were easily stained with dyes, such as methyl green, known to have strong affinity for desoxyribonucleic acid compounds, but the cells did not retain dyes, such as trypan blue, which have an affinity for mononucleotides. In this respect, cells of *E. coli* exposed to bacteriostatic concentrations of cepharantine react similarly to those exposed to streptomycin (Pratt and Dufrenoy; Antibiotics, J. B. Lippincott Co., 1949).

[1] Samples of a pharmaceutical preparation of cepharantine were kindly provided by Mrs. Fumiko Murayama. The compound is available commercially from the Kaken Drug Company., Ltd., Tokyo, Japan.

ATTEMPTS TO REPLACE THE ADDED CARBON DIOXIDE REQUIRED BY SOME STRAINS OF BRUCELLA ABORTUS[1]

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The requirement for an increased atmosphere of carbon dioxide by newly isolated strains of *Brucella abortus* not only complicates the culture of these organisms but also is of interest in the general problem of carbon dioxide utilization. That the requirement is for carbon dioxide per se has been demonstrated by Wilson (Brit. J. Exptl. Path., **12**, 88, 1931). Ajl and Werkman (Arch. Biochem., **19**, 483, 1948; J. Bact., **57**, 579, 1949) recently have shown that certain compounds associated with the Krebs cycle or their metabolic precursors may substitute for carbon dioxide in the metabolism of *Escherichia coli* or

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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