evoked counterclaims of contamination from unwashed hands and stopcock grease (Anderson and Chargaff, J. Biol. Chem., 85, 509, 1930). Wherever careful precautions were taken, no sterols have been found except in Lactobacillus arabinosus and L. pentosus (Guirard et al., Arch. Biochem., 9, 361, 1946); in E. coli (Dauchy et al., Compt. rend. soc. biol., 150, 1974, 1956); and in Azotobacter chroococcum (Sifferd and Anderson, Z. physiol. Chem. Hoppe-Seyler's, 239, 270, 1936).

Major effort in the search for bacterial sterols has been concentrated on a few genera, notably Bacillus (Lemoigne et al., Bull. soc. chim. biol., 31, 1587, 1949), Escherichia (Williams et al., J. Bacteriol., 37, 301, 1939), Lactobacillus (Guirard et al., Arch. Biochem., 9, 361, 1946) and Mycobacterium (Anderson and Chargaff, J. Biol. Chem., 85, 509, 1930). Miyoshi (Sei-i-kwai Med. J., 49, 47, 1930) analyzed the lipids of Shigella, Staphylococcus, Pseudomonas, and Vibrio. Aerobacter (Zambruno, Bull. soc. ital. biol. sper., 22, 337, 1946), Corynebacterium (Gubarev and Bakulenko, Biokhimiya, 10, 285, 1945), and Azotobacter (Sifferd and Anderson, Z. physiol. Chem. Hoppe-Seyler's, 239, 270, 1936) have also been examined.

From this list, it is apparent that, in addition to mycobacteria, only representatives of the eubacteria have been investigated. To determine whether other major groups of bacteria contain sterols, cultures of Micromonospora sp., Streptomyces griseus, Sphaerotilus natans, and Rhodospirillum rubrum were cultivated in appropriately defined media to yield approximately 30 to 40 g wet weight. The nonsaponifiable lipids were extracted (Klein, J. Bacteriol., 69, 620, 1955), and assayed for sterols using a modified Lieberman-Burchard test (Idler and Baumann, J. Biol. Chem., 203, 389, 1953), digitonin precipitation, and column chromatography (Johnston and Bloch, J. Am. Chem. Soc., 79, 1145, 1957).

Micromonospora sp. gave a positive test equivalent to approximately 0.001 per cent (calculated as ergosterol) of the wet weight. This material behaved with digitonin and on columns like known sterols. We, therefore, conclude that Micromonospora contains traces of steroid, pending final characterization of this material. R. rubrum presented difficulties because of its high concentration of pigments, which were largely removed by chromatography. The resulting lipids yielded a positive Lieberman-Burchard test indicating a maximum sterol content (calculated as ergosterol) of about 0.001 per cent on a wet weight basis. Since this material did not yield a precipitate with digitonin, it is probable that the positive test was due, at least in part, to contaminating pigments. S. natans and S. griseus yielded negative results in tests that could detect as little as 0.00001 per cent of the wet weight as sterol.

USE OF A TETRAZOLIUM SALT FOR AN EASILY DISCERNIBLE SULFIDE-MOTILITY REACTION

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The hanging-drop technique employed for the detection of bacterial motility is tedious. Young cells are required, and, as it is difficult to observe motility when a limited number of cells within a culture exhibit motion, results are often confusing.

With semisolid media, motility is macroscopically manifested by a diffuse zone of growth spreading from the line of inoculation. The effects are cumulative, and localized outgrowths appear when only a small proportion of motile cells are involved, eliminating the possibility of overlooking motility. Growth and diffusion in semisolids, however, may be so slight as to necessitate comparison with a control tube for proper interpretation.

Colorless solutions of various tetrazolium salts are reduced to insoluble, pigmented formazans in the presence of viable cells. Kuhn and Jerchel (Ber. deut. chem. Ges., 74B, 941, 1941) called attention to the fact that tetrazolium solutions could be used to stain yeast and bacteria. Wood

We routinely exploit the sulfide reaction in our diagnostic protocol. Hajna’s (Pub. Health Lab., 8, 36, 1950) sulfide-mobility medium is preferred. Reactions are rendered easily discernable when 0.005 per cent 2,3,5-triphenyltetrazolium chloride is incorporated into the medium and autoclaved at 117°C (10 lb) for 15 min. Color develops in regions where bacteria are present.

Nonmotile, nonsulfide producing organisms reveal a red line along the route of inoculation. Nonmotile, sulfide producing cultures form a black line. Motile, nonsulfide producing cultures develop a diffuse pink cloud throughout the medium. Motile, sulfide producing cultures blacken the entire tube.

EFFECTS OF AZASERINE ON AZOTOBACTER AGILIS

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Azaserine has been shown to inhibit the growth of various microorganisms, carcinomas, and fungi (Stock et al., Nature, 173, 71, 1954; Ehrlich et al., Nature, 173, 72, 1954). Levenberg et al. (J. Biol. Chem., 225, 163, 1957) have demonstrated that it competes with glutamine to block one of the steps in the de novo formation of purines. Barker et al. (J. Am. Chem. Soc., 78, 4632, 1956) have postulated that azaserine inhibits transamination reactions in Scenedesmus sp., and Aaronson (J. Bacteriol., 77, 548, 1959) has found that glutamine, arginine, and glutamic acid reverse the inhibitory effect of azaserine on the growth of Gaffkya homari. The effect of azaserine on transamination and other reactions in the nitrogen metabolism of Azotobacter agilis (Azotobacter vinelandii, Wisconsin strain 0) was tested in the present studies in a search for responses related to nitrogen fixation.

Azaserine inhibited the growth of A. agilis at a concentration of 1 × 10^{-5} M, regardless of the source of nitrogen. The inhibition was not reversed by glutamine, added after 3 hr preincubation of the culture with azaserine, but was partially reversed when glutamine was added with the azaserine.

A study of the incorporation of N^{15}H_4 into glutamic and aspartic acids (table 1) showed that azaserine markedly inhibited N^{15} incorporation by cell-free preparations of A. agilis with or without added α-ketoglutarate. This result led

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**TABLE 1**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Incubation Time</th>
<th>Added Azaserine</th>
<th>Glutamic acid</th>
<th>Aspartic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 min</td>
<td>0.0</td>
<td>0.069</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>1 × 10^{-5}</td>
<td>0.017</td>
<td>0.025</td>
</tr>
<tr>
<td>2</td>
<td>2 hr</td>
<td>0.0</td>
<td>3.298</td>
<td>4.377</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>1 × 10^{-5}</td>
<td>0.355</td>
<td>0.417</td>
</tr>
</tbody>
</table>

* temperatures, 30°C; pH, 7.0. Acids were separated on Dowex 1-acetate.
  a Ten ml of cell-free sonic extract of A. agilis containing approximately 85 mg of protein per ml were used in a total volume of 20 ml.
  c Thirty and four-tenths atom per cent N^{15} excess supplied as 9.5 × 10^{-3} m (N^{15}H_4)_2SO_4.
  d No added α-ketoglutarate in reaction mixtures.
  a-Ketoglutarate, 5.5 × 10^{-3} m, in reaction mixtures.