NOTES

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ALPHA-NAPHTHOL COLOR TEST FOR DIHYDROXYACETONE AND HYDROXYMALEIC ACID[1]

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Received for publication May 27, 1942

In the course of other studies we tested a bacterial culture for acetylmethylcarbinol by Barritt's modification of the Voges-Proskauer reaction (1936). Instead of the expected red, a blue color developed. Suspecting the presence of either glyceraldehyde or dihydroxyacetone, we tested these substances with the same reagent and found that dihydroxyacetone gave a blue color. We have been unable to locate any reference to a similar observation.

The reaction occurs when, to 1 ml. of aqueous solution of the substance to be tested, there is added, first, 0.5 ml. of freshly prepared 6 per cent alcoholic alpha naphthol and, second, 0.2 ml. of 40 per cent potassium hydroxide. In the presence of more than 0.1 milligram of dihydroxyacetone a yellow color soon changes to green and, in the course of a minute or so, this gives way to blue. The blue color is stable for hours. There is no characteristic visible absorption spectrum. The pigment is soluble in polar organic solvents, but not in benzene or ether.

With 1 mgm. pyruvic acid or 25 mgm. acetoacetic ester a blue color also develops. No color is given by glucose, glyceraldehyde, lactic acid, glucuronic acid, acetaldehyde, or acetone. Beta naphthol cannot be substituted for alpha naphthol.

Hydroxymaleic acid, M.P. 144°, prepared according to Wohl and Claussner (1907), yields a pink color which, in concentrations of less than 0.5 mgm. per ml., is slow to develop. With more than 1 mgm. per ml. there is a preliminary formation of the green and blue phases seen with dihydroxyacetone, which pass through purple to a red which has no characteristic visible absorption spectrum. There is no fluorescence of this red substance, in contrast with that formed from diacetyl.

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Heating hastens and intensifies the color development. Extraction by amyl alcohol during the blue phase separates the pigment into alcohol-soluble blue and water-soluble red. Extraction later reveals the blue unchanged, but the red gives way to yellow. Late extraction with benzyl alcohol results in all pigment going into the alcohol with a red color.

Conclusion: With alpha naphthol and potassium hydroxide a blue color is given by dihydroxyacetone, pyruvic acid and acetoacetic ester. Hydroxymaleic acid first turns blue, then red.

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HYDROGEN IN THE METABOLISM OF AZOTOBACTER[1]

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Received for publication July 24, 1942

Phelps and Wilson (1941) found that cultures of *Azotobacter vinelandii* possess an active hydrogenase, the enzyme which activates molecular hydrogen. Because of the possible significance of this finding for nitrogen fixation by *Azotobacter*, we recently tested other species to determine if they too have the enzyme, using O_2 as the hydrogen acceptor according to the technique already described (Wilson, Lee, and Wilson, 1942). The results were:

	A. VINELANDII	A. AGILE	A. CHROOCOCCUM
Q_{O_2} (N)	—	325	118
Q_K (N)	3635	4990	1500
	4365	4180	1500

For this experiment *Azotobacter* cells from 40-hour cultures were used, 0.11 mgm. cellular nitrogen per flask. The Q_{O_2} (N) (mm.^{3} O_2 per hour per mgm. N) was estimated in 96 per cent He, 4 per cent O_2; the Q_K (N) (mm.^{3} total gas uptake per hour per mgm. N), in 96 per cent H_2, 4 per cent O_2. No substrate was added. In this particular trial the hydrogenase activity in *A. chroococcum* was less than in the other two species, but in other experiments it had a Q_K (N) equal to that typical of *A. vinelandii*—about 4000.

The possession of hydrogenase by *Azotobacter* is somewhat unexpected since

[1] Support for this work was furnished in part by grants from the Rockefeller Foundation and the Wisconsin Alumni Research Foundation.