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13081 P

Preparation of Cell-free Solutions of Hydrogenase.

M. BOVARNICK. (Introduced by Augustus B. Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

In certain bacterial species, including *B. coli, Clostridium sporogenes,* and others, enzyme systems have been described which catalyze the reduction by molecular hydrogen of various substrates such as methylene blue, nitrate, fumarate, oxygen, and some amino

acid.[1,2] It has been shown[3,4] that the enzyme of *B. coli* which activates molecular hydrogen, hydrogenase, catalyzes in a completely reversible manner the reaction

$$H_2 \rightleftarrows 2H + 2\epsilon$$

The enzyme preparations used in previous studies have been suspensions of intact growing or resting bacterial cells; extraction of the enzymes involved resulted in loss of activity.[5]

As a basis for further study of this enzyme the observations reported in this paper seemed of interest, namely, that the system catalyzing the reduction of methylene blue by hydrogen is quite stable, and that active dry preparations and cell-free solutions can readily be secured, as evidenced by data in the accompanying table.

An 18-hour broth culture of *B. coli communior,* No. 142, was washed twice with saline by centrifugation and resuspended in 0.1 M phosphate buffer, pH 7.0. The final volume was one-thirtieth that of the original culture. Active dry powders were obtained by pouring this suspension into 20 volumes of cold acetone and rapidly filtering by suction. Cell-free solutions were obtained by filtering a 16-day autolysate through Mandler filters. Other methods of lysing the cells, such as alternate freezing and thawing, and prolonged exposure to high salt concentration followed by rapid dilution, have yielded solutions that were active but difficult to filter.

The reactions were carried out at room temperature and at pH 7.0

TABLE I.
Catalysis of Reduction of Methylene Blue by Hydrogen.

Preparation	Gas	Methylene blue (1 ml)	Time required for decoloration
Washed *B. coli* suspended in 4 ml phosphate buffer	H	1/5000	8 min
Same	N	"	>90 "
Washed *B. coli* susp. heated 100°C 20 min	H	"	> 5 hr
Acetone-dried preparation suspended in 4 ml phosphate buffer	H	1/1000	20-25 min
Same	N	"	> 6 hr
Suspension of acetone-dried preparation heated 100°C 20 min	H	"	> 6 "
Mandler filtrate	H	1/5000	25 min
" "	N	"	>12 hr
" " heated 100°C 20 min	H	"	>12 hr

[1] Stephenson, Marjory, and Stickland, L. H., *Biochem. J.*, 1931, **25**, 205.

[2] Hoogerheide, J. C., and Kocholaty, Walter, *Biochem. J.*, 1938, **32**, 949.

[3] Green, D. E., and Stickland, L. H., *Biochem. J.*, 1934, **28**, 898.

[4] Farkas, A., Farkas, L., and Yudkin, J., *Proc. Roy. Soc. London, Series B,* 1934, **115**, 373.

[5] Stickland, L. H., *Biochem. J.*, 1929, **23**, 1187.

(0.1 M phosphate buffer), either in modified Thunberg tubes or, more conveniently, by bubbling the gases through the solutions in test tubes provided with two-holed rubber stoppers bearing inlet and outlet tubes. Amounts of the enzyme preparation were used that corresponded to 4 ml of the original phosphate suspension. The gases used were deoxygenated by passing over hot reduced copper filings.

In the presence of active preparations the dye was reduced under an atmosphere of hydrogen but not under nitrogen. Heating for twenty minutes at 100°C destroyed the activity of the enzyme.

Summary. Stable dry powders and cell-free solutions that possess hydrogenase activity have been prepared from cultures of *B. coli communior.*

13082

Effect of Gramicidin on Metabolism of Bovine Spermatozoa.*

GERTRUDE HENLE AND CHARLES A. ZITTLE. (Introduced by Stuart Mudd.)

From the Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia.

Gramicidin,†,‡ a bactericidal agent for gram-positive organisms,[1] seems to have a wider range of activity than the name indicates. It acts as a hemolytic agent in low concentrations,[2,§] and it is highly

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† The preparation of crude and purified gramicidin, and purified tyrocidine, used in these studies were obtained through the kindness of Dr. R. J. Dubos, who independently suggested this investigation. We are indebted also to Dr. Dubos for reading this paper before publication.

‡ Crude gramicidin has recently been renamed tyrothricin by Hotchkiss and Dubos[4] and the name gramicidin retained for one of the purified components. The other principal component has been purified also and named tyrocidine; it exhibits bactericidal activity against both Gram positive and Gram negative organisms.

1 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1, 11; Dubos, R. J., and Cattaneo, C., *ibid.*, 249; Dubos, R. J., *Ann. Int. Med.*, 1940, **13**, 2025.

2 Heilman, D., and Herrell, W. E., PROC. SOC. EXP. BIOL. AND MED., **1941, 46**, 182.

§ Hotchkiss and Dubos[5] recently have stated that tyrocidine is hemolytic but that purified gramicidin is not hemolytic. Further studies[6] have confirmed the hemolytic effect of gramicidin in saline or buffer solutions[2] but have shown that hemolysis is completely inhibited by small amounts of glucose.