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Preparation and Properties of Soluble Bacterial Enzymes Which Decompose Creatinine.

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Several years ago, soil bacteria capable of producing adaptive enzymes which specifically decompose creatine and creatinine were isolated.[1, 2] This report presents preliminary observations on the manner of preparation and properties of soluble enzymes prepared from one species (NC) of these bacteria.

When the washed cells are desiccated by various methods a portion of the enzymic system which decomposes creatinine is rendered soluble. This soluble portion can be obtained by adding water to the dried suspension, centrifuging, and collecting the clear supernatant solution. The methods that have been used include grinding with abrasive materials, such as pumice-stone, desiccation with phosphorus pentoxide and sulphuric acid, and desiccation with alcohol-ether, or acetone-ether.

The method of assay employs 0.5 mg of neutral creatinine-hydrochloride as substrate, 0.5 cc of 0.5 M phosphate buffer pH 7.0, the substance being tested, and water to 5 cc. The suspension is incubated at 38° to 40°C for 30 minutes. The undecomposed creatinine is determined colorimetrically after the addition of alkaline picrate.

The activity of the preparation obtained under the above conditions represents about 10% of the activity of the original cells when assayed under the same conditions. The properties of the extract differ in certain respects from the original cells. Sodium cyanide 10^{-3}M, and thymol inhibit the cells but not the extract. Toluene and formaldehyde depress the activity of the cells and extract. Sodium fluoride, 10^{-3}M, does not inhibit the cells or extract.

Studies on the purification of the enzymes are in progress.

1 Miller, B. F., and Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 335.

2 Dubos, R. J., and Miller, B. F., *J. Biol. Chem.*, 1937, **121**, 429.