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The production of tyrosine by a putrefactive anaërobe.

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In caring for our collection of anaërobic cultures which now numbers 69 strains distributed among 15 clearly recognizable species and 4 strains as yet unidentified, we have observed that certain ones habitually form white crystalline products in the deep brain medium that is used for preserving the stock cultures; namely 3 strains of *B. bifermentans*, 4 of *B. centrosporogenes* (a new species shortly to be described for the first time), 1 of *B. histolyticus*, and especially an unplaced culture, herewith designated No. 106, that resembles *B. sporogenes* in certain properties but differs in its striking crystal formation. All of these are actively putrefactive anaërobes.

We have failed to observe such crystals in 6 strains of *B. Welchii*, 3 of *B. Novyi*, 2 of *B. butyricus*, 19 serologically homologous strains of *B. sporogenes*, 5 heterologous strains of *B. sporogenes*, 2 strains of *B. botulinus* Type A, 3 of *B. botulinus* Type B, 7 of *Vibrion septique*, 7 of *B. tetani*, 3 of *B. putrificus*, and 1 each of *B. Chauveauii*, *B. sphenoides*, *B. tertius*, and *B. tetanomorphus*. Of this list *B. sporogenes* and *B. botulinus* are actively putrefactive, *B. tetani* and *B. putrificus* less strikingly so.

References to crystals supposed on microscopic grounds to be tyrosine in cultures of putrefactive anaërobes are scattered through the literature, a review of which is reserved for a more detailed report. So far as we are aware, no one else has recovered tyrosin in a state of high purity from a pure culture of any single bacterium.

Our studies to date show that culture No. 106 produces crystals, macro- and microscopically resembling tyrosin, in ground meat,

brain, salmon, milk and suspended casein mediums not containing fermentable carbohydrates, *i.e.*, monosaccharides in excess. The early stages of incubation are marked by clouding and vigorous gas production. The crystals begin to appear in 4–6 days at 37° C. along with a visible liquefaction of the protein as well as odorous evidence of putrefaction. Meat and brain mediums are blackened presumably owing to the precipitation of iron sulfide by the action of sulfuretted hydrogen upon the iron freed by proteolysis. Milk, salmon and casein mediums are not blackened, although sulfuretted hydrogen is produced, except upon the addition of iron ions, as by the inclusion of an iron nail. These facts fail to support a suggestion that the blackening of certain proteins by putrefactive anaërobes parallels the supposed action of a tyrosinase in transforming tyrosin into melanin in the animal body.

The recovery of the crystals in a pure form involves the removal of the water-soluble constituents by washing the partially digested culture mediums with cold water, extraction of the crystals by boiling water, with or without the addition of ammonia, followed by hot filtration to remove undissolved proteins, concentration of the filtrate by boiling, crystallization from the concentrated filtrate by cooling, removal of the crystals by cold filtration and repeated clarification with animal charcoal in boiling water alternated with crystallization in the cooled filtrate. Impurities soluble in cold water are removed by washing at each cold filtration and the crystals are dried after rinsing with 95 per cent. alcohol followed by ether.

Quantitative methods of extraction are yet to be devised; there is considerable loss at each step excepting in the treatment with ether. The sample displayed, about 0.8 gram, represents the purified product from several liters of culture.

The crystals are identified as tyrosine by their physical and chemical properties. To the naked eye they appear as snowy white flakes with a silken sheen, the individual needles barely visible. These flakes may be readily suspended in alcohol or distilled water; in the latter particularly the characteristic crystals may be seen with a hand lens as colorless double-pointed needles. When allowed to crystallize slowly from somewhat dilute hot

water or ammonia solutions, they readily form the sheaf-like bundles so characteristic of tyrosin.

They are soluble in boiling water, N/10 ammonia, and dilute mineral acids, slightly soluble in dilute acetic acid and relatively insoluble in cold water, cold and hot absolute alcohol, ether, toluene, acetone, benzine, carbon disulphide, glycerine and chloroform. They are not decomposed in aqueous solution by heating in the Arnold sterilizer at 100° C. or in the autoclave at 15 lbs. pressure for 1 hour.

They give Pirie's, Hoffmann's and Denige's tests.[1]

The senior author is now engaged in perfecting the method of extraction and in studying the crystal formation of other anaërobes.

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A method for the preparation of cystin.

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A number of years ago Folin[2] described an improved method for the preparation of cystin which has come into general use. It is based on the fact that the solubility of cystin is a minimum in solutions possessing an acidity between P_H 4–5. To obtain the optimum acidity for the precipitation of cystin, the HCl used to hydrolyze the protein is neutralized by the addition of sodium acetate. Although good yields of the amino acid are obtained by this method it nevertheless is not economical for the production of cystin in quantity since large amounts of relatively expensive materials are required. Neutralization of HCl with sodium acetate results in the simultaneous precipitation of humin which later necessitates the repeated use of large quantities of charcoal to effect its removal.

In the method described below the HCl is in large part recovered by vacuum distillation. Use is then made of commercial finishing lime to neutralize the remaining HCl, to precipitate

[1] Hammerstein-Mandel, "A Text-book of Physiological Chemistry," J. Wiley & Sons, N. Y., 1912, p. 150.

[2] Folin, O., *J. Biol. Chem.*, 1910, viii, 9–10.