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The Isolation of a Beta Bios.

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In 1924 Eddy, Kerr and Williams[1] reported the isolation of a crystalline Bios M.P.223. As tests of the physiological activity of this product proceeded, it became evident that this substance alone could not account for all the yeast growth stimulatory power of its source, yeast autolyzate. Search was therefore begun for another bios and for convenience Bios M.P.223 will be referred to hereafter as Alpha-bios. By manipulation of the yeast autolyzate it has now been possible to fraction it into at least 3 specifically distinct bios-containing fractions. One fraction yields a homogenous product not hitherto described and which is here designated as Beta-bios. A third fraction yields a concentrate whose behavior toward precipitants suggests Lucas and Miller's Bios II[2] and which we will call here Gamma-bios.

The evidence of the existence of Beta-bios which led to the development of the fractionation methods for its isolation was first obtained by studying the behavior of yeast autolyzate and Alpha-bios solutions under electrodialysis. Using a 14 compartment carbon cell with parchment paper septa and yeast autolyzate, yeast stimulatory activity was sharply concentrated in 2 separate compartments of quite different pH. No such separation occurred when the cell was filled with pure Alpha-bios solution.

The detailed method of fractionation will be reported later. The essential feature of the separation of Beta-bios is the formation of an insoluble barium salt of this substance preliminary to the separation of Alpha and Gamma bioses. This barium salt is then decomposed with sulfuric acid. Basic impurities are eliminated by silver and mercuric sulfate and phosphotungstic acid. Non-nitrogenous acids are removed by extraction with acetone.

The Beta-bios isolated by our procedure is very hygroscopic. When dehydrated with acetone and the aid of the vacuum desiccator it is a fine white granular powder decomposing at 100° C. on prolonged heating. It dissolves to form a colorless solution in acids such as HCl, but takes on a bright yellow color in neutral solvents such as water and an intense yellow turning to brown in alkalies. It gives the following reactions:

Molisch test = negative. Fehling-Benedict = negative even after boiling 10 hours with 10% HCl.

Ninhydrin test = positive. Folin phenol test = positive. Millon test = doubtful.

Ppt. by basic lead acetate but not by normal lead acetate.

Indol test negative but positive after fusion of product with KOH.

Decomposed at 110° C. with evolution of CO_2.

Fused with KOH evolves ammonia and skatole, the latter even when fused in copper tube with an atmosphere of hydrogen.

Does not ppt. with phosphotungstic acid.

S, P, Halogens and metals absent.

Quantitative elementary analysis:

	%C	%H	%N*	
Product 1	42.76	6.52	6.18	5.47
Product 2	42.58	6.42		
Product 3	42.65	6.60		

* By micro-Kjeldahl.

Evidence of homogeneity of product rests to date on the fact that repeated isolations have always produced a compound with identical physical, chemical and physiological properties and constancy of elementary analysis. At present we are investigating a synthetic compound built upon the theory of Beta-bios construction. This synthetic product to date exhibits physiological activities similar to that of Beta-bios but details of this work are reserved for a later communication.

The physiological activity has been studied to determine critical concentration;[3] effect on 6 different pure strains of yeast; and effect of combination with different culture media such as Clark's, Nageli's, Medium F, etc. The effect varies somewhat with different yeast and culture media but at a conc. of .05-.075 milligrams per cc. maximum effects are demonstrable amounting to at least ten fold growth in 48 to 72 hours incubation at 31° C. In these tests the Funk-Dubin method[4] was used, and in most tests R. J. Williams' culture medium.[5]

[1] Eddy, Kerr and Williams, *J. Am. Chem. Soc.*, 1924, xlvi, 2846.

[2] Lucas, G. H. W., *J. Phys. Chem.*, 1924, xxviii, 1180.

[3] Peskett, G. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 340.

[4] Funk and Dubin, *J. Biol. Chem.*, 1920, xliv, 487.

[5] Williams, R. J., *J. Am. Chem. Soc.*, 1927, xlix, 227.