BIOTIN AND PARA-AMINOBENZOIC ACID AS GROWTH FACTORS FOR THE ACETONE-BUTANOL ORGANISM, CLOSTRIDIUM ACETOBUTYLICU M

Sir:

Rubbo and Gillespie [Rubbo and Gillespie, *Nature*, **146,** 838 (1940)] have recently reported that *p*-aminobenzoic acid (p. a. b.) is a growth factor for nine strains of *Cl. acetobutylicum*. They state that it is the only factor required by the organism. We are unable to confirm this conclusion.

In a previous paper [Oxford, Lampen and Peterson, *Biochem. J.*, **34,** 1588 (1940)] we reported that *Cl. acetobutylicum* on a medium of glucose, asparagine and Speakman's salts requires the addition of biotin and of an unidentified factor from yeast. The basal was identical with that of Rubbo and Gillespie except that 0.11% of salts was added instead of the 1.32% which they used. Asparagine–ammonium sulfate or ammonium phosphate were also used as nitrogen sources.

In later experiments we have found that p. a. b. will replace the yeast factor. This activity together with the close agreement between the properties of the two make it evident that the active substance in our earlier preparations was either p. a. b. or some equivalent compound. However, growth does not occur on the addition of p. a. b. alone to the basal medium. If biotin is added also, growth is optimal. This result has been obtained with strains S9 from our collection and nos. 824 and 862 of the American Type Culture Collection. No. 862 is one of the strains used by Rubbo and Gillespie. All strains required both biotin and p. a. b. Table I illustrates the effect of the two factors on the S9 strain.

TABLE I

EFFECT OF BIOTIN AND P. A. B. ON *Cl. Acetobutylicum* S9 (WISCONSIN COLLECTION)

Biotin (γ/cc.)	P. A. B. (γ/cc.)	Evelyn reading
.....	.....	97
0.00154	.....	98
.00154	0.00005	87.5
.00154	.00010	83
.00154	.00020	69.5
.00154	.00040	55
.00154	.00080	35
.00154	14.3	44
.....	0.01	98
.00001	.01	90
.00002	.01	81
.00005	.01	70
.00010	.01	55
.00020	.01	30

Growth was determined by measuring the turbidity in an Evelyn photoelectric colorimeter. The biotin employed was the crystalline methyl ester obtained through the generous coöperation of Professor V. du Vigneaud.

Our only explanation of the discrepancy between our findings and those of Rubbo and Gillespie is that the natural constituents of their medium may have contained biotin. We have found that some grades of glucose contain appreciable quantities of this factor.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN J. O. LAMPEN
MADISON, WISCONSIN W. H. PETERSON

RECEIVED JULY 14, 1941

THE PREPARATION OF NICOTINIC ACID FROM PYRIDINE

Sir:

The recent report by Gilman and Spatz [Gilman and Spatz, THIS JOURNAL, **63,** 1556 (1941)] on the preparation of 3-cyanoquinoline from 3-bromoquinoline and the hydrolysis of the cyano compound to the corresponding acid prompts the publication of a parallel synthesis in the pyridine series on which we have been working. Since 3-bromopyridine may be prepared by the direct bromination of pyridine [Englert and McElvain, *ibid.*, **51,** 863 (1929); Wibaut, *et al.*, *Rec. trav. chim.*, **51,** 381 (1932)], the synthesis now reported makes nicotinic acid readily available from pyridine. The following is the procedure by which 3-cyanopyridine (nicotinonitrile) was prepared. To 6.25 g. (1 mol) of 3-bromopyridine in a Claisen flask set for vacuum distillation was added 5.5 g. (1.5 mol) of cuprous cyanide ["Organic Syntheses," Coll. Vol. I, p. 38]. The mixture, which warmed spontaneously, was heated to 165–170° in an oil-bath for one hour. The resulting black viscous reaction product was then heated under about 30 mm. pressure with a smoky burner flame until no more volatile material came over. The nitrile that distilled over solidified in the receiver. After recrystallization from ligroin (b. p. 60–68°) the yield of product that melted at 49–50° [Fischer, *Ber.*, **15,** 63 (1882)] amounted to 2.1 g. (50%).

3-Cyanopyridine is readily converted to nicotinic acid by hydrolysis. The following procedure was found to be satisfactory. A solution of 3.6 g. of 3-cyanopyridine and 4 g. of sodium hydroxide in 40 ml. of 70% alcohol was refluxed