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

for three hours. The solvent was then removed by evaporation and the residue dissolved in 25 ml. of water. This aqueous solution after cooling to 0° was carefully neutralized with the calculated amount of hydrochloric acid. The precipitated nicotinic acid after recrystallization from water amounted to 3.8 g. (90%) and melted at 231–232°.

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RECEIVED JULY 14, 1941

THE CONCENTRATION OF "FOLIC ACID"

Sir:

Using *Streptococcus lactis R* as a test organism, we have obtained in a highly concentrated and probably nearly pure form an acid nutrilite with interesting physiological properties.

Four tons of spinach have been extracted and carried through the first stages of concentration. A considerable portion of this material has been subjected to an extended process involving essentially successive adsorptions on and elutions from charcoal, followed by successive precipitations with lead and silver salts and chromatographic adsorption on fullers' earth.

The material contains nitrogen, no sulfur or phosphorus and has a molecular weight of about 500 as determined by diffusion of the active principle and possesses high physiological activity.

This acid, or one with similar chemical and physiological properties, occurs in a number of animal tissues of which liver and kidney are the best sources. It is widespread in the biological kingdom. Mushrooms and yeast are good sources. It is especially abundant in green leaves of many kinds, including grass. Because of this fact, and since we have obtained what appears to be a nearly pure chemical entity, we suggest the name *folic acid* (Latin, folium—leaf). Many commercially canned greens are nearly lacking in the substance.

The basal medium used for the microbiological test was the same as described in another publication [E. E. Snell and H. K. Mitchell, *Proc. Nat. Acad. Sci.*, **27**, 1 (1941)] except that guanine, adenine, xanthine and uracil were added in amounts of 50 γ each per tube. These latter substances increase the sensitivity of the test but are inactive singly or collectively. Growth responses are determined by the thermoelectric turbidimeter of Williams, *et al.* [R. J. Williams, E. D. McAlister and R. R. Roehm, *J. Biol. Chem.*, **83**, 315 (1929)] and a growth curve may be illustrated as follows.

γ "folic acid" prep. per ml.	Turbidity reading (galv. defl.)
0.0	9.7
.000025	14.0
.000075	19.8
.000175	24.8
.00025	27.5
.0005	32.0

The concentrated substance stimulates the growth of *L. delbrückii* and *L. casei* with similar conditions and dosages.

"Folic acid" stimulates *L. casei* under the same conditions as the factor reported by Snell and Peterson [E. E. Snell and W. H. Peterson, *J. Bact.*, **39**, 273 (1940)] and recently reported to be isolated by Stokstad [E. L. R. Stokstad, *J. Biol. Chem.*, **139**, 475 (1941)]. A possible identity of the two substances is thus indicated, but chemical evidence shows dissimilarity since Stokstad reports a considerable phosphorus content in the factor he isolated while this element is absent from "folic acid." Another marked difference lies in the degree of biological activity. "Folic acid" in the purest form obtained produces approximately a half maximum growth in our microbiological test at a level of 0.00012 γ/ml. while this effect was obtained by Stokstad under his testing conditions, at about 0.014 γ/ml.

Indications have been obtained that the substance may have vitamin-like properties for animals. In a series of six rats on a control diet the average gain was 64 g. per 21 days. Five rats of the same litter gained an average of 71.5 g. (correcting for sex differences) when 50 γ of a "folic acid" preparation per rat per day was given. Assays of the tissues of the animals suggest bacterial production in the intestine.

DEPARTMENT OF CHEMISTRY HERSCHEL K. MITCHELL
UNIVERSITY OF TEXAS ESMOND E. SNELL
AUSTIN, TEXAS ROGER J. WILLIAMS

RECEIVED JUNE 20, 1941

AN ADDITION REACTION OF ALKALI-TREATED SILK, INVOLVING A NEW SYNTHESIS OF CYSTINE

Sir:

We have recently reported (in press) conclusive evidence of a striking lability toward alkali which serine and threonine show when (and only when) in combined form. At the same time, we