

## A NEW BIOS FACTOR IN LIVER EXTRACT

*Sirs:*

The known bios factors to date are inositol, thiamine,  $\beta$ -alanine, pantothenic acid, vitamin B<sub>6</sub>, biotin, and biotic acid. That there may be other unknown yeast growth-stimulating substances is suggested in a recent report by Williams *et al.*<sup>1</sup>

By fractionating liver extract we have been able to obtain a fraction which, when added to Williams' yeast growth medium, strongly stimulated the growth of a certain strain of yeast.<sup>2</sup> The detailed description of the preparation of this fraction which contained 1 mg. of N per cc. will be reported subsequently. It is based on the further fractionating of the phenol eluate prepared as described by Subbarow and Jacobson<sup>3</sup> in their isolation of the antipericious anemia fraction of liver extract. The phenol eluate was subjected to acetone-ether precipitation, the filtrate extracted with alcohol, and the alcoholic solution, freed of alcohol, was precipitated with acetone. Most of the activity was found in the acetone extract.

We believe that this fraction contains a substance or substances distinct from the hitherto described bios factors. Inositol, thiamine,  $\beta$ -alanine, and vitamin B<sub>6</sub> separately or combined were inactive on the growth of our strain of yeast; pantothenic acid<sup>4</sup> had only slight effect. In the presence of 0.04  $\gamma$  of thiamine, 0.04  $\gamma$  of vitamin B<sub>6</sub>, 0.005 mg. of inositol, and 1.0  $\gamma$  of  $\beta$ -alanine per cc. of medium, the amount of yeast growth after 18 hours was identical with that in the presence of medium alone. Pantothenic

<sup>1</sup> Williams, R. J., Eakin, R. E., and Snell, E. E., *J. Am. Chem. Soc.*, **62**, 1204 (1940).

<sup>2</sup> Generously supplied by Haffenreffer Brewing Company, Strain 1101.

<sup>3</sup> To be published. The charcoal eluate described by Subbarow, Jacobson, and Fiske (*New England J. Med.*, **214**, 194 (1936)) was precipitated by ammonium reineckate and the precipitate was extracted with hot water. Reinecke acid was removed from the hot water-insoluble fraction, which was then adsorbed on charcoal and eluted with phenol.

<sup>4</sup> Generously supplied to us by Dr. R. J. Williams.

acid in amounts up to 1.0  $\gamma$  per cc. of medium produced only a 20 per cent increase in growth.

In addition, we have been able to obtain evidence that our material differs from biotin. This evidence is as follows: The probable identity of biotin with vitamin H has been reported by György *et al.*<sup>5</sup> Our present preparation was assayed by Dr. György for its vitamin H activity and was found to contain 1 vitamin H unit per mg. of N. A potent vitamin H preparation (containing 1000 units per cc.) obtained from Dr. György was tested on our yeast and when used in amounts varying from 0.01 to 100 units of vitamin H gave much less growth (300 to 630 per cent increase in growth above that on the medium alone) than 0.01 vitamin H unit of our material alone (2440 per cent increase in growth). When 0.01 vitamin H unit of our material was added to 10 units of Dr. György's preparation, growth was greatly enhanced; *i.e.*, growth was more than 4 times that obtained with 100 units of Dr. György's material alone. We believe therefore that the active principle in our material differs from vitamin H and biotin.

Our active material is heat-stable, stable to acid hydrolysis, fairly stable to alkaline hydrolysis, soluble in organic solvents such as ether, alcohol, and benzene, precipitated by phosphotungstic acid, adsorbed by charcoal, and not destroyed by  $\text{HNO}_2$  at room temperature.

*Medical Research Laboratories*  
*Beth Israel Hospital*  
*Departments of Medicine and Biochemistry*  
*Harvard Medical School*  
*Boston*

BENJAMIN ALEXANDER  
YELLAPRAGADA SUBBAROW

Received for publication, June 25, 1940

---

<sup>5</sup> György, P., Melville, D. B., Burk, D., and du Vigneaud, V., *Science*, **91**, 243 (1940).