

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°.

DEPARTMENT OF CHEMISTRY
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S. M. McELVAIN
M. A. GOESE

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 nutritive 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. def.)
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.

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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