

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.

DEPARTMENT OF CHEMISTRY
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HERSCHEL K. MITCHELL
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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

suggested good reasons for believing that the serine which disappeared under these conditions was dehydrated to a peptide of dehydroalanine.

Some of the reasons have been discussed [B. H. Nicolet, *THIS JOURNAL*, **53**, 3066 (1931); *J. Biol. Chem.*, **95**, 389 (1932); *J. Wash. Acad. Sci.*, **28**, 84 (1938)] for believing that such a peptide should be especially activated for addition reactions. We are accordingly studying the reaction of various sulfur compounds on suitable proteins in the presence of alkali.

We report here specifically on the reaction of benzyl mercaptan with whole silk, in the presence of alkali. Insofar as our theory of the reaction mechanism is correct, a certain amount of addition to the dehydroalanyl residues of the protein would be expected, with formation of an occasional S-benzylcysteine unit in the peptide chain.

After hydrolysis of such a product, the technique of reduction in liquid ammonia should permit the rather quantitative conversion to cysteine of any S-benzylcysteine present.

Silk was chosen as a particularly suitable protein complex because of its conspicuously low cystine content and its conspicuously high serine content. Dried whole silk (3.923 g.), 1.98 g. of benzyl mercaptan neutralized with 16 cc. of 1 *N* sodium hydroxide, and 112 cc. of 0.1 *N* sodium hydroxide, were boiled for one hour in a nitrogen atmosphere. After acidification and hydrolysis

an aliquot of the hydrolyzate was reduced with sodium in liquid ammonia by the technique cited. Cysteine was then determined in the hydrolyzate and in the reduced hydrolyzate by the specific Sullivan method. The values before and after reduction were 0.4 and 3.3%, respectively.

The difference between the two values quoted must represent (as a minimum) the extent of the original addition of benzyl mercaptan. Incidentally, this is of course a new cystine synthesis.

We are engaged in other experiments of a similar nature, in which (among other compounds) cysteine and sodium sulfide will be tested for their additive powers. [We already have evidence that at least a 1% increase in the cystine content of casein may be produced by 14 days incubation of this protein in an excess of 2% sodium sulfide solution.] We believe that we shall be able to throw considerable light on the mechanism by which lanthionine [M. J. Horn, D. B. Jones and S. J. Ringel, *J. Biol. Chem.*, **138**, 141 (1941)] is formed in the course of alkali treatment of wool and other proteins. And the way in which selenium enters the protein molecule [M. J. Horn and D. B. Jones, *ibid.*, **139**, 649 (1941)] may also come to seem quite simple.

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RECEIVED JULY 10, 1941

NEW BOOKS

Organic Chemistry. By FRANCIS EARL RAY, University of Cincinnati. J. B. Lippincott Co., 220 N. Michigan Avenue, Chicago, Illinois, 1941. x + 706 pp. Price, \$4.00.

In the preface the author describes this text as "an attempt to lead the student from the most elementary beginnings of the subject to the point where he can read with profit much of the current literature." This book should be eminently successful in accomplishing this aim. It proceeds from a clear and simple explanation of the introductory material to a more complete coverage of the field than is customarily found in texts of this kind. This great abundance of material has been carefully chosen and organized in a manner that gives sufficient emphasis to the methods of the science.

A few of the many excellent features of the book may be

mentioned. Students and teachers will find that the summaries of specific and general methods of synthesis at the end of each chapter are extremely useful. In addition to providing a review for the student, they often give a critical comparison of the available methods. Too often the student learns reactions without obtaining any idea of their practical value. The illustration of molecular structure by reproducing, side by side, photographs of models of the Stuart type and the older variety is particularly effective. The chapters dealing with diazonium compounds and with polynuclear hydrocarbons are especially good. The tables of physical constants which also contain the melting points of derivatives suitable for identification, and the author's excellent treatment of nomenclature, are also worthy of note.

The book is as modern as a text in this rapidly advancing