affect secondary teachers in service. The proposed regulations would become effective for those who receive the provisional college certificates subsequent to September 1, 1942.

We believe recommendation (a) to be of high importance. Under the certification "science" a person may actually be certified to teach the sciences, with as little as three semesters in the science he may be asked to teach, a condition we believe to be indefensible.

Recommendations (b) and (c) are concerned with correcting the present practice of allowing certification in the sciences by groups of sciences which thereby permits certification of inadequately trained persons. If a person is poorly trained in physics and chemistry both, he is no better trained by certifying him in natural science.

Recommendation (e) differs very little from a similar recommendation circulated under date of January 12, 1939, by the Department of Public Instruction and sent to school officials throughout the state. The essential difference is that this committee believes that positive safeguards must be set up to insure that subject-matter training go hand in hand with professional training. It is felt that it is just as important to know what you teach as how to teach it. Particularly it is desired to emphasize that certification in more than one science should occur only upon completion of adequate training in the subject-matter (18 semester hours) in each of the sciences concerned.

This committee believes that now, when the need for more adequate science instruction is so clear, is the time to determine upon new standards. It is true that the scarcity of teaching personnel may force temporary measures under emergency conditions. But a permanent policy may nevertheless be set up now. The undersigned respectfully urge upon you the need for taking constructive action in this connection.

The membership of this committee and the societies represented are as follows: Dr. M. H. Trytten, *Chairman,* National Defense Research Committee—Pennsylvania Conference of College Physics Teachers; W. H. Michener, *Secretary,* Carnegie Institute of Technology —Association of Physics Teachers of Western Pennsylvania and Environs; Dr. E. L. Haenisch, Villanova College—Pennsylvania Chemical Society; Dr. John C. Johnson, Edinboro State Teachers College—Pennsylvania Academy of Science; Dr. C. O. Oakley, Haverford College—Philadelphia Section of the Mathematical Association of America; Dr. F. W. Owens, Pennsylvania State College—Allegheny Mountain Section of the Mathematical Association of America; Dr. F. C. Stewart, Pennsylvania State College—Allegheny Mountain Section of the Society for the Promotion of Engineering Education.

SPECIAL ARTICLES

THE GROWTH-STIMULATING EFFECT OF BIOTIN FOR THE DIPHTHERIA BACILLUS IN THE ABSENCE OF PIMELIC ACID[1, 2]

In a study of the accessory growth factors for the diphtheria bacillus, Mueller[3] discovered that pimelic acid stimulated the growth of the Allen strain and some of the Park 8 strains.[4] Pimelic acid proved to be specific in this effect in so far as the higher and lower homologues of this dicarboxylic acid were inactive. In an attempt to detect pimelic acid in connection with other work in this laboratory, it occurred to us that the effect of pimelic acid on the growth of the diphtheria bacillus might be utilized as the basis of a microbiological assay method for this compound. We have found that cultures of the Allen strain[5] which were recently transferred from Loeffler serum medium to a liquid medium grew very little in the absence of pimelic acid, whereas the addition of increasing amounts of pimelic acid within certain limits resulted in proportional increments of growth. The procedure gives promise as a method for the bio-assay of pimelic acid.[6] The idea of utilizing the diphtheria organism for this purpose has also led us to the recognition of an interesting relationship between pimelic acid and biotin.

Several years ago we tested vitamin H (biotin)

[1] The authors wish to express their appreciation to the S.M.A. Corporation for a research grant which has aided greatly in this work. They also wish to thank Mr. W. O. Frohring and the Research Staff of the S.M.A. Corporation and Dr. R. Major and the Research Staff of Merck and Co., Inc., for supplies of biotin.

[2] After this work had been initiated by us we learned that Dr. E. E. Snell reported at the annual meeting of the Federation of American Societies of Experimental Biology held at Boston that Dr. R. E. Eakin in Professor Roger J. Williams's laboratory had found that a larger amount of biotin was synthesized by *Aspergillus niger* when pimelic acid and cystine were added to the medium.

[3] J. H. Mueller, *Jour. Biol. Chem.*, 119: 121, 1937; and *Jour. Bact.*, 34: 163, 1937.

[4] J. H. Mueller, *Proc. Soc. Exp. Biol. and Med.*, 36: 706, 1937.

[5] This strain was made available through the generosity of Professor J. Howard Mueller.

[6] The liquid medium employed was the same as that employed by Mueller (*Jour. Bact.*, 36: 499, 1938). The culture was transferred from the solid Loeffler medium to the liquid medium, which contains pimelic acid and was incubated at 34–35° C. At the end of 48 hours a loop-full (2 mm loop) of the pellicle was transferred to a second tube of liquid medium. Solutions to be tested together with the pimelic acid-free medium were inoculated with a loop-full of the pellicle which formed in the second tube after incubation for 24 hours at 34–35° C. Micro-Kjeldahl determinations were used to determine the amount of bacterial nitrogen produced in 64-hour cultures incubated at 34–35° C., according to the procedure used by Mueller (*Jour. Bact.*, 29: 383, 1935).

preparations for their effect on the growth of the diphtheria bacillus, and under the conditions of our experiment we found that biotin had no effect on the growth of this organism. Recently Landy *et al.*[7] reported that *Corynebacterium diphtheriae* (Park 8) grows in the absence of free biotin in the medium. While we were considering the utilization of the diphtheria organism for the assay of pimelic acid it occurred to us that perhaps pimelic acid might be a precursor for the bio-synthesis of biotin by these strains of the diphtheria bacillus. If this were the case biotin might support the growth of these organisms in a pimelic acid-free medium. This possibility was explored experimentally and we wish to report that in the absence of pimelic acid we find that biotin is an accessory growth factor for the Allen strain diphtheria bacillus.

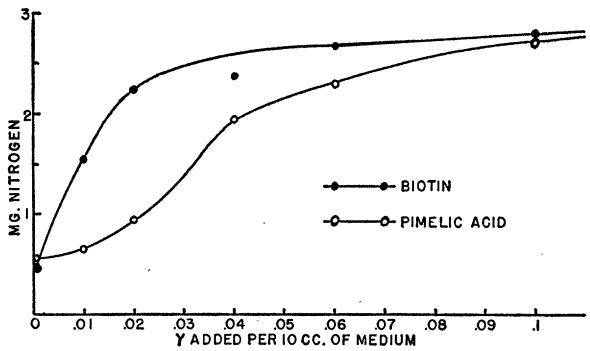

FIG. 1. Curves showing the effect of biotin and pimelic acid on the growth of the Allen strain diphtheria bacillus. Growth is expressed as milligrams of bacterial nitrogen per 10 cc of medium. Maximum growth was obtained by 1.5 γ of either biotin or pimelic acid, which produced respectively 4.5 and 4.7 mg of nitrogen.

Chart I shows the amount of growth obtained with both biotin and pimelic acid from 0.01 to 0.10 γ per 10 cc of medium. Under the conditions employed in these experiments maximum growth was obtained with approximately 1.5 γ of each, pimelic acid producing slightly more growth than biotin at this maximum level. Biotin, however, was more effective than pimelic acid at low concentrations. The addition of biotin to a medium containing a maximum amount of pimelic acid did not increase the growth obtained and undoubtedly explains the previous results which indicated a lack of an effect on the growth of diphtheria bacillus on the part of biotin.

It is worth noting that avidin prevents the growth-stimulating effect of biotin for the diphtheria bacillus when biotin is added to a pimelic acid-free medium, but does not prevent the growth-stimulating action of pimelic acid. It was also found that pimelic acid was unable to replace biotin in its growth-stimulating effect on yeast.

These experimental results may be interpreted on the basis of the pimelic acid being utilized by this diphtheria bacillus for the synthesis of biotin. The results obtained are highly suggestive of this but can not be regarded as conclusive proof. These findings raise the interesting possibility that other organisms which are capable of growing without biotin in the medium may be able to utilize pimelic acid in the same manner. It should also be pointed out that pimelic acid may have to be taken into consideration in nutritional studies in which the synthesis of biotin by bacteria in the intestinal tract may play a rôle.

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A BIOSYNTHESIS OF BIOTIN[1,2]

PIMELIC acid, unlike other growth factors for bacteria and yeast, has been found significant for only a single type of organism, the diphtheria bacillus.[3] However, the close relationship between the B-vitamins and the nutrilites for lower forms of life suggests that pimelic acid is a compound of general biological importance.

A possible physiological role of pimelic acid occurred to us when du Vigneaud, Hofmann and Melville reported tentative structural formulae for biotin.[4] These investigators have carried out chemical degradations which indicate that the biotin molecule probably contains the side chain-$CH_2CH_2CH_2CH_2COOH$. The presence of such a radical in the structure of biotin suggested that pimelic acid might serve as a precursor in biological syntheses of biotin. Experimental studies we are reporting indicate this to be the case.

It seemed desirable to choose for the biosynthesis studies an organism whose rate of growth is not affected by either biotin or pimelic acid. *Aspergillus niger,* an easily cultured mold satisfying these conditions, was selected. As biotin does not stimulate the growth of this mold, it was assumed that it possessed

[1] The material reported in this note was included in a discussion by Dr. E. E. Snell at the annual meeting of the Federation of American Societies of Experimental Biology at Boston on April 2.

[2] This study was aided by a grant from Standard Brands Incorporated to Dr. R. J. Williams (University of Texas), and by a grant from the John and Mary R. Markle Foundation to Dr. Tom D. Spies (Nutrition Clinic, Hillman Hospital).

[3] J. H. Mueller, *Jour. Biol. Chem.,* 119: 121, 1937; *Jour. Bact.,* 34: 163, 1937.

[4] V. du Vigneaud, K. Hofmann, D. B. Melville, *Jour. Am. Chem. Soc.,* 64: 188, 1942.

[7] M. Landy, D. M. Dicken, M. M. Bicking and W. R. Mitchell, *Proc. Soc. Exp. Biol. and Med.,* 49: 441, 1942.