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.

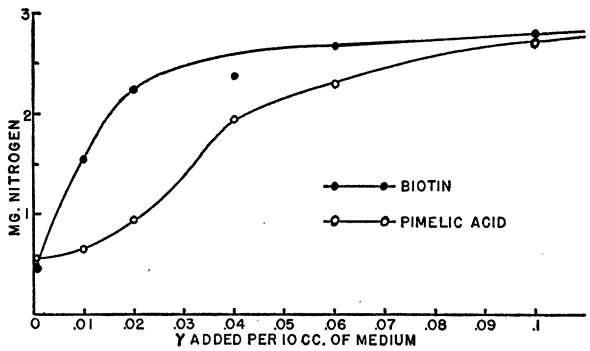


FIG. 1. Curves showing the effect of biotin and pimelic acid on the growth of the Allen strain diphtheria bacillus. Growth is expressed as milligrams of bacterial nitrogen per 10 cc of medium. Maximum growth was obtained by 1.5 γ of either biotin or pimelic acid, which produced respectively 4.5 and 4.7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

VINCENT DU VIGNEAUD
KARL DITTMER
ELEANOR HAGUE
BARBARA LONG

DEPARTMENT OF BIOCHEMISTRY,
CORNELL UNIVERSITY MEDICAL COLLEGE

## 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.

the mechanism necessary for the synthesis of biotin. It was necessary that pimelic acid have no effect on the growth of the organism used; otherwise, increased biotin production in cultures containing pimelic acid could be attributed to an increased growth of the cultures rather than to a conversion of pimelic acid into biotin.

The biotin free medium for yeast,[5] adjusted to pH 5.2, was used for culturing the mold. Sterilized 12 cc cultures (2 cc of addendum plus 10 cc of medium) were each inoculated with 2 drops of a suspension of *Aspergillus niger* spores. After 72 hours' incubation at 30° C., the cultures were autoclaved and filtered, and the biotin content of the filtrate determined by the yeast assay method.[5]

Results of a typical experiment, tabulated in Table I, demonstrate the activity of pimelic acid in promoting the synthesis of biotin.

TABLE I

| Culture | Addendum per culture | Biotin content of filtrate of culture |
|---|---|---|
| 1 | None | 0.006 microgram/culture |
| 2 | None | 0.007 |
| 3 | 1 mg pimelic acid | 0.096 |
| 4 | 1 mg pimelic acid | 0.108 |
| 5 | 1 mg cysteine | 0.011 |
| 6 | 1 mg cysteine | 0.016 |
| 7 | 1 mg cystine | 0.010 |
| 8 | 1 mg cystine | 0.012 |
| 9 | 1 mg pimelic acid + 1 mg cysteine | 0.192 |
| 10 | 1 mg pimelic acid + 1 mg cysteine | 0.180 |
| 11 | 1 mg pimelic acid + 1 mg cystine | 0.216 |
| 12 | 1 mg pimelic acid + 1 mg cystine | 0.180 |

In spite of the difference in the amount of biotin produced, there were no visible differences in the growth of the cultures.

In subsequent studies, it was found that the maximum production of biotin could be obtained with pimelic acid concentrations of 20 micrograms per 12 cc culture.

The lower homologues of pimelic acid, succinic, glutaric and adipic acids, and an isomer, β-methyl adipic acid, were tested and found inactive. The higher homologues, suberic and azelaic acids, however, have activity comparable to pimelic acid. The biotin active substance produced from any of the active dibasic acids react in the usual manner with avidin.[6]

Cysteine or cystine, sources of organic sulfur, were found to enhance the effect of pimelic acid. A study of the supplementary action of these and other sulfur-containing compounds has given erratic results, but this problem is being investigated further.

ROBERT E. EAKIN
ESTHER A. EAKIN
HILLMAN HOSPITAL, BIRMINGHAM, ALA., AND
UNIVERSITY OF TEXAS

[5] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[6] The physiological relationship between pimelic acid and biotin has been demonstrated independently by du Vigneaud, Ditmer, Hague and Long, who have shown that biotin is a growth stimulant for the diphtheria bacillus in the absence of pimelic acid.

## TETRAPLOIDY IN ANTIRRHINUM MAJUS INDUCED BY SANGUINARINE HYDROCHLORIDE

IN the fall of 1941, Dr. Glenn A. Greathouse, discussing his work on the influence of alkaloids on the growth of fungi, mentioned that the alkaloid sanguinarine had produced peculiar swellings in the hyphae of certain fungi. This observation led the author to try the effect of sanguinarine on seedlings of *Antirrhinum majus* to determine whether it would produce polyploidy in a manner similar to colchicine.

In December, 1941, 100 seedlings of Antirrhinum (snapdragon, variety White Prosperity) were treated by placing a drop of 0.2 per cent. sanguinarine hydrochloride solution on the terminal growing point of each. At the same time, 100 seedlings of the same variety were treated with 0.2 per cent. colchicine, another 100 seedlings were treated with 0.2 per cent. lycorine (tried because, like colchicine, it is derived from monocotyledonous plants), and another 100 seedlings were left untreated for a check.

The toxic effect of the sanguinarine was very obvious within 24 hours after treatment, practically all the seedlings showing some dead tissue where the drop had been applied. At first the growth of the seedlings was greatly retarded, but after several weeks normal growth was resumed and the plants were examined for abnormalities of the leaves or stems. Eighteen of the plants were selected as appearing somewhat abnormal, and these were repotted for growing to maturity. While these plants appeared to have larger and thicker leaves than normal, the leaves had none of the roughened or wrinkled appearance characteristic of the seedlings treated with colchicine. Of these 18 plants, 9 were lost, due to an error in handling, but the remaining 9 were grown to maturity, and chromosome counts were made from propionocarmine smears of the pollen-mother-cells. Five of the plants were found to be tetraploids, and the remaining four diploids. Two of the tetraploids had some diploid branches, which had emerged below the point of treatment. Because some of the plants were lost, we can only say with certainty that tetraploidy was induced in at least 5 per cent. of the treated plants. This compared favorably with the results from 100 seedlings treated with colchicine, in which 4 tetraploids were found (a much higher percentage of tetraploidy has been induced by colchicine, however, using the same method of treatment, but repeating it 3 or 4 times at 3-day intervals). No tetraploids were found