SYNTHESIS OF VITAMIN B BY MICROORGANISMS

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Studies on the synthesis of vitamins have led to no general agreement as to the ability of specific microorganisms to produce vitamin B or as to the extent of this synthesis among the lower plant forms.

This lack of agreement may be attributed in part to the failure of investigators to keep clearly in mind the characteristics and properties that define a vitamin and in part to the marked differences in methods which have been employed.

There is, on the one hand, the group which has adhered rigidly to the conception of a vitamin as defined by Drummond (1924), i.e., a substance of unknown constitution neither fat, protein, carbohydrate, nor mineral salt, necessary for the life and well-being of a suitable organism. On the other hand, there is a group which has disregarded the requirements of Drummond and has used as the criterion of the presence of vitamin B, the ability of a substance to stimulate multiplication of a specific microorganism. The yeast growth test of Williams (1919) employs the multiplication of Saccharomyces cerevisiae.

If we exclude from analysis results which are not in strict conformity with the definition of Drummond, we are still confronted by the use of a multiplicity of methods all purporting to detect the presence of vitamin B. The animal used may have been the rat, mouse or pigeon. In certain cases a few milligrams have constituted the entire quantity of microorganisms fed per rat per day and the ability of an organism to synthesize vitamin B has been decided upon the results obtained in such experiments. Again, the period of feeding has varied from two to several
weeks. Generally, no cognizance has been taken of the manner of feeding, or the time required by the animals to become accustomed to the taste of the microorganisms. In feeding certain microorganisms, the animals may continue to lose weight for as long as a week. In certain cases the organisms have been fed apart from the basal ration, whereas, in other cases they have been intimately mixed with the constituents of the basal ration. Again, too little attention has been given to the freedom of the culture medium from vitamin. At the most, it should not contain more than traces of vitamin. Vitamin-free synthetic media are to be preferred.

For the purpose of this investigation Drummond's definition of a vitamin is accepted; no other conception is justifiable in the light of present knowledge and no adequate proof has been offered that vitamin B, as defined by Drummond, has any effect on the rate of multiplication of microorganisms. In fact, Werkman (1927) has shown by an analysis of experimental data on multiplication of Azotobacter chroococcum and Rhizobium leguminosarum that vitamin B does not stimulate multiplication of these organisms.

The purpose of this investigation was (1) to determine whether vitamin B is synthesized by certain bacteria, one torula and forms intermediate between molds and bacteria, (2) to choose these organisms from biologically separate groups of the lower plant forms so as to give an indication of the general occurrence of vitamin B synthesis among microorganisms, (3) to determine the variation, if any, in the ability of different strains of the same species to synthesize the B vitamin, (4) to make a quantitative study of the production of the vitamin, (5) to determine the effect of drying of the organisms on their vitamin content.

Feeding the microorganisms in testing for the vitamin has been quantitative so far as practicable. As much as 15 grams of organisms per rat per day have been fed. Care has been taken to accustom the rats to the diet and experiments have been continued until definite results were secured. The culture media have been synthetic or thoroughly extracted with 95 per cent hot alcohol to insure suitability for use. The uninoculated medium was always fed to control animals.
SYNTHESIS OF VITAMIN B BY MICROORGANISMS

LITERATURE

Among the workers using rats in determining the presence of vitamin B, were Wollman (1921) and Wollman and Vagliano (1922) who tested the Bulgarian bacillus and Amylomucor B for vitamins A and B. They concluded that neither vitamin A nor B was present. Slanetz (1923) using mice found that Azotobacter chroococcum, Bacterium lactis-acidi, Bacillus mycoides, Serratia marcescens, Rhizobium leguminosarum, Bacillus subtilis Micrococcus agilis and three other soil forms produced neither A nor B.

Cunningham (1924) fed young rats in order to test for both A and B in three strains of the tubercle bacillus. She found neither.

Pacini and Russel (1918) in some work referred to by McCollum and Simmonds (1925) as “not convincing” claim to have shown stimulation of the growth of rats by extracts of typhoid bacilli. Bierry and Portier (1918) injected under the skin and into the peritoneal cavity living cultures of some normal intestinal bacteria. Their animals showed improvement within twenty-four to forty-eight hours.

Damon (1921) has probably tested more organisms for vitamin B synthesis than any other worker who has published. His first results with para B, Bacterium coli and Bacillus subtilis were negative. In a later publication (1923) he concluded that Pfeiffer’s bacillus and the timothy bacillus synthesized vitamin B but that Bacillus adhaerens and Friedlander’s pneumobacillus were negative. Damon (1924) believed that a substance analogous to vitamin B was produced by Mycobacterium smegmatis, Mycobact. phlei and Mycobact. moelleri. Hunter (1923) concluded that Azotobacter synthesized vitamin B. Kuroya and Hosoya (1923) concluded that Bacterium coli was capable of synthesizing B. Hoet, Leclef and Delarue (1924) using both rats and pigeons found Torula rosea, and Mycoderma cerevisiae negative but Monilia candida positive.

Funk’s pigeon test has been used extensively to determine the presence of vitamin B. McCollum and Simmonds (1925) gave evidence to support the view that “the pigeon test as ordinarily
carried out is without value for the specific purpose for which it has been so widely used."

Cooper (1914) fed *Bacterium coli* to pigeons on a polished rice diet but found no evidence of vitamin B. Weill, Arloing and Dufourt (1922) fed the colon bacillus and three spore formers from the intestinal tract of pigeons to pigeons on a polished rice diet. The birds died in what was considered normal time for polyneuritic pigeons.

Scheunert and Schieblich (1922, 1923) fed cultures of various bacteria to pigeons. They concluded that there was some effect due to vitamins in certain bacteria and no effect with other organisms. Eijkman, Van Hoogenhuije and Derks (1922) concluded from their experiments that *Bacterium coli* contains no antineuritic factor. They used pigeons in their work.

William's test for vitamin B depends upon the assumption that the increased multiplication of yeast cells upon the addition of alcoholic extracts of various substances is a measure of water-soluble B and that only. Souza and McCollum (1920) and Fulmer, Nelson and Sherwood (1921) have presented evidence to the effect that the yeast growth test is unreliable.

Robertson (1924) using William's yeast growth test concluded that *Bacterium coli*, *Serratia marcescens*, *Bact. proteus*, *Pseudomonas pyocyanea*, *Bacillus subtilis* and *Sarcina lutea* synthesized a vitamin.

Thjotta (1921) obtained evidence of a growth promoting substance for the influenza bacillus from *Bacillus proteus*, *Bacillus ozaenae* and Friedlander's pneumobacillus. Damon (1921), however, did not confirm these results.

**METHODS AND MATERIAL**

The authors have felt that the only adequate test for the presence of vitamin B is the effect of a substance upon the growth of young 50-gram white rats. Weighings of the rats receiving the substance to be tested, of control rats receiving the B deficient ration, and of rats receiving the basal ration made complete by the addition of 5 per cent wheat embryo or yeast, were made and growth curves over a period of several weeks compared. Metal
SYNTHESIS OF VITAMIN B BY MICROORGANISMS

cages with a small quantity of shavings were used. The cages were cleaned weekly.

The basal ration consisted of alcohol extracted casein 18 parts, corn starch or dextrin, 73.3 parts, salt mixture 3.7 parts, salt-free butter fat 5 parts. The salt mixture was that of McCollum and Davis (1915) modified by the addition of 0.002 gram of KI and the substitution of ferric citrate for the lactate. The casein was continuously extracted with hot 95 per cent alcohol for seventy-two hours.

Young 45- to 50-gram rats were kept on this ration until definite symptoms of vitamin deficiency were observed before feeding of the organisms was started.

The microorganisms were generally fed moist in a separate container; at other times, they were mixed with the basal ration as indicated in the individual experiment. The organisms were generally grown on media in 18- by 40-inch pans. The media used are indicated under the experimental results with each organism. They were synthetic where practicable. Where peptone or agar was used, each had been continuously extracted with hot 95 per cent alcohol for never less than one week. The media were always fed to control animals as a check.

EXPERIMENTAL RESULTS

Torula rosea

Torula rosea from the stock culture collection of the department was grown on medium E of Nelson, Fulmer and Cessna (1921), modified by the use of alcohol extracted agar and sucrose. Surface growth from the pans was scraped off after three days and fed moist. In the feeding of Torula rosea mixed with the basal ration, it was found that the rats consistently refused to eat appreciably of the mixture.

When the torula was fed separately, the rats are it readily. This may account for the fact that Torula rosea is reported in the literature as not synthesizing vitamin B. By actual determination it was found when the torula was mixed in the basal ration,
the rats received approximately 2 grams per rat per week, an amount too small to supply the vitamin need of the animal.

In figure 2 the animals received the mixed ration from the third until the end of the fifth week. Beginning with the sixth week the torula was fed separately at the rate of 8 grams per rat per day. One animal was in a moribund condition and died. The remaining two began to gain in weight immediately. A gain of approximately a gram per rat per day body weight is recorded for each day torula was fed apart from the basal ration.
SYNTHESIS OF VITAMIN B BY MICROORGANISMS

Oöspora lactis

No record of the synthesis of vitamin B by Oöspora lactis has been found. We have found it a rich source of the vitamin.

The organism was grown on a synthetic medium:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grams</th>
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<tbody>
<tr>
<td>K₂HPO₄</td>
<td>2.0</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>2.0</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>0.5</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.1</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>0.1</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose (extracted)</td>
<td>15.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0</td>
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This medium gave a heavy pellicle growth within four days, which could be removed en masse. Quantitative feeding experiments were made with this organism to determine the requirements of rats. One of several experiments is given in figure 3. The organism was fed at the rate of 2.5 grams per rat per day during the sixth week. It was then fed ad libitum during the seventh week when the rats consumed approximately fifteen grams per rat per day. During the eighth week it was again fed in amounts of 2.5 grams and ad libitum during the ninth and
tenth weeks. When fed at the rate of 2.5 grams per rat per day the animals made no gain and if larger quantities had not been tried Oöspera lactis would have been considered incapable of B synthesis. Larger quantities fed to the same animals gave excellent gains. Oöspera lactis is a good source of vitamin B, distinctly better than Torula rosea.

**FIG. 3. EFFECT OF Oöspera lactis UPON THE GROWTH OF VITAMIN B DEFICIENT RATS**

Organism feeding started at ↓

_Bacillus adhaerens_

_Bacillus adhaerens_ was chosen primarily because we were desirous of employing in certain experimental work an organism incapable of synthesizing vitamin B and this one had been so reported in the literature.

The organism was grown on extracted peptone agar in large petri dishes. The composition of the medium was:

<table>
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<th>Grams</th>
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</thead>
<tbody>
<tr>
<td>Alcohol extracted peptone</td>
<td>7.0</td>
</tr>
<tr>
<td>Alcohol extracted agar</td>
<td>15.0</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>2.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0</td>
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</tbody>
</table>
SYNTHESIS OF VITAMIN B BY MICROORGANISMS

The growth was scraped from the medium after forty-eight hours incubation and fed separately. Three grams per rat per day were given to the rats in the experiment shown in figure 5. In this particular experiment after feeding the organisms for two weeks they were omitted from the ration. A drop from 16 to 2.6 grams occurred in the average gain per rat per week. Bacillus adhaerens is an excellent synthesizer of vitamin B.

![Graph showing growth of Actinomyces](http://jb.asm.org/

**Actinomyces**

**Fig. 4. Effect of Actinomyces upon the growth of vitamin B deficient rats**

Organism feeding started at ↓

**Bacterium coli**

Three strains of Bacterium coli were tested in this experiment to determine the relative potencies of the different strains. Four grams per rat per day were fed separately. The results demonstrated that Bacterium coli is capable of synthesizing vitamin B and that no significant differences exist in the ability of the different strains to perform the synthesis. A growth
curve showing the average increase in weight of three rats fed strain no. 24 is shown in figure 5.

**Bacillus subtilis**

The organism was grown on extracted peptone agar, scraped off and fed apart from the basal ration. In the experiment graphed in figure 5 the rats received 6 grams per rat per day until the eighth week when they received 1 gram per rat per day.

![Diagram of weight increase over time](image)

**Fig. 5. Effect of Bacteria upon the Growth of Vitamin B Deficient Rats**

Organism feeding started at ↓

In order to demonstrate that the marked loss of weight resulting was due to insufficient vitamin, the rats were again fed 6 grams per rat per day during the ninth week. Although *Bacillus subtilis* serves as an excellent source of vitamin B, one gram per rat per day of the microorganism is insufficient to maintain body weight.

Drying the bacterial growth at 37°C. for two days had no detectable effect on the vitamin potency.
**SYNTHESIS OF VITAMIN B BY MICROORGANISMS**

**Bacillus mycoides**

Quantitative feeding results with *Bacillus mycoides* are given to demonstrate the effects of feeding various amounts of an organism. It is apparent that if 2 grams or less per rat per day of mycoides were fed, the conclusion might be drawn that the organism is unable to synthesize vitamin B, whereas, our results

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**Fig. 6. Effect of Bacteria upon the Growth of Vitamin B Deficient Rats**

Organism feeding started at ↓
demonstrate that mycoides is a good source of vitamin B when compared with other organisms. In the results of the experiment given in figure 7, groups of rats were fed simultaneously 0.5, 1.0, 2.0, 4.0 and 8.0 grams of *B. mycoides* per rat per day. The group receiving 0.5 gram per rat per day lost weight just slightly less rapidly than the rats receiving no organisms; while the group receiving 1.0 gram per rat per day in two week's time averaged a slight loss of weight. The groups receiving 2.0, 4.0 and 8.0 grams gained in order of increasing quantity fed.
During the third week of the feeding of the organism (fig. 8) the rats that had been receiving 8.0 grams were given 2.0 grams and vice versa. The rats that had received 2.0 grams per rat per day had made an average gain of 2 grams per rat per week.

When fed 8.0 grams per rat per day they made an average gain of 24 grams in one week. This marked increase can be accounted for only as due to the increase in the amount of the organism fed.

The rats that had been given 8.0 grams per rat per day made
an average gain for the two weeks of 12 grams per week. When reduced to 2.0 grams the average gain per week was 7 grams.

Organisms dried at 37°C and at 100°C for forty-eight hours were fed in order to determine the effect of desiccation on the vitamin potency. The results indicated no effect of drying at 37°C and only a slight effect at 100°C.

*Azotobacter chroococcum*

This organism was grown on Ashby’s medium modified by the use of alcohol extracted sucrose. The animals ate readily of the bacterial growth when mixed with the basal ration or when fed separately. In figure 6 are shown the results of feeding 6 grams per rat per day separately. Two grams per rat per day were found to be inadequate to provide sufficient vitamin B for increase in body weight.

*Rhizobium leguminosarum*

*Rhizobium* was grown on Ashby’s medium modified as for *Azotobacter*. The strain happened to be one isolated from alfalfa roots. Synthesis of vitamin B by this organism is indicated by the growth curve in figure 6.

*Actinomyces (species unknown)*

An unknown soil actinomyces was grown on the following medium:

<table>
<thead>
<tr>
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<th>Grams</th>
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<tbody>
<tr>
<td>K2HPO4</td>
<td>1.0</td>
</tr>
<tr>
<td>KNO3</td>
<td>2.0</td>
</tr>
<tr>
<td>Calcium succinate</td>
<td>10.0</td>
</tr>
<tr>
<td>Extracted agar</td>
<td>17.0</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>1.8</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0</td>
</tr>
</tbody>
</table>

Considerable difficulty was experienced in obtaining sufficient growth to feed. Long periods of incubation were necessary so that the growths scraped from large plate cultures were stored for several days before the experiment began in order to have sufficient growth to complete the feeding. Six grams per rat per day
to 2 rats constituted the only work done with actinomycetes. The growth curve is shown in figure 4. The results indicate a vitamin B synthesis by the organism.

**SUMMARY AND CONCLUSIONS**

The synthesis of vitamin B by such biologically separated genera of microorganisms as *Torula*, *Oospora*, *Actinomyces* and four genera of the order *Eubacteriales* reveals a general occurrence of vitamin B synthesis among widely separated groups of the lower plant forms. Vitamin B, whatever its structure chemically may be, is a constituent prevalent in microorganisms.

Specifically the following organisms were found to produce the growth promoting vitamin for white rats: *Torula rosea*, *Oospora lactis*, *Bacillus adhaerens*, *Bacterium coli*, *Bacillus subtilis*, *Bacillus mycoides*, *Azotobacter chroococcum*, *Rhizobium leguminosarum* and *Actinomyces* (species unknown).

The results showed no marked differences in the ability of three strains of *Bacterium coli* to synthesize vitamin B.

Drying at 37°C. or at 100°C. for forty-eight hours does not materially diminish the vitamin potency of the bacterial mass.

Several reasons are suggested to account for the discrepancies present in the literature dealing with the synthesis of vitamin B by microorganisms. Our conceptions of a vitamin are not in accord and thus differently defined the term vitamin assumes an ambiguous and obscured meaning until it is in direct conflict with the original conception intended.

After limiting by definition the meaning of the term vitamin to the legitimate conception expressed by Drummond we find a multiplicity of methods employed to determine the synthesis of a vitamin by microorganisms. Important among these are (1) quantity of organism fed, (2) period of feeding and time allowed for the rats to become accustomed to the bacterial diet, (3) manner of feeding organisms to the rats, (4) species of animal used as an indicator.
REFERENCES


COOPER, E. A. 1914 On the protective and curative properties of certain food stuffs against polynestis induced in birds by a diet of polished rice. Jour. Hyg., 14, 12.

CUNNINGHAM, RUBY L. 1924 An attempt to determine the presence of vitamin A and B in tubercle bacilli. Amer. Rev. Tuberc., 9, 487.

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DAMON, SAMUEL R. 1924 Acid-fast bacteria as a source of vitamin B. Jour. Path. and Bact., 27, 163.


KUBOTA, Dr. Masahiko, AND HOSOYA, Dr. Seigo 1923 The synthesis of water-soluble vitamin by coli bacillus grown on synthetic medium. Scientific reports from the Government Institute for Infectious Diseases, Tokyo Imperial Univ., 2, 287.


SYNTHESIS OF VITAMIN B BY MICROORGANISMS


WERKMAN, C. H. 1927 Vitamin effects in the physiology of microorganisms. J. Bact., 14, 335.

