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The Effect of Vitamin B₁ on the Growth of *Paramecium multimicronucleatum*

Irene Alma Herbst
INTRODUCTION

THE EFFECT OF VITAMIN B₁ ON THE GROWTH OF PARAMECIUM MULTIMICRONUCLEATUM.

By Irene Alma Herbst

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INTRODUCTION

The effect of Vitamins on the growth and development of plants and animals has created a great deal of interest in the scientific world.

So rapidly has vitamin research progressed and so fast has the knowledge regarding the chemistry of vitamins accumulated that it is sometimes hard to realize that the vitamins at first regarded as imponderable medical curiosities and accessory food substances are now looked upon as essential nutritive factors.

Today there are several factors recognized in vitamins. Vitamin A was reported first in 1913 by McCollum and Davis, and Osborne and Mendel to be a fat soluble substance necessary for normal growth.

In 1795 the British navy in an effort to control scurvy by administering lemon juice to sailors recognized the value of Vitamin C. However, an investigation of this factor was not carried out until 1907 when Holst and Froelich began their studies on the role of Vitamin C in the control and cure of scurvy.

Vitamin D because it is found together with Vitamin A in nature at first did not receive the attention due to it as a separate factor. Even today the two vitamins are often associated together.
It is probable that Vitamin D is not essential to some growth of epithelial cells and not sufficient to support epithelial cell growth by itself, but is a powerful stimulant to cell growth in the presence of Vitamin A."

There are other vitamin factors such as Vitamin E, and Vitamin K which have not yet received complete recognition but which are steadily making a place for themselves in clinical practice.

After Vitamins A, C, and D had been identified and established as separate nutritive factors there still remained a complex group of several factors to be analysed. Vitamin B as it was known before 1926 was a complex substance composed of a number of components which had certain chemical characteristics.

In the year 1926 Vitamin B was divided into two constituents. These two fractions were designated as B and G or B₁ and B₂. Upon further experimentation seven or eight vitamins were isolated from these two factors. Elvehjem classifies them as follows:

1. Vitamin B₁-Thiamin-Antineuritic factor
2. Riboflavin-Flavin factor
3. Nicotinic acid-P-P factor-antipellagra factor
4. Vitamin B₆-Rat antidermatitis factor
5. Chick antidermatitis factor
6. Factor W
7. Vitamin B₄ "2

It is with the first of these, Vitamin B₁ or Thiamin Chloride, that this thesis is concerned. Vitamin B₁ is water soluble, and thermal labile. It is the only

1 McLaughlin, R.R. : Medical Times 67:303 (1939)
2 Elvehjem, C. A. : Symposium on Nutrition. May 26, 1936
known vitamin to contain an amino group.

By means of isolation techniques Ohdake, Seidell and Windaus ascertained the chemical composition of Vitamin B₁ to be C₁₂ H₁₆ N₄ SO₂⁻. In 1935 due to cleavage experiments of Williams the following chemical formula was derived:

\[ \text{N} = \text{C} - \text{NH₂} \cdot \text{HCl} \quad \text{C} = \text{C} - \text{CH₂} \text{CH₂ OH} \]
\[ \text{CH₃} \quad \text{CH} \quad \text{CH} \quad \text{N} \quad \text{CH} \quad \text{Cl} \]

Vitamin B₁ is considered to be essential for growth of microorganisms, of the roots of plants and of animals. The assimilation of Thiamin in the body is as follows. Thiamin unites with two molecules of phosphoric acid to form a pyrophosphate which is identical with cocarboxylase. This coenzyme affects the decarboxylation of pyruvic acid to acetaldehyde. By aiding in this oxidation process Thiamin prevents the accumulation of lactic and pyruvic acid in the brain, heart and blood which would in turn hinder carbohydrate metabolism and internal respiration.

The purpose of this experiment is to determine the effect of Vitamin B₁ on the size and number of paramecium in solutions of various concentrations.
MATERIALS AND METHODS

In each of four glass culture bowls was placed 500 cc. tap water and a few handfuls of wheat straw. To these cultures various amounts of Vitamin B₁ solution were added.

The B₁ solution was prepared as follows. A .4 gram capsule was divided into half. This .2 gram capsule was dissolved in 200 cc. water which made a solution of one part per 100 or 200 milligrams pure B₁ in 200 cc. water. To this solution was added 8000 cc. water to make a solution of one part per thousand.

To the four culture bowls as prepared above were added ½ cc B₁ solution; 1 cc B₁ solution and 5 cc B₁ solution. One culture jar remained without any B₁ and was used as a control.

To these jars were added equal amounts of Paramecium multimicronucleatum.

The cultures were left undisturbed for two days. On the third day the count was started. With a dropper three drops from various sections of the culture were taken. Each drop was equal to 1/50 of a cc.

A small drop of formaldehyde was placed on the slide with the drop from the culture to kill the paramecium so as to make it easier to measure them. The paramecium in a drop were measured with a micrometer taking three paramecium from different sections of the slide. The number of paramecium on a slide
were then counted. This was repeated three times for each culture.

The count was taken of the four culture jars on alternating days until six counts had been taken over a period of two weeks.

RESULTS

Number of Individuals

The experiment shows that the $\frac{1}{2}$ cc. B concentration has no characteristic effect on the number of individuals. The curve is not comparable at any time with the normal or control curve. With the 1 cc. concentration the greatest increase was obtained. Although the 1st day there were more paramecium in the $\frac{1}{2}$ cc. B than in the control the rate of increase for the next 3 days was slower. However from the fourth day on the rate of increase was greater in the control although the two solutions followed the same line of deviation on alternating counts. When a count was taken on the paramecium in the 1 cc which showed an increase over the last count the count in the control would show a decrease in the last count and vice versa.

In the five ml. solution for the last six days the count never reached the standard set by the control.
After the sixth day the count increased rapidly and from then on was higher than the control.

(Table I. Graph I and II)

Size Of Individuals.

As regards to the size of the individuals the experiment shows that for the first two days there is a decrease in size for the control while the decrease in the \( \frac{1}{2} \) cc. lasted two days, the 1 cc. four days and the 5 cc. three days. However the rate of decrease in the control and five cc. corresponds, and the rate of decrease of the \( \frac{1}{2} \) cc. and the 1 cc. corresponds.

(Table II. Graph III and IV)

DISCUSSION

Number Of Individuals.

The \( \frac{1}{2} \) ml. \( B_1 \) is detrimental to the growth in that there is just enough \( B_1 \) in the solution of this concentration to prevent normal increase in number because the paramecium must adapt themselves to its presence and when this adaptation has taken place there is not enough \( B_1 \) supplied in the solution to meet their requirements.

The 1 cc. provides a balance between the amount of adaptation needed and the concentration of the solution and a good working medium is established. There is neither too much nor too little \( B_1 \).
In the 5 cc. B₁ solution because of the high concentration it takes the paramecium a long time, six days, to adapt themselves but when once adapted there is enough B₁ to supply their needs and to bring their rate of increase above standard.

Size Of Individuals:

In an analysis of the results in regards to size of protozoans in a culture there must be kept in mind the periods of increase and decrease in a regular protozoan culture. As comparable with this regular increase and decrease this experiment shows that the 1 ml. B₁ solution is the best solution for concentration. (It must be noted that the sizes of the paramecium at the first count varied considerably.)

While the ½ ml solution for the first three days shows a rate of decrease parallel to the rate of decrease in the 1 ml. the increase in size is not in proportion to the decrease as it is in the 1 ml. solution.

The 5 ml. and the control solutions show no regularity whatsoever. These two solutions are similar in their rate of decrease for the first three days but there the similarity stops. From
the third day the 5 ml. B₁ decreases at a rate equal to that of the 1 ml. and from the sixth day to the eighth day their rates of increase are the same.

The control after running parallel to the 5 ml. for the first 3 days changes its rate and increases at a rate similar to that of the ½ ml. from the third to the eighth day. All the other solutions show that the maximum size was reached at the 8th day and from then on decrease began and continued at various rates. However in the control the rate of decrease was very small and lasted only two days at which time there was an abrupt change to increase and on the 13th day the maximum increase was reached.

From this it can be seen that the only solution with any regularity in its rates of increase and decrease in size is the 1 ml. B₁ solution.

SUMMARY

The 1 ml. B₁ to 500 ml. tap water wheat straw culture was the solution of the best concentration for both the number and size of individuals.

The 1 ml. B₁ solution gave the greatest number of individuals and the rate of increase
and decrease closely followed the control.

The 1 ml. B₁ solution presented the only regularity in the rate of increase and decrease in the size of the individuals.

The other concentrations of Vitamin B₁ were detrimental to the growth of paramecium in so far as there either was not enough Vitamin to outweigh the harm it did in making adaptation necessary or there was too much Vitamin to permit regular adaptation at a sufficient rate.
### TABLE I

**Number of Individuals**

<table>
<thead>
<tr>
<th>DAYS</th>
<th>CONTROL</th>
<th>1/2 c.c.B.</th>
<th>1 c.c. B.</th>
<th>5 c.c. B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>5</td>
<td>11</td>
<td>9.3</td>
</tr>
<tr>
<td>3</td>
<td>29.3</td>
<td>11.6</td>
<td>19.3</td>
<td>24.3</td>
</tr>
<tr>
<td>6</td>
<td>13.3</td>
<td>12.6</td>
<td>42</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>34.6</td>
<td>14.5</td>
<td>31</td>
<td>15.6</td>
</tr>
<tr>
<td>10</td>
<td>27.6</td>
<td>8.6</td>
<td>71</td>
<td>51.3</td>
</tr>
<tr>
<td>13</td>
<td>9.6</td>
<td>22</td>
<td>42</td>
<td>36.6</td>
</tr>
<tr>
<td><strong>TOTAL AVERAGES</strong></td>
<td><strong>20.73</strong></td>
<td><strong>12.38</strong></td>
<td><strong>36.05</strong></td>
<td><strong>25.65</strong></td>
</tr>
</tbody>
</table>
Graph I

No. of Individuals

Days

- CONTROL
- 0.5 ML.
- 1 ML.
- 5 ML.
TABLE II

Size of Individuals MM

<table>
<thead>
<tr>
<th>DAYS</th>
<th>CONTROL</th>
<th>½ cc B</th>
<th>1 cc B</th>
<th>5 cc B</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>.184</td>
<td>.195</td>
<td>.204</td>
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<td></td>
<td>3</td>
<td>.175</td>
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<tr>
<td></td>
<td>6</td>
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<td>.203</td>
<td>.200</td>
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<tr>
<td></td>
<td>13</td>
<td>.216</td>
<td>.201</td>
<td>.202</td>
</tr>
<tr>
<td>TOTAL AVERAGES</td>
<td>19.11</td>
<td>.2023</td>
<td>.2070</td>
<td>.1983</td>
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GRAPH III

Size of Individuals

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<th>Size (mm)</th>
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<tbody>
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<td>0.170</td>
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<td>0.185</td>
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<td>0.190</td>
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<tr>
<td>0.195</td>
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<td>0.200</td>
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<td>0.210</td>
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<td>0.220</td>
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<tr>
<td>0.225</td>
</tr>
</tbody>
</table>

Days

- CONTROL
- 1/2 c.c.
- 1 c.c.
- 5 c.c.
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Major Professor

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Date _______________