VITAMIN SYNTHESIS IN PLANTS AS AFFECTED BY LIGHT SOURCE.*

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An inspection of the literature dealing with the subject of vitamins furnishes evidence to indicate that they are probably chemical compounds in nature, losing their potency easily through changes (oxidative, hydrolytic, or splitting in nature) whose exact nature has never been fully established. There is much to indicate that they are compounds existing in an active and an inactive form mutually interchangeable through processes not yet known. It is commonly conceded that the vitamin potency of most substances decreases with age, heat, and exposure to light, due to changes previously suggested. Articles pertaining to this subject are too commonly known and too numerous to be listed here. Vitamin C is undoubtedly most susceptible to this deterioration with vitamins A, D, and B following in order of instability. Granting that the vitamins are unstable and susceptible to some form of change as suggested, and postulating the existence of an active and inactive form, the question follows: By what methods are the compounds first activated or synthesized? It is to be deduced from the findings of others and from an inspection of data accumulated, that the actuation accompanies living processes which take place primarily in the plant, and probably is in some way dependent upon light rays; possibly it is directly related to the photosynthesis taking place and likewise dependent upon the chlorophyll content of the plants.

It is generally conceded that vitamin C rapidly increases in the seed during germination. Chick and Delf (1) definitely demon-

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Light Effect on Vitamin Synthesis

Honeywell and Steenbock (2) demonstrated that this increase was dependent upon the presence of light, while Eggleton (3) particularly emphasizes the necessity of light. Vitamin A was reported by Coward and Drummond (4) as synthesized by green-colored plants. Wilson (5) was of the opinion that germination produced it even in etiolated seeds, while Harrow and Krasnow (6) confirm Coward and Drummond. Widmark (7) reports that plants that lose their chlorophyll content likewise lose the power to synthesize vitamins. Coward (8) has since reported that some is formed even when seeds germinate in darkness; and Moore (9), in a carefully planned piece of work, affirms this finding. Very recently Evans and Hoagland (10) have reported that Canadian field peas synthesize vitamin E on germinating, while Stepp (11) has not been able to show any such increase in vitamin D in any germination process.

The present investigation was undertaken in an attempt to ascertain as far as possible the following points: (1) Is vitamin synthesis or actuation in plants dependent upon light rays, or is it a function of germination? (2) If light proves to be the determining factor, are waves of certain lengths responsible for the change? (3) Are the vitamins which are most susceptible to destruction, likewise most readily actuated? (4) From a commercial standpoint, would it be better to use vegetables grown in the open air or in the greenhouse? (5) Would it be possible to increase the vitamin content by artificial illumination with some definite light wave?

EXPERIMENTAL.

Much of the early work in this investigation was concerned with a study of the nature of germination of seeds and their relative rate of growth when sprouted in complete darkness, in sunlight, and under glass of various colors, as well as under the Mazda, arc, and ultra-violet lamps. Similar observations have been recently published by others in very complete form, in articles dealing particularly with the effect of light upon the germination of seeds, and need only be briefly mentioned here in so far as they are concerned in this problem. Those interested in a more complete account should read the reports from the Boyce Thompson Insti-
tute of Plant Research, as well as others in the literature pertaining to that field. In this work, not only the physical appearance of the sprouts was observed, but also the rate of photosynthesis was measured under the various conditions by a modification of the Spoehr method. The seeds used were wheat and yellow milo maize. In all cases the seeds were treated with diluted formaldehyde to destroy any spores, then thoroughly washed, and quickly air-dried. The germinating medium was blotting paper in some of the work, but more frequently fine sand was used which had been digested first with alkali, and then with acid, and finally with much water. The germinating temperature approximated 25°, and the various lots were grown simultaneously. To summarize very briefly, it was observed that the etiolated sprouts seemed to grow fastest; that is, they were longer and thinner in structure. Leaves were slower in forming, and were lacking in chlorophyll. The sun-grown sprouts were much more sturdy, and quickly produced normal leaves. Those under the ultraviolet lamp grew well at first and resembled the sun-grown, but soon began to show red streaks in the leaves and shortly afterward growth ceased entirely. Those under the Mazda and arc lamps compared more nearly with those grown in the sunlight and under glass. It was noted that as the wave-lengths became shorter or the light more intense, the plants became more squat in form and more highly colored. In an attempt to gauge the actual conditions more closely, the photosynthetic rates of lots similar in all respects save the light waves, were measured by the CO₂ exchange. It was observed that those plants grown in darkness always produced the greatest amount of CO₂ in a given length of time, and that the amount decreased as the shorter rays were increased. It was observed that sunlight through quartz glass produced less CO₂ than through ordinary glass. This introduced a question that has had considerable bearing upon the problem as well as upon some of the previously published work; that is, what effect would this differing metabolic rate have upon the final weight of the seedlings and the amounts of feed eaten by the animals in the biological tests to be described? In order to answer this question, careful observations were made at frequent intervals. Given amounts of seeds were weighed out and germinated under similar conditions, save the nature of the light, for the same
length of time, then carefully dried and reweighed. A typical example may be used as an illustration.

<table>
<thead>
<tr>
<th>Light Effect</th>
<th>Weight after germinating 8 days.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>gm.</td>
</tr>
<tr>
<td>Ultra-violet light</td>
<td>gm.</td>
</tr>
<tr>
<td>Mazda lamp</td>
<td>gm.</td>
</tr>
<tr>
<td>Arc lamp</td>
<td>gm.</td>
</tr>
<tr>
<td>Sunlight</td>
<td>gm.</td>
</tr>
</tbody>
</table>

If in feeding tests one were to use equal weights of these dried samples, it is obvious that different amounts of the original grains were being used and the assumption must be made that constituents of the seed are being used up simultaneously at the same rate. That such an assumption is not well founded is proved by chemical study of the resulting products, which will be reported separately at a later date. On the other hand, to assume that the loss all comes from the non-vitamin content and that the vitamin thereby becomes concentrated as suggested by some is dependent upon an assumption, data for which are not available and which our data seem to contradict. Likewise, even air-drying the seedling, which would be necessary for weighing amounts, is subject to criticism, as some of the vitamins must be injured. After accumulating data for over 2 years it became evident to us that all attempts to solve this problem by the use of dried products have been open to serious criticism, and our work has been completely repeated, using green seedlings taken directly from the germinators kept under conditions as similar as possible, fed to test animals by hand daily in like numbers, and the results noted. Even this procedure does alter the non-vitamin content slightly and assumes that no vitamin is consumed by the seed in growth. This introduces many obstacles. It is difficult to keep germinators at the same degree of temperature and at the same humidity. New lots of seeds must be constantly started. Moulds must be carefully controlled, it having been previously found by the writer (unpublished data) that mould growing on seed synthesizes vitamin. The germinating medium (digested sand) must be free from vitamin itself.
In the biological tests of the vitamin content of the seedlings, rats were used for vitamins A and B, and guinea pigs for vitamin C. All animals used were from our own colonies. All precautions usually observed in this type of work were observed; namely, all animals were normal individuals, caged so as to be comparable as to litter origin, age, weight, and sex. They were kept in cylindrical metallic cages frequently cleaned and sterilized. They were fed from special feed cups so made as to measure the amount of basal ration eaten. They were fed and watered daily and weighed biweekly. The basal ration for the rats for vitamin A determinations consisted of:

<table>
<thead>
<tr>
<th></th>
<th>per cent</th>
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</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>73.5</td>
</tr>
<tr>
<td>Casein</td>
<td>18</td>
</tr>
<tr>
<td>Salt Mixture 185*</td>
<td>3.5</td>
</tr>
<tr>
<td>Yeast</td>
<td>5.0</td>
</tr>
</tbody>
</table>


The dextrin was starch hydrolyzed with 1 per cent citric acid for 2 hours. The casein was carefully water-washed for several weeks. The dextrin was prepared from starch by hydrolyzing for vitamin A determination, and was further alcohol-extracted and dried at 100°. In the work on vitamin A, 5 per cent ground Fleischmann's yeast was used as a source of vitamin B. The vitamin B-short rations consisted of:

<table>
<thead>
<tr>
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<th>per cent</th>
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</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td>75</td>
</tr>
<tr>
<td>Casein</td>
<td>18</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>3.5</td>
</tr>
<tr>
<td>Salts</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The basal vitamin C ration used for the guinea pigs consisted of:

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Ground corn</td>
<td>100 parts</td>
</tr>
<tr>
<td>&quot; oats</td>
<td>100 &quot;</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1 part.</td>
</tr>
<tr>
<td>NaCl</td>
<td>1 part.</td>
</tr>
<tr>
<td>Tankage</td>
<td>2 parts.</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1 cc. per day</td>
</tr>
</tbody>
</table>

Alfalfa hay was autoclaved in live steam at 30 pounds pressure for 3 hours and fed *ad libitum*. While this ration may not be entirely free from vitamin C, it produces much more uniform
results and keeps the animals from other disturbances; scurvy appeared at about the 22nd day.

With both rats and guinea pigs, control lots were always used, and when it was found that variations occurred here, the entire series was discarded. In the case of the rats, the animals were first kept upon the basal rations until the characteristics of vitamin shortage became evident, and then the seedlings were added in such amounts as to bring the animals just back to normal. The preliminary work used to determine these amounts is omitted to save space. It might be added that the dextrin and casein were exposed to the ultra-violet rays to safeguard the vitamin D requirements. However, the stunting from the lack of this exposure, as reported by Steenbock and Coward (12) was not observed, possibly due to the fact that the animals were in a room receiving the brilliant illumination of this climate.

**Vitamin A Studies.**

The results obtained in the case of vitamin A were the most satisfactory. It was observed, however, with lots fed seed grown under certain colored lights, that the results were somewhat open to question and these have not been included in this report. When various trials revealed similar results, the figures have been averaged, and the growth curve of the test animal given in Chart I represents the average of fifteen or more animals fed at various times of the year. Curves are shown for growth of animals fed seeds germinated: (1) in open sunlight; (2) under the ultra-violet light, with 3 hours exposure per day; (3) under the Mazda light, with 12 hours exposure per day; (4) in darkness as complete as possible; and ungerminated. The curves speak for themselves.

Undoubtedly light causes a synthesis of vitamin A, and the intensity of the light and the shortness of the wave seem to accelerate the synthesis. From other data not shown in the curves, it becomes evident that seeds germinated under quartz glass are richer in this substance than seeds grown under glass, other conditions being similar. While the difference is slight, the data would indicate that seeds grown in the open sunlight should always be slightly superior. Whether a longer exposure to the ultra-violet light would have caused a greater synthesis was not determined, as it was found that long exposure seemed to be injurious to the
CHART I.
growth of the plant itself. There is no question of the ability of light from the Mazda lamp and also the arc to produce a similar synthesis. Since a photometer was not available, the completeness of the illumination cannot be expressed in exact terms; but the full light of a 100 kilowatt lamp was directed upon the seeds at short range, producing a brilliant illumination.

From our observations, germination in the dark always slightly increased the growth of the rats. Like Moore (9), we cannot say whether this was due to the germination itself, or to the slight amount of light that seeds, kept even under the most careful conditions, must obtain. A possibility not mentioned by these authors might be the stimulation of appetite due to new products formed in the green feed even though vitamin was entirely absent, resulting in a temporary increase of food eaten. In these studies both wheat and yellow milo maize were used. The curve represents the results of feeding four seeds of milo per rat per day, added at the point where the animal growth curve had ceased.

*Vitamin B Studies.*

The investigation of vitamin B synthesis was carried on in a similar fashion, although the literature gave no encouragement; in fact, there are hints which suggest that germination may be injurious to this vitamin. Growth curves in this case, as illustrated in Chart II, represent the average of twelve or more animals. The seeds were both milo maize and wheat, as shown in the separate drawings. However, in this case the number of seeds was increased to an amount found advisable in the preliminary work. Eight seeds per day per rat, and later as much as twenty-four seeds per day, were added of the milo, while eight seeds, and later twenty-five seeds, of the wheat were used. The results as indicated in the chart, show that the average growth curves of the various lots fed the milo are so nearly the same that no conclusion can be drawn, but that the formation of vitamin in the plant comes at a time later in its development and evidently is not a function of the nature of the light during the germination and early seedling period. In the case of the wheat, there was a slight increase in weight in some rats receiving the green germinated seeds; the gains were small and irregular, however, and would not justify
CHART II.
Light Effect on Vitamin Synthesis

an assumption of much synthesis. Those grown under other lights show no increase.

_Vitamin C Studies._

Data obtained from the use of guinea pigs are always more tedious to obtain and subject to more criticism. The conclusion to be drawn, however, comes from observations of many lots of animals observed over a period of 2 years, only the most typical being used to illustrate what was thought to be generally characteristic. The lots fed at various periods of the year did not compare so favorably as in the case of the rats, probably due to other factors introduced by varying temperatures. Several rations recommended by various workers did not prove so satisfactory as the one indicated in this paper, while scurvy in no case developed as rapidly as reported by others. The general condition of the animals seemed to be better on this ration. Wheat, barley, and milo maize were used in the experiments in 10 and 15 grain amounts per animal per day. The results are summarized in Table I. An examination of the data will indicate that germinated seeds always increase the growth, and improve the appearance of the animals, and delay scurvy development. The check lots, which are not listed, invariably succumb to scurvy, showing little or no benefit from an equal amount of the dried seed. The seeds germinated, and permitted to grow to seedlings about 2 inches tall in the sunlight, always furnished the most potent source of vitamin; those grown under the Mazda light were somewhat less satisfactory. Those grown under the ultra-violet light, exposed for about 3 hours per day, in no case proved as satisfactory, probably due to the fact that the seedlings turned red and failed to develop normally. The seeds germinated in the dark, while possessing much less vitamin than the light-grown seedlings, did permit the animals to live after all the group being fed dried seeds had succumbed to scurvy. While the results obtained with the ultra-violet light do not prove that the short wave-lengths are responsible for the synthesis, the fact that open sunlight is more favorable for vitamin formation than light filtered through glass, indicates that it in some way assists in vitamin formation.
### TABLE I.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight..................</td>
<td>days 70</td>
<td>gm. 48</td>
<td>gm. 300</td>
<td>gm. 445</td>
<td>gm. 145</td>
<td>Good shape.</td>
</tr>
<tr>
<td>Dark.......................</td>
<td>70</td>
<td>50</td>
<td>350</td>
<td>465</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Mazda lamp.................</td>
<td>70</td>
<td>51</td>
<td>270</td>
<td>430</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Sunlight..................</td>
<td>70</td>
<td>52</td>
<td>220</td>
<td>365</td>
<td>145</td>
<td>Good shape.</td>
</tr>
<tr>
<td>Dark.......................</td>
<td>70</td>
<td>53</td>
<td>270</td>
<td>255</td>
<td>15</td>
<td>Scurvy.</td>
</tr>
<tr>
<td>Mazda lamp.................</td>
<td>70</td>
<td>54</td>
<td>295</td>
<td>340</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>(Winter test.) Germinated yellow milo seedlings (10 gm. per day).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunlight..................</td>
<td>60</td>
<td>55</td>
<td>210</td>
<td>340</td>
<td>130</td>
<td>Good shape.</td>
</tr>
<tr>
<td>Mazda lamp.................</td>
<td>60</td>
<td>56</td>
<td>310</td>
<td>395</td>
<td>85</td>
<td>Fair</td>
</tr>
<tr>
<td>Dark.......................</td>
<td>60</td>
<td>57</td>
<td>350</td>
<td>300</td>
<td>50</td>
<td>Scurvy.</td>
</tr>
<tr>
<td>Germinated wheat (15 gm. per day).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunlight..................</td>
<td>54</td>
<td>28</td>
<td>250</td>
<td>325</td>
<td>75</td>
<td>Good shape.</td>
</tr>
<tr>
<td>Ultra-violet light.........</td>
<td>54</td>
<td>29</td>
<td>210</td>
<td>270</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Mazda lamp.................</td>
<td>54</td>
<td>30</td>
<td>410</td>
<td>250</td>
<td>Died.</td>
<td>Scurvy.</td>
</tr>
<tr>
<td>Dark.......................</td>
<td>54</td>
<td>31</td>
<td>260</td>
<td>310</td>
<td>50</td>
<td>Fair shape.</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>170</td>
<td>210</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(LOSS.) Germinated yellow milo seedlings (10 gm. per day).

Sunlight.................. | 60 | 33 | 380 | 410 | 50 | Good shape. |
| Mazda lamp................. | 60 | 34 | 250 | 260 | 10 | |
| Dark....................... | 60 | 35 | 275 | 240 | 35 | Poor shape. |
| (Loss.) Died. Scurvy. | | 36 | 195 | | |
| Dark....................... | 60 | 37 | 330 | | Died. | Scurvy. |
| | 38 | 325 | 320 | 5 | |

Germinated wheat (15 gm. per day).

Sunlight.................. | 54 | 23 | 210 | 306 | 96 | Good shape. |
| Ultra-violet light......... | 54 | 24 | 185 | 230 | 45 | |
| Mazda lamp................. | 54 | 25 | 320 | 370 | 50 | Fair shape. |
| Dark....................... | 54 | 26 | 200 | 185 | Died. | Poor |
| | 27 | 210 | 255 | 45 | Fair |
| Mazda lamp................. | 54 | 28 | 250 | 325 | 75 | Good shape. |
| Dark....................... | 54 | 29 | 210 | 270 | 60 | Fair |
| | 30 | 410 | 250 | Died. | Scurvy. |
| | 31 | 260 | 310 | 50 | Fair shape. |
| | 32 | 170 | 210 | 40 | |
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TABLE I—Concluded.

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</tr>
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<tbody>
<tr>
<td>Germinated wheat (15 gm. per day).—Continued.</td>
<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td></td>
<td></td>
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<td>Sunlight</td>
<td>51</td>
<td>40</td>
<td>300</td>
<td>385</td>
<td>85</td>
<td>Good shape. “ “</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>180</td>
<td>220</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultra-violet light</td>
<td>51</td>
<td>42</td>
<td>300</td>
<td>275</td>
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<td>Scurvy.</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>225</td>
<td>225</td>
<td></td>
<td>Poor shape.</td>
<td></td>
</tr>
<tr>
<td>Mazda lamp</td>
<td>51</td>
<td>46</td>
<td>300</td>
<td>350</td>
<td>50</td>
<td>Good shape. “ “</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>250</td>
<td>290</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>51</td>
<td>44</td>
<td>225</td>
<td>240</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>350</td>
<td>305</td>
<td>Died.</td>
<td>Scurvy.</td>
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</tbody>
</table>

Germinated wheat (10 gm. per day).

<table>
<thead>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight</td>
<td>57</td>
<td>17</td>
<td>350</td>
<td>388</td>
<td>36</td>
<td>Fair shape. “ “</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>290</td>
<td>350</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultra-violet light</td>
<td>57</td>
<td>21</td>
<td>270</td>
<td>320</td>
<td>24</td>
<td>Fair shape. “ “</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>340</td>
<td>322</td>
<td>18</td>
<td>Scurvy.</td>
<td></td>
</tr>
<tr>
<td>(Loss.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mazda lamp</td>
<td>57</td>
<td>15</td>
<td>310</td>
<td>350</td>
<td>40</td>
<td>Fair shape. “ “</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>320</td>
<td>370</td>
<td>50</td>
<td></td>
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</tr>
<tr>
<td>Dark</td>
<td>57</td>
<td>30</td>
<td>410</td>
<td>250</td>
<td>Died.</td>
<td>Scurvy.</td>
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<tr>
<td></td>
<td>31</td>
<td>260</td>
<td>310</td>
<td>50</td>
<td></td>
<td>Fair shape. “ “</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>170</td>
<td>210</td>
<td>40</td>
<td></td>
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</tr>
</tbody>
</table>

RESULTS.

1. The amount of vitamin A formed in seedlings seems to be a factor depending upon the light rather than upon changes taking place in the process of germination. Some increase is always found in etiolated seedlings.

2. The quantity of vitamin A synthesized is dependent upon the intensity of illumination, length of exposure, and evidently the relative amount of shorter wave-lengths, and follows closely the rate of growth of the plant.
3. Vitamin B is evidently not increased in the germination and early growth of the seedlings, its formation coming at a later period of development of the plant.

4. Vitamin C is formed more rapidly than either of the other vitamins studied. There is evidence that germination alone, even in the dark, produces a considerable amount of vitamin C. It increases in light-grown seedlings to an extent that is greater than can be accounted for on the basis of the increased growth of the seedlings, and its production is evidently accelerated like vitamin A, by increased intensities of light.

5. Evidence is furnished that plants grown in the open sunlight under intense illumination should be slightly superior sources of vitamin.

**BIBLIOGRAPHY.**

VITAMIN SYNTHESIS IN PLANTS AS AFFECTED BY LIGHT SOURCE
V. G. Heller