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Vitamin K in Germinating Peas

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A survey of the literature reveals only two instances of vitamin K formation in germinating seeds. Dam and Glavind [1] studied the effect of light on the formation of vitamin K in germinating peas and used the biological method for determining the vitamin. The plant material was dried and fed to test animals (chickens) either as such or as a petroleum ether extract. The vitamin amount was reported in Dam units and obtained by measuring the clotting time of blood. The findings showed that the vitamin K content of peas germinating in the presence of light for sixteen days was approximately treble the amount of vitamin K of seedlings kept a corresponding time in darkness. Dam *et al.*[2] later carried out a similar experiment with the germinating seeds of *Picea canadense* whose sprouts exposed to light were analyzed. Also the vitamin K-content of plants germinating in light was approximately double that of sprouts kept in darkness. In the latter case some vitamin K had formed in the darkness since the vitamin content after 14 days of germination was higher than in seeds.

The present paper relates to an experiment in which the vitamin K was determined colarimetrically by means of the redox method, the objective being to follow daily the vitamin K formation in peas and simultaneously compare the values obtained with those reported by Dam. The germinations were carried out both in the presence and absence of light.

EXPERIMENTAL

Vitamin K was determined according to the method of Scudi and Buhs [3] which is a colorimetric adaptation of the principles employed by Trenner and Bacher [4]. This method involves a catalytic hydrogenation of the vitamin K-quinone to the hydroquinone stage with Raney nickel catalyst in butanol solution, and use of phenosafranine as an indicator. Hydroquinone is reoxidized with 2,6-dichlorophenol indephenol in the

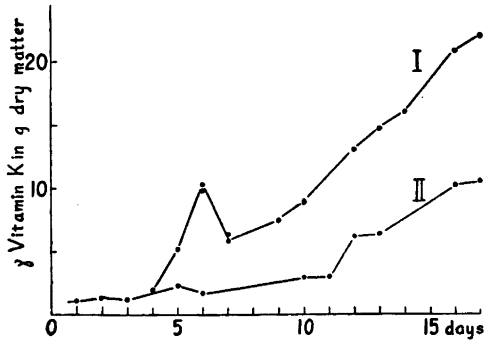

Fig. 1. Vitamin K content of peas germinating in (I) presence of light, (II) absence of light.

absence of air, and the decrease in colour is measured. Interfering colors present are removed by extracting the hydroquinone, after reduction with Claisen's alkali, in a special apparatus. The method is not specific since all quinones present give the same reaction.

The seedlings were allowed to germinate in damp quartz sand kept in a photothermostat. Two 200-watt lamps gave light during nine hours of the day. Of the 50 seedlings the best developed 40—45 were chosen for each determination. The seedlings were rinsed, dried between blotting paper and weighed. They were then cut into small pieces which were dried in a thermostat at 60° C for 12 to 18 hours and subsequently in a vacuum desiccator having nitrogen pressure of about 12 mm. The dried preparation was weighed and extracted in the dark with petroleum ether. Reduction in the modified apparatus of Scudi and Buhs lasted for 15 to 30 min, in some cases still longer, despite the relative rapidity of the hydrogen current.

The vitamin K content of peas does not change notably during the first four days, but from this time on the phylloquinone amount begins to increase in seedlings grown in light. Immediately after the epicotyl is exposed to light, the vitamin K amount in the seedlings increases five-fold during the fifth and sixth days, then decreases slightly during the seventh day but after that the vitamin amount of the seedlings exposed to light increases steadily until the seventeenth day.

In etiolated seedlings an increase in vitamin K content begins approximately at the end of the ninth germination day. In peas sixteen days old the vitamin content of those exposed to light was 20.8 and of those grown in the dark 10.1 γ per gram dry-material (Fig. 1).

DISCUSSION

Thayer *et al.*[5] define the activity of phylloquinone in a mg as 1000 Thayer-Doisy units, which corresponds to 10 Dam units. Hence, 1 Dam unit equals 0.1 γ of vitamin K. The 16 days old peas in the experiment of Dam and Glavind[1] consequently contained 33.5 γ and 9.5 γ vitamin K corresponding to our values of 20.8 γ and 10.1 γ. Our values for etiolated peas conform with

those of Dam and Glavind, but the ones for peas germinating in the presence of light are considerably lower. For removal of carotinoids the reduction prior to determination proper had to be repeated twice in our experiments which process probably caused some losses in green plants. Nor is the method quite specific as fairly large amounts of tocoferylquinone affect the result (Scudi and Buhs [6]).

Figure 1 is elucidative in showing the effect of light on the vitamin K formation. Vitamin K forms also in the absence of light and consequently its biosynthesis is not bound to chlorophyll formation as assumed by Dam *et al* [2]. Dam regards his findings of vitamin K in peas germinating in the dark so low that they hardly exceed the amount found in the seed itself. He explains that the vitamin K increase in the seedlings of *Picea canadense* germinating in the absence of light is due to chlorophyll-synthesis which, as is well known, is noted in evergreens despite the absence of light. The parallelism between vitamin K synthesis and chlorophyll synthesis is according to Dam, due to phytol occuring in both molecules. True, Dam himself remarks [2] that in the absence of light phytyl forms in connection with protochlorophyll which in turn can develope in the etiolated parts of plants. On the other hand, there is no evidence as to whether the material defined colorimetrically or by animal tests is, in reality, phylloquinone. In the animal tests also other naphtoquinone derivatives are active, and in the method employed all the quinones give the same reaction.

The vitamin K maximum occuring on the sixth day in peas germinating in light is interesting and deserves attention. As vitamin K accumulates in the chloroplasts [2] the great increase in vitamin K content can, during the time mentioned, be connected with the likewise intensive chlorophyll synthesis. Here mention is made of the fact that when peas germinate under conditions identical with those used in our experiments catalase, according to Virtanen *et al.*[7], also attains an activity maximum on the sixth day of germination. In a like manner this enzyme has greatly increased in the chloroplasts and its activity generally is proportional to the intensity of photosynthesis [8,9].

SUMMARY

The formation of vitamin K in peas germinating in the presence and absence of light has been studied. Vitamin K is also formed in the absence of light although in quantities considerably smaller than in the presence of light.

Between the fourth and sixth day the seedlings which developed in the presence of light were observed to increase greatly in vitamin content after

the exposure of the epicotyl. The maximum attained on the sixth day was followed by a slight drop and then by a gradual increase in the vitamin content beginning from the seventh day. Etiolated seedlings showed an increase in vitamin K only after the eleventh day.

The vitamin K content of ungerminated but swollen seeds was 1.2 γ per gram of dry matter. In 17 days the vitamin K content increased to 22.0 γ in the presence of light and to 10.3 γ in the absence of light.

REFERENCES

1. Dam, H., and Glavind, J. *Biochem. J.* **32** (1938) 485.
2. Dam, H., Glavind, J., and Nielsen, N. *Z. physiol. Chem.* **265** (1940) 80.
3. Scudi, J., and Buhs, R. *J. Biol. Chem.* **141** (1941) 451.
4. Trenner, N., and Bacher, F. *J. Biol. Chem.* **137** (1941) 745.
5. Thayer, S., Binkley, S., MacCorquodale, D., Doisy, E. Emmet, A., Brown, R., and Bird, O. *J. Am. Chem. Soc.* **61** (1939) 2563.
6. Scudi, J., and Buhs, R. *J. Biol. Chem.* **143** (1942) 665.
7. Virtanen, A. I., Kärkelä, A., and Rautanen, N. *Suomen Kemistilehti* **B 17** (1944) 21.
8. Krossing, G. *Biochem. Z.* **305** (1940) 359.
9. Nakamura, H. *Japan. J. Botany* **11** (1941) 221.

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