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## PRELIMINARY STUDIES ON RIBOFLAVIN (VITAMIN B<sub>2</sub>) CONTENT OF PLANT MATERIALS

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It will probably be agreed that the last decade has brought forth much startling and important knowledge in regard to the chemistry and physiology of the vitamins and there is every indication that there is still much to be done. One of the most interesting and intensive phases of this work has been the isolation and identification of the vitamins of the "B-complex." Aside from the common feature of water solubility, each one seems to be universally distributed in living, growing organisms which probably indicates that most of them, at least, are indispensable to all forms of life. Several members of this group which have been most studied, thiamin, riboflavin and nicotinic acid, have been shown to exert their influence as prosthetic groups for enzymes or as co-enzymes in cellular respiration. The author of a recent review (1) has stated that over 700 papers were written on the water soluble vitamins during 1941. However, even a cursory reading of the text reveals that the proportion of papers dealing with plant-vitamin relationships (excluding determinations on foods) is exceedingly small.

Green plants are known to be the primary seat of vitamin synthesis not only for themselves (wherein they are not vitamins according to definition) but for all animals dependent upon them directly or indirectly for food. The study of vitamin relationships in plants should be a

rich field of physiological endeavor but has been slow getting started mainly because of a lack of adequate methods for their determination. A recent publication by Williams and co-workers (2) presents microbiological assay methods which require little outlay for equipment and appear to be reasonably accurate.

The present investigator has applied the microbiological method for riboflavin to plant materials with a view of learning something about the occurrence, synthesis and possible functions of the substance in plants. One phase of the problem which is considered to be important is the relation of mineral nutrition to vitamin content of cereal grasses and is being studied at the present time in the laboratory at the University of Illinois.

The study of riboflavin in plants seems to have been almost untouched up to the present time but experiments with other vitamins can serve as a guide. Riboflavin content of several plant materials is presented and is used to illustrate possibilities for further research. Bonner (3) has studied the thiamin distribution and transport in tomato plants and reports that the thiamin content, on the basis of gammas per gram of dry weight, is highest in the youngest leaves and falls off with each successive leaf to a low figure in the oldest. The accompanying data on tobacco and tomato show no difference between the riboflavin content of young

TABLE I

Plant	Part	Riboflavin in gammas per gram dry tissue
Tobacco.....	Growing point and youngest leaves.....	10.88 ± 0.1
	Leaves, mature.....	11.15 ± 0.1
	" , senescent.....	8.00 ± 0.8
Tomato.....	Growing point and youngest leaves.....	23.90 ± 1.0
	Leaves, growing rapidly.....	23.71 ± 1.5
	" , mature.....	20.73 ± 1.0
	" , senescent.....	14.37 ± 1.0
Oat (Flowering stage).....	Roots.....	9.73 ± 0.5
	Leaves.....	23.38 ± 2.0
	Stem..... (includes leaf sheaths)	4.35 ± 0.4
	Spikelets.....	14.90 ± 0.1
Coleus.....	Leaf margins (chlorophyll).....	21.63 ± 0.9
	Leaf centers (no chlorophyll).....	22.17 ± 1.3
Magnolia.....	Flower petals.....	15.76 ± 1.3
Spinach..... (Var: Old Dominion)	Reproductive, male.....	11.58 ± 0.3
	" , female.....	16.49 ± 1.0
	Vegetative, male.....	8.75 ± 1.0
	" , female.....	10.48 ± 1.0

and mature leaves but shows a low value for senescent leaves. This may be due to destruction or removal of riboflavin from necrotic cells. By means of girdling experiments, Bonner was able to conclude that thiamin is apparently synthesized in the mature leaves and transported up or down the stem to the growing leaves and to the roots. This technique might be profitably applied to the other B vitamins.

The data on the oat plant indicates that the leaf is the place of synthesis of riboflavin. Its occurrence in the stem might be in part due to transport to the flowers and to the roots. The difference between the concentration in tobacco and tomatoes is interesting but should be confirmed because the tobacco samples were not analysed until several months after they were dried. They were stored at room temperature in light-tight containers.

As early as 1935, Kuhn and Kaltschmitt (4) investigated the possible relationship between riboflavin and chlorophyll in the green leaf but came to no definite conclusion. The results of analysis of variegated clove leaves and of magnolia petals would lead one to believe that there is no necessary relationship between chlorophyll and riboflavin content. It is especially interesting if one assumes that the only difference between

the cells of the margin and those of the center is presence or absence of chlorophyll.

Loehwing's (5) investigation on the chemistry of sex expression in spinach suggested that there might be a difference in vitamin content between male and female because of apparent differences in metabolic activity. The female plants, which seem to have a higher rate of nitrogen metabolism, also show a higher riboflavin content. This conforms to the previously reported (6) relation between protein synthesis and riboflavin content in oats.

The list of vitamins of the "B-complex" now includes 7 known chemical compounds and the list is probably not complete. The application of some of the above-mentioned methods to the study of each of these factors in plants offers a field of fundamental and productive research.

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