THE EFFECT OF BENZIMIDAZOLE ON THE BIOSYNTHESIS OF CHLOROPHYLL*

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In a previous report (Wang and Waygood, 1959) from this laboratory, evidence was presented that benzimidazole is capable of stimulating the formation of chlorophyll in detached wheat leaves as well as preventing its destruction in light and in darkness. Recently, we have made further studies on the mode of action of benzimidazole in maintaining the rate of chlorophyll synthesis in detached wheat leaves and some of the observations are presented here.

The primary leaves of greenhouse grown Khapli seedlings were excised at the two-leaf stage and floated on water or on benzimidazole solution (100 p.p.m.) in petri plates for two days. The leaves were then placed in a growth chamber (20°C.) and subjected to a light period (650 ft.c) of eight hours daily. At the end of the treatment, 10 to 15 leaves, weighing approximately 0.6 gram of fresh weight, were sampled. A one-centimeter section was cut off at the base of each excised leaf and discarded prior to sampling. Procedures used in feeding carbon-14 labelled compounds to the excised leaves and in the extraction of the leaf tissues are being published elsewhere (Wang, in press). The alcohol-free extract was partitioned with chloroform to remove pigments, essentially chlorophyll and carotenoids. The organic layer was removed, evaporated, and then made to a volume of 1.0 ml., while the aqueous layer was saved for further

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fractionation and analysis. Aliquots of 10 µl were taken for the determination of radioactivity.

The effect of benzimidazole on the incorporation of carbon-14 labelled compounds into the chlorophyll of detached wheat leaves is shown in Table 1. The amount of radioactivity from experiments with glycine-2-C^{14} in the benzimidazole treated leaves was approximately double that of the water control.

TABLE 1

Effect of benzimidazole on the incorporation of carbon-14 labelled compounds into chlorophyll (chloroform fraction) in detached wheat leaves.

Experiment No.	Compound fed	Radioactivity (c.p.m.)		Control/Benzimidazole
		Water control	Benzimidazole	
1	Glycine-2-C^{14}	11,200	27,500	0.407
2	Glycine-2-C^{14}	14,500	36,500	0.397
3	Glycine-2-C^{14}	4,980	8,300	0.600
4	Glycine-2-C^{14}	11,760	26,200	0.449
5	Glycine-2-C^{14}	16,460	31,300	0.525
6	Glycine-2-C^{14}	15,000	31,000	0.415
7	Succinic-2,3-C^{14} acid	8,200	21,000	0.390
8	Succinic-2,3-C^{14} acid	7,400	37,300	0.198
9	Glutamate-C^{14}	2,200	2,600	0.846
10	Glutamate-C^{14}	1,000	1,100	0.910
11	Urea-C^{14}	3,500	2,700	1.295
12	Urea-C^{14}	2,300	2,800	0.822

Furthermore, results from experiments with succinate-2,3-C^{14} showed an even lower ratio of water control to benzimidazole treated. Benzimidazole, however, showed no effect on the incorporation of labelled glutamate and urea. Evidently the rate of incorporation of glycine and succinate was greatly enhanced by benzimidazole in the wheat leaves detached for two days.

Recent studies on the biosynthesis of porphyrins in red blood cells have given evidence of the formation of porphyrin with glycine and succinate as the earlier precursors (Radin et al., 1950, Shemin et al., 1953, 1954, 1955). Shemin (1955) gave a detailed scheme for the intermediates and the possible reactions involved in the biosynthesis of porphobilinogen in blood cells. Granick (see Rabinowitch, 1956) speculated that the glycine and succinate cycle also operates in plants. Recently Saloman et al. (1950) and Della Rosa et al. (1953) reported that acetate and glycine were incorporated into the chlorophyll of Chlorella and assumed as early precursors. Our results obtained with glycine and succinate are in accord with Granick's suggestion and with the findings of Della Rosa et al. However, more recent evidence indicates that glycine may not be the only nor the essential earlier amino-containing precursor in wheat leaves.

When a comparison was made on the incorporation of carbon-14 glycine between the immediately detached leaves and those floated on water or on benzimidazole solution, it was found that benzimidazole maintained the normal rate of incorporation even after a four day period of detachment (Table 2). In contrast, the amount of radioactivity from glycine in the two-day water control was only half the value of immediately detached leaves. After four days on water, the rate of incorporation of glycine in the detached leaves decreased further to a value of approximately one quarter of that of the immediately detached leaves, whereas those treated with benzimidazole showed only a slight decrease. However, the latter value is even higher than that from the immediately detached older primary leaves of the same age which had been left intact on the plants for the same period. In view of the previous and the present evidence, it is reasonable to assume that benzimidazole either functions as or can take the place of a natural factor which is necessary in the biosynthesis of chlorophyll in wheat leaves. Despite the fact that benzimidazole has not yet been proven to be a naturally occurring factor, its importance in the maintenance of the normal physiology and of the rust resistance of the detached wheat leaves (Samborski et al., 1958; Wang, 1959) remains.

The authors are fully aware that the radioactivity in the chloroform frac-

tion should not be regarded solely as due to chlorophyll synthesis, since compounds other than chlorophyll such as carotenoids are also present in this fraction. Paper chromatography of this fraction, however, revealed that neither carotenoids

TABLE 2

Effect of benzimidazole on the incorporation of glycine-2-C^{14} into chlorophyll (chloroform fraction) in detached wheat leaves

Pretreatment (day)	Radioactivity (c.p.m.) Water Control	Benzimidazole	Control/Benzimidazole
0	30,000	30,500**	1.017
0	34,600	30,500**	0.882
0	17,000*		
2	14,600	30,500	0.415
4	8,400	21,400	0.392

* Older primary leaves were obtained from plants having the same age as those in the four-day pretreatment.

** An average of five experiments with detached wheat leaves which were pretreated with benzimidazole for two days.

nor chlorophyll a and b were labelled in five hours of feeding. The radioactivity was found to reside mainly in two major unknown compounds having a pale greenish yellow colour. Studies have been made on the significance of these compounds and their possible role as well as on alanine which may be the primary early precursor in the biosynthesis of chlorophyll in wheat leaves. The results of these studies will be published elsewhere.

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