INHIBITION OF CHLOROPHYLL SYNTHESIS BY 5-FLUOROURACIL AND 5-FLUORODEOXYURIDINE

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The role of iron in chlorophyll synthesis has not been elucidated. Recent findings (Fuwa et al., 1960; Wacker, 1962; Winder and O'Hara, 1962) that metals may play an important role in the structure and function of nucleic acids led us to investigate the effect of 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FDU) on the synthesis of chlorophyll in regreening leaves of iron-deficient bush bean. Both FU and FDU are known inhibitors of nucleic acid synthesis.

Methods. Seeds of bush bean (Phaseolus vulgaris, var. Improved Tendergreen, mosaic resistant) were germinated in sand. Eight-day-old seedlings were transferred to Hoagland #2 nutrient solution, devoid of iron, and allowed to grow for eight days. The first trifoliate leaves showed typical iron deficiency symptoms at this time. Representative samples of five plants per treatment were placed in a solution of 5 ppm iron, as Fe^{59}EDDHA* (S. A. 19,500 cpm/μmole) for 24 hours. A 300 watt Champion bulb, placed two feet from the plants, was used as a supplementary light source. 5-Fluorouracil, 5-fluorodeoxyuridine, uridine, and thymidine, dissolved in water containing ca. 0.1% Tween 80, were applied by immersing the first trifoliate leaves in the solution and allowing them to drain. Chlorophyll content of the leaves was determined by the method of Arnon (1949).

Results. The data of table 1 show the effect of the various metabolites on chlorophyll synthesis in the first trifoliate leaves. Treatment of iron-deficient bush

*Fe^{59} ethylenediamine di(o-hydroxyphenyl acetate).

bean for 24 hours with iron increased the chlorophyll content of the first trifoliate leaves by 320 μgm per gm fresh weight (FW) over the control. Both FU and FDU inhibited chlorophyll synthesis although FDU was far more effective. Chlorophyll synthesis was completely inhibited by 10^{-3}M FDU, whereas 10^{-3}M FU reduced it to 55%. At the 5 x 10^{-3}M level an equimolar concentration of uridine, applied simultaneously, failed to reverse the inhibition caused by either FU or FDU. However, simultaneous application of an equimolar concentration ($5x10^{-3}$M) of thymidine reduced the inhibition by FU from 69% to 30% and the inhibition by FDU from 100% to 25%. The amount of Fe^{59} per gm FW was essentially the same in all treatments.

Table 1

The Effect of 5-Fluorouracil and 5-Fluorodeoxyuridine on the Synthesis of Chlorophyll in Regreening Bush Bean Leaves During 24-hour Treatment

Leaf treatment	Fe^{59} in leaves cpm/gm FW	Chlorophyll μgm/gm FW	Chlorophyll synthesized	
			μgm/gm FW	%
-	-	490	0	0
-	22000	810	320	100
10^{-3}M FU	21400	665	175	55
$5x10^{-3}$M FU	21800	590	100	31
$5x10^{-3}$M FU + $5x10^{-3}$M Uridine	20900	585	95	30
$5x10^{-3}$M FU + $5x10^{-3}$M Thymidine	22300	715	225	70
10^{-3}M FDU	20500	490	0	0
$5x10^{-3}$M FDU	22200	490	0	0
$5x10^{-3}$M FDU + $5x10^{-3}$M Uridine	21700	490	0	0
$5x10^{-3}$M FDU + $5x10^{-3}$M Thymidine	20700	730	240	75

Discussion. The importance of metals in the structure and function of nucleic acids had not been recognized until quite recently. As Wacker (1960) pointed out, the introduction of metal deficiencies in appropriate organisms can be a rewarding approach

to the understanding of their mode and locus of action. FU and FDU have been shown to be inhibitors of the metabolic pathways of nucleic acid synthesis in a number of instances (Cohen et al., 1959; Harbers et al., 1959; Bonner and Zeevaart, 1962; and Zeevaart, 1962). The inhibition of DNA synthesis has been shown to be due to the inhibition of thymidylate synthetase. RNA synthesis is affected by the incorporation of FU into the RNA molecule in place of uracil, possibly resulting in "abberant RNA" with altered physiological expression. In the present experiments, FDU was a far more effective inhibitor of chlorophyll synthesis in regreening bush bean than FU. Furthermore, this inhibition can be reversed by the simultaneous application of thymidine but not uridine, suggesting that FU and FDU both suppress DNA synthesis which is apparently necessary for the synthesis of chlorophyll. Whether FU is incorporated into "abberant RNA" in addition to inhibiting DNA synthesis remains to be determined. Further investigations are in progress.

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