Chlorophyll biosynthesis in wheat leaves

The suggestion[1] that the pathway of biosynthesis of the dihydroporphyrins in higher plants is similar to the known pathway for the synthesis of porphyrins in animals[2] has been supported by the experiments of SALOMAN *et al.*[3], DELLA ROSA *et al.*[4], BRZESKI AND RÜCKER[5], and SHLYK *et al.*[6]. WANG *et al.*[7], however, having failed to detect any radioactivity in the chlorophylls of mature excised primary leaves of wheat following administration of [2-^{14}C]glycine suggest that glycine is a poor precursor of these compounds in wheat. During investigations of the biochemical changes occurring in cold-hardening winter-wheat plants, we have studied chlorophyll biosynthesis and present here the results of preliminary experiments in which [2-^{14}C]-glycine was fed to both mature and immature intact primary leaves of wheat. The data show that the apparent inefficiency of glycine as a chlorophyll precursor in the experiments of WANG *et al.*[7] was probably a reflection of a very low turnover rate of both chlorophylls *a* and *b* in mature wheat leaves.

Triticum aestivum L. emend Thell. var. Kharkov 22 M.C. was grown at 21° for both 5 and 13 days by which times the young plants had still-expanding primary leaves while the older plants had fully-expanded primary leaves. The illuminated plants were fed [2-^{14}C]glycine (New England Nuclear Corp.) through the roots for 4 h. The primary leaves were frozen and ground in liquid nitrogen and extracted with cold acetone. Chlorophylls *a* and *b* were purified from the extracts by solvent partition and chromatography on icing-sugar columns. The purity of the products was established by examination of their absorption spectra[8] and by conversion to the corresponding pheophytins which were purified by chromatography on icing sugar. To determine the incorporation of ^{14}C into phytol, the chlorophylls were also degraded to the corresponding pheophorbides[8], which were freed of unreacted pheophytins by partition between 1 % NaOH and diethyl ether[10].

The molar extinction coefficients of ZSCHEILE AND COMAR[8] were used in determining chlorophylls *a* and *b* and pheophytins *a* and *b*. The same extinction coefficients were used for the pheophorbides as for the corresponding pheophytins[11]. Radioactivity determinations (to within 2 % standard error) were made on infinitely thin layers on glass planchets in a windowless, methane, gas-flow counter operating in the proportional region at an absolute efficiency of 50 %.

TABLE I

INCORPORATION OF ^{14}C FROM [2-^{14}C]GLYCINE ($1.72 \cdot 10^9$ DISINTEGRATIONS/MIN/MG CARBON) INTO THE DIHYDROPORPHYRINS OF 5- AND 13-DAY-OLD PRIMARY LEAVES OF KHARKOV 22 M.C. WHEAT

Compound	*Specific activity**	
	5-day-old	*13-day-old*
Chlorophyll *a*	6.52	0**
Pheophytin *a*	5.21	0
Pheophorbide *a*	5.75	–
Chlorophyll *b*	4.54	0***
Pheophytin *b*	1.84	0
Pheophorbide *b*	2.28	–

* In disintegrations/min/mg carbon $\times 10^{-5}$.
** Less than 160 disintegrations/min/mg carbon
*** Less than 640 disintegrations/min/mg carbon.

The data in Table I show that mature (13-day) primary leaves synthesized virtually no chlorophyll in 4 h. The residue from the acetone extract of these leaves contained several times as much ^{14}C as that from the younger leaves. The low dilution of the ^{14}C in the chlorophylls of the young (5-day) leaves indicates that the methylene carbon of glycine is an efficient precursor of both chlorophylls *a* and *b*, and the similarities between the specific activities of the corresponding pheophytins and pheophorbides indicate that this carbon is also a precursor of phytol in wheat leaves. The loss of radioactivity on conversion of the chlorophyll *b* to pheophytin *b* shows that the former compound was impure. The loss during the corresponding conversion of chlorophyll *a* is probably within the experimental error.

Our data also suggest that it is highly improbable that chlorophyll *a* is formed from chlorophyll *b*, but the opposite possibility is not ruled out. Further experiments are in progress.

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N-Methyladrenaline, a new catecholamine in the adrenal gland

In a study on the properties of the enzyme that converts noradrenaline to adrenaline, it was observed that the incubation of the soluble supernatant fraction obtained from beef adrenal glands with adrenaline and [^{14}C-*Me*]-S-adenosylmethionine formed a compound that had the same R_F values as N-methyladrenaline. This led to an investigation of the normal occurrence of N-methyladrenaline in the adrenal gland.

Frozen adrenal glands from cows were homogenized with 5 vol. 0.4 *N* $HClO_4$ in a Waring blender and the resulting suspension was centrifuged. To the supernatant solution was added 0.1 vol. each of 1 % disodium ethylenediamine and 1 % freshly prepared ascorbic acid and the pH carefully adjusted to 8.4 with 0.5 *N* K_2CO_3. After centrifugation the clear supernatant fluid was passed over a column of aluminium