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 14C as that from the younger leaves. The low dilution of the ¹⁴C 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 [14C-Me]-S-adenosylmethionine formed a compound that had the same RF 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 HClO₄ 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 K₂CO₃. After centrifugation the clear supernatant fluid was passed over a column of aluminium oxide (Woelm, neutral) and the catechols absorbed and eluted as described by Weil-Malherbe and Bone¹. The eluates were evaporated to a small volume in vacuo and an aliquot subjected to two-dimensional paper chromatography (ascending) with Whatman No. I filter paper, using butanol-acetic acid-water (4:1:1) as the first solvent system, and phenol-water (0.85:0.15) saturated with SO₂ as the second. After the paper was washed several times with benzene and dried, it was sprayed first with 0.44 % K₃Fe(CN)₆ in phosphate buffer, pH 7.8, and then with ferric sulfate². One of the blue spots appearing had the same R_F values (0.40, 0.72) as authentic N-methyladrenaline.

Additional evidence for the identity of N-methyladrenaline was obtained by forming an O-methyl derivative. The concentrated eluate from the alumina column was distributed along a starting line of Whatman No. 1 filter paper and subjected to ascending chromatography using the phenol-water-SO₂ solvent system. The paper was washed as described above and a strip of an area corresponding in R_F value of 0.60 to 0.80 was cut out and a 3-O-methyl derivative made enzymically with catechol-O-methyl transferase³ as follows: The paper was cut into small pieces and added to a beaker containing a soluble supernatant fraction obtained from 500 mg rat liver, 0.2 ml 0.5 M MgCl₂, 100 mµmoles [14C-Me]-S-adenosylmethionine (46,000 counts/min) and 2 ml 0.5 M phosphate buffer, pH 7.9. After 2-h incubation, the reaction mixture was adjusted to pH 10.0 with NaOH and borate buffer and extracted twice with 5 vol. of a mixture containing toluene-isoamyl alcohol (3:2). The organic phase was evaporated to a small volume and co-chromatographed with synthetic N-methylmetanephrine (N-methyl-3-O-methyladrenaline) in 4 solvent systems. Radioactive peaks were found that coincided in R_F values with authentic N-methylmetanephrine. The following were the solvent systems used and the R_F values obtained; butanol-acetic acid-water (4:1:1), R_F 0.68; isopropanol-ammonia (8:1), R_F 0.90; butanol-methanol-ammonia (8:1:1), R_F 0.81; butanol-ethanol-water (4:1:1), R_F 0.46. An unidentified radioactive peak whose R_F values differed from those of N-methylmetanephrine was also present.

A compound having the same R_F values as N-methyladrenaline was also found in the adrenal glands of the monkey, rat, rabbit and guinea pig.

When N-methyladrenaline was administered intravenously to a rat, large quantities of N-methylmetanephrine (free and conjugated) were found in the urine.

From the observations described above, the following scheme for the formation and metabolism of N-methyladrenaline is suggested:

Adrenaline -- N-methyladrenaline -- N-methylmetanephrine

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