chromatographed on a Perkin Elmer 900 gas chromatograph with a ^3H -foil electron-capture detector. The mean concentration of total 4-hydroxy-3-methoxyphenylethylene glycol was found to be 95 (S.E.M. ± 9 ; 21 animals) ng/g for whole brain and 82 (S.E.M. ± 7 ; 13 animals) ng/g for cerebral cortex. Electrical stimulation of the left locus coeruleus for 1 h produced a significant ($P < 0.005$) increase in total 4-hydroxy-3-methoxyphenylethylene glycol of 70% in whole brain and a significant ($P < 0.0025$) increase of 60% in pooled left and right cerebral cortices. The separate analysis of left and right cerebral cortices suggested that the increase in 4-hydroxy-3-methoxyphenylethylene glycol was ipsilateral.

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Effects of Diphenyleneiodonium and Trialkyltin Compounds on Photophosphorylation and Chloride-Hydroxide Exchange in Isolated Chloroplasts

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Work in this laboratory has shown that trialkyltin compounds mediate a Cl^- - OH^- exchange across mitochondrial and chloroplast membranes (Selwyn *et al.*, 1970; Watling & Selwyn, 1970). It has also been established that these compounds also produce a direct inhibition of phosphorylation in mitochondria (Aldridge & Street, 1964, 1971; Sone & Hagihara, 1964; Stockdale *et al.*, 1970). Under some conditions the Cl^- - OH^- exchange also produces, indirectly, inhibition of mitochondrial phosphorylation, respiration and adenosine triphosphatase activity (Coleman & Palmer, 1971; Dawson *et al.*, 1972; Selwyn *et al.*, 1972).

Holland & Sherratt (1971) have reported that

diphenyleneiodonium compounds mediate an anion- OH^- exchange across mitochondrial membranes but have little effect as direct inhibitors of mitochondrial phosphorylation. Observation of chloroplast volume changes, passive in NH_4Cl or on illumination in NaCl , shows that diphenyleneiodonium sulphate is effective in mediating a Cl^- - OH^- exchange across chloroplast membranes.

Kahn (1968) has reported that tributyltin is an inhibitor of photophosphorylation in chloroplasts from *Euglena* and spinach. In view of the complex effects found with mitochondria, the inhibition of photophosphorylation has been investigated in relation to the mediation of Cl^- - OH^- exchange.

Inhibition of photophosphorylation was estimated from the effect on these compounds on the stimulation by ADP of O_2 evolution from pea (*Pisum sativum*) chloroplasts at pH 7.6. Tributyltin and triphenyltin were most effective ($0.3\ \mu\text{M}$ and $0.6\ \mu\text{M}$ respectively), tripropyltin ($23\ \mu\text{M}$) and diphenyleneiodonium ($80\ \mu\text{M}$) were moderately effective, but trimethyltin ($0.3\ \text{mM}$) and triethyltin ($0.8\ \text{mM}$) were poor inhibitors. This order of effectiveness bears little relation to the order of effectiveness in mediating the Cl^- - OH^- exchange. It is also different from the pattern of inhibition of mitochondrial phosphorylation, where triethyltin, tripropyltin and tributyltin are all highly effective.

When electron transfer was uncoupled with NH_4Cl the trialkyltin compounds and diphenyleneiodonium sulphate inhibited O_2 evolution but only at concentrations much higher than those required to inhibit photophosphorylation. When carbonyl cyanide *m*-chlorophenylhydrazone was used as the uncoupler even higher concentrations of inhibitor were required, and in some cases no significant inhibition was observed at the highest usable concentration.

These observations confirm a direct inhibition of photophosphorylation by tributyltin, triphenyltin and tripropyltin, and the effectiveness of the butyl and phenyl compounds suggests a very hydrophobic region for the site of action.

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Oxidation of Oxalate and Formate by Leaf Peroxisomes

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The association of oxalate oxidase (oxalate-oxygen oxidoreductase, EC 1.2.3.4) and formate dehydrogenase (formate-NAD⁺ oxidoreductase, EC 1.2.1.2) with particulate fractions of leaf homogenates has been reported (Srivastava & Krishnan, 1962; Arnon & Whatley, 1954; Mazelis, 1960). The activities of these enzymes in a peroxisome fraction prepared from leaves of spinach beet (*Beta vulgaris* L.) by a modification of the method of Tolbert *et al.* (1969) have been investigated by the release of ¹⁴CO₂ from ¹⁴C-labelled substrates.

Oxalate oxidase activity was demonstrated by ¹⁴CO₂ release from 1 mM-oxalate, containing [¹⁴C]-oxalate, in 50 mM-citric acid-KH₂PO₄ buffer, pH 4.0. The reaction was linear over 60 min, stimulated by 0.03 mM-FMN or -FAD, and completely suppressed under an atmosphere of N₂. It was characterized by a sharp optimum at pH 4.0. The addition of unlabelled formate did not diminish the release of ¹⁴CO₂.

Formate oxidation was measured by ¹⁴CO₂ release from 0.1 mM-formate, containing [¹⁴C]formate, in KH₂PO₄-K₂HPO₄ buffer, pH 6.0 (0.1 M with respect to phosphate). The course of the reaction was linear over 2 h and suppressed under N₂. However, whereas under air neither NAD⁺ nor glyoxylate affected the reaction significantly, under N₂ the rate was restored to that in air by these reagents; NADP⁺ and glycollate were both ineffective. NADH production in air could not be demonstrated spectrophotometrically, but transfer of ³H from [³H]formate to NAD⁺ was observed, although this was equivalent to less than 20% of the CO₂ released. NADH oxidase activity in the preparations could be demonstrated. NADH inhibited the oxidation of formate competitively (cf. Peacock & Boulter, 1970).

Formate oxidation was stimulated in air by H₂O₂ addition, and, under N₂, H₂O₂ restored the activity to at least the rate in air. pH-activity curves with H₂O₂ and with NAD⁺ under N₂ showed distinct curves with peaks at pH 5.0 and 7.0 respectively.

These results are consistent with the oxidation of formate in air and under N₂ proceeding by different reactions. Under air, H₂O₂, released by endogenous

reactions in the peroxisomes, oxidizes formate by the peroxidatic action of catalase (Chance, 1952). In the absence of O₂, formate is oxidized through formate dehydrogenase, dependent on a source of NAD⁺. Formate decarboxylation does not participate in the oxidation of oxalate.

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Oxidative Decarboxylation of Glycollate and Glyoxylate by Leaf Peroxisomes

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The oxidation of glycollate and glyoxylate has been implicated in the photorespiration of illuminated leaves, since the CO₂ released has been shown to arise from the carboxyl group of glycollate (Zelitch, 1966). It has been proposed that glycine is an essential intermediate (Tolbert, 1971; Kiski *et al.*, 1971), but reactions involving a more direct decarboxylation of glyoxylate and glycollate have been investigated with peroxisome preparations from leaves of spinach beet (*Beta vulgaris* L.).

The release of ¹⁴CO₂ from [1-¹⁴C]glycollate, [2-¹⁴C]glycollate, [1-¹⁴C]glyoxylate and [2-¹⁴C]glyoxylate, with 1-5 mM substrate concentrations in KH₂PO₄-K₂HPO₄ buffer (0.1 M with respect to phosphate), was studied over a range of pH values. ¹⁴CO₂ release from the [1-¹⁴C]glyoxylate was up to 9 times that from [2-¹⁴C]glyoxylate, although the difference with the corresponding glycollates was less marked. These reactions were inhibited under N₂. When H₂O₂ was added, considerable stimulation of ¹⁴CO₂ release from [1-¹⁴C]glyoxylate, and some from [2-¹⁴C]glyoxylate, was observed under air or under N₂ at pH 5.0; the effect on release from labelled glycollate was slight. With 1 mM-oxalate in air, stimulation from [1-¹⁴C]glyoxylate was again observed at pH 5, this presumably being due to H₂O₂ produced by oxalate oxidase (Leek & Butt, 1972).

¹⁴CO₂ release from [1-¹⁴C]glyoxylate and [1-¹⁴C]glycollate was not affected by a pool of unlabelled formate, but that from the 2-¹⁴C-labelled acids was significantly decreased. Addition of unlabelled