The fruit-bodies that had been inhibited by polyoxin D were considerably softened, and appeared very similar to fruit-bodies that had been incubated overnight with cell-wall-hydrolytic enzymes (e.g. chitinase, EC 3.2.1.14, or laminarinase, EC 3.2.1.6). This softening was not observed with the other metabolic inhibitors mentioned above. It is suggested that polyoxin D interrupts concomitant enzymic wall loosening and cell-wall synthesis during morphogenesis by specifically inhibiting the synthesis.

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Inhibition of Electron Transport in Isolated Turnip (*Brassica napus*) Mitochondria by Inhibitors of Protein Synthesis

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The concept of autonomy of subcellular organelles relies largely on the results of experiments in which inhibitors are used to differentiate between cytoplasmic and organelle protein synthesis. The interpretation of these experiments or those obtained with whole cells and cell homogenates is dependent on the specificity of the inhibitor chosen. Published evidence suggests that this specificity is lacking for some inhibitors of protein synthesis. Thus D-serine (a competitive inhibitor of L-serine) and cycloheximide inhibit ion uptake but not O₂ uptake by plant tissues (Ellis *et al.*, 1964; Macdonald & Ellis, 1969; Ellis & Macdonald, 1970), suggesting an uncoupling action. Chloramphenicol has been shown to inhibit ox heart mitochondria at the level of phosphorylation site I when NAD-linked substrates were being oxidized (Freeman & Haldar, 1967). Ca²⁺ uptake by isolated maize mitochondria is also inhibited by chloramphenicol (Stoner *et al.*, 1964).

The present study describes the effects on tightly coupled turnip mitochondria of a range of protein-synthesis inhibitors. Lincomycin, puromycin, mitomycin C, actinomycin D and D-serine had no significant effect, at concentrations that inhibit protein synthesis, on electron transport in either State 3 or State 4 with malate or succinate as substrate. Cycloheximide (4 µM) caused a 20% inhibition of O₂ uptake

in State 3 with malate but not with succinate as substrate, indicating action in the region of site I. No uncoupling action was observed with any of the inhibitors during the relatively short incubations used in these experiments.

D-threo-Chloramphenicol was, however, a potent inhibitor of electron transport, but, contrary to previous reports, inhibition of phosphorylation was minimal. Inhibition was greatest with malate as substrate in State 3. The effects on State 4 in the absence of uncouplers and on succinate and NADH oxidations were less marked. L-threo-Chloramphenicol, which is inactive on protein synthesis (Rendi & Ochoa, 1962), was a more effective electron-transport inhibitor than the D-threo-isomer. D- and L-erythro-Chloramphenicol were relatively ineffective inhibitors of electron transport.

Oxidized–reduced difference spectra, obtained at –196°C 5 and 15s after the addition of D-threo-chloramphenicol to 330 µM, support the conclusion that the inhibitor has two sites of action on the electron-transport chain: a sensitive site in the region of site I, and one associated with the antimycin A-sensitive site.

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The Enzymic Hydrolysis of Malonated Flavone Glycosides

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Apiin (apigenin 7-apiosyl glucoside), the most abundant glycoside of parsley (*Petroselinum hortense*), may, with the less abundant graveobioside B (chrysoeriol 7-apiosyl glucoside), account for 7% of the dry weight of the leaf (Hahlbrock & Wellmann, 1970). Crude 'apiin', now known to be principally a mixture of the above glycosides, was first observed as a gelatinous precipitate formed when hot-water extracts of parsley leaves are cooled (Rump, 1836; cited by Hudson, 1949). This relative insolubility of the major glycoside constituents of parsley in aqueous

media led us to consider that they could occur in the living leaf in the form of a soluble precursor.

Parsley leaf extracts prepared in water kept at boiling point do not gel on cooling. Gel formation is initiated by addition to the cooled extract of either parsley leaf press juice or a protein fraction precipitated by 30–55% saturation with respect to $(\text{NH}_4)_2\text{SO}_4$. The activity is thermolabile and was assumed to be enzymic. It was also found that gel formation is accompanied by acid production. Diffusible free acids in the boiling-water extracts were removed by prolonged dialysis against water and, after concentration at reduced pressure, the extract was passed down columns of Sephadex G-10. Brown phenolic materials, which neither gelled nor produced acid in the presence of the enzyme, appeared in the first column fractions and were discarded. The later yellow glycoside fractions were concentrated, and acid production was followed by autotitration with NaOH at constant pH. At completion of the enzyme reaction 95% of the total flavone, determined spectrophotometrically (Mabry *et al.*, 1970), could be recovered as a gelatinous pellet in the centrifuge and 0.66 acid equiv. had been generated/mol of total flavone.

Chromatography of the gel fraction on paper showed it to consist principally of apiin and graveobioside B. Examination of the organic acid content of the supernatant fluid by paper chromatography suggested that malonate is produced in the enzyme reaction, and this was confirmed by g.l.c. and mass spectrometry of trimethylsilyl and propyl esters of the acid.

Malonate as a major constituent of leaves is known to occur sporadically in the Umbelliferae, Leguminosae and Graminae (Bentley, 1952), and from *Trifolium* spp. Beck & Knox (1971) have isolated and characterized three flavone glycosides malonated on the 6"-position of the glucose moiety. In preliminary studies we have isolated an enzyme from leaves of another legume, *Cicer* sp. (chick pea), that liberates malonate from crude glycoside fractions from the same plant and is also active towards the parsley extracts. Parsley enzyme is likewise active towards *Cicer* glycosides. In contrast, spinach (*Spinacea oleracea*) and *Phaseolus vulgaris* do not possess a similar enzyme, nor do their glycoside fractions produce malonate in the presence of the pea or parsley enzymes.

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Configuration of Soil Arabinose

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Arabinose and xylose are as abundant in soil polysaccharide as galactose or mannose, yet the pentose formed when readily available carbohydrate is metabolized by soil micro-organisms is only one-tenth of the hexose (Cheshire *et al.*, 1971). It is possible that the pentoses are present in the residues of plant material. By using ^{14}C -labelled rye straw it has been shown that about 30% of both the glucan and the xylan in the straw can persist in soil for 14 months (Cheshire *et al.*, 1972).

This could explain why there is so much of the pentose xylose in the soil, but plants generally contain much less arabinose than xylose. Another means of distinguishing the source of the arabinose is by its configuration. Arabinose in plants is in the L-form, except in a few species not found in such northern latitudes as Britain. Arabinose in micro-organisms occurs in both D- and L-forms, depending on the type of organism.

Treatment of an arable soil derived from a granitic till by cold 12M- H_2SO_4 followed by dilution to 0.5M- H_2SO_4 and heating released (i) 1.5 mg of arabinose/g of soil. Hot 0.5M- H_2SO_4 alone released (ii) 1.3 mg/g of soil. A polysaccharide, extracted from the same soil by dilute alkali, hydrolysed by heating with 0.5M- H_2SO_4 yielded (iii) 0.17 mg/g of soil.

In each case the arabinose was isolated by adsorption chromatography on a charcoal–Celite column and by partition chromatography on Whatman no. 1 paper. The sugar was shown to be pure by g.l.c. of the alditol acetate derivative.

The yields of pure arabinose were (i) 13%, (ii) 2.0% and (iii) 2.9%. Measurement of the optical rotation showed that the arabinose was composed of (i) 90%, (ii) 87% and (iii) 93% of the L-form.

This strongly suggests that the greater part of the arabinose is directly derived from plant sources, although the only firm conclusion that can be drawn is that at least 10% of the soil arabinose has a microbial origin.

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