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The Intestinal Absorption of 3-*sn*-Phosphatidylethanolamine in the Rat

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1,2-Di[1- ^{14}C]palmitoyl-*sn*-glycero-3-[^{32}P]phosphoryl[2- ^3H]ethanolamine (Owen *et al.*, 1971) was fed in olive oil by gastric intubation to albino rats with thoracic-duct fistulae.

Analysis of the intestinal contents 1 or 2 h after feeding showed that at least 15% of the fed compound had been degraded to monoacyl-*sn*-glycero-3-phosphorylethanolamine and palmitate. Up to 90% of the ^{32}P and ^3H radioactivities in the clear aqueous supernatant, after centrifugation of the intestinal contents, was recovered as monoacyl-*sn*-glycero-3-phosphorylethanolamine.

Up to 4.5% of the administered ^{14}C , 6% of the administered ^{32}P and 6% of the administered ^3H were recovered in the lymph phospholipids. Of the administered ^{14}C 52% was recovered in the lymph diacylglycerols and triacylglycerols. ^{32}P and ^{14}C were recovered in the lymph ethanolamine phosphoglyceride (3-*sn*-phosphatidylethanolamine and monoacyl-*sn*-glycero-3-phosphorylethanolamine), choline phosphoglyceride, phosphoinositide and sphingomyelin fractions. ^3H was recovered in the lymph ethanolamine phosphoglyceride fractions only.

The ^{14}C , ^{32}P and ^3H relative specific radioactivities of the lymph 3-*sn*-phosphatidylethanolamine fraction rose to maxima of 25, 50 and 50 respectively when the specific radioactivities of the administered trilabelled 3-*sn*-phosphatidylethanolamine were 100 d.p.m./ μg of lipid P.

The isotopic proportions ^{14}C : ^{32}P : ^3H of the lymph 3-*sn*-phosphatidylethanolamine fraction remained constant at 1.0:1.0:1.0 throughout lymph collection when the administered trilabelled compound had isotopic proportions 2.0:1.0:1.0. The ^{14}C : ^{32}P ratio of the major lymph phospholipid fraction, 3-*sn*-phosphatidylcholine, rose to a maximum of 7.0:1.0.

These results suggest that the administered 3-*sn*-phosphatidylethanolamine was absorbed from the intestinal lumen as monoacyl-*sn*-glycero-3-phosphorylethanolamine and in part subsequently reacylated (Stein & Stein, 1966) within the mucosa. A small fraction of both the ^{14}C - and ^{32}P -labelled metabolites of the administered compound were incorporated into other phospholipid species.

When 1,2-di[1- ^{14}C]palmitoyl-*sn*-glycero-3-[^{32}P]phosphorylcholine was fed to rats under similar con-

ditions to those described above, ^{14}C and ^{32}P radioactivities were recovered in lymph choline phosphoglycerides only; no detectable label was found in other lymph phospholipid species. This latter result is in agreement with previous work (Scow *et al.*, 1967; Nilsson, 1968).

The generous financial support of the Medical Research Council and the Wellcome Trust are acknowledged.

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The Effect of Polyoxin D on Morphogenesis in *Coprinus cinereus*

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Polyoxin D, a nucleoside antibiotic (Isono *et al.*, 1967), is a powerful competitive inhibitor of chitin synthetase (UDP-*N*-acetylglucosamine–chitin *N*-acetylglucosaminyltransferase, EC 2.4.1.16) in *Neurospora crassa* (Endo *et al.*, 1970). It has little effect on respiration or on synthesis of DNA, RNA and protein in test fungi (Sasaki *et al.*, 1968).

Polyoxin D inhibited the chitin synthetase activity in the microsomal preparation from homogenized stalks of fruit-bodies of the toadstool *Coprinus cinereus* (Schaeff. ex Fr.) S. F. Gray (= *Coprinus lagopus sensu* Buller). In an enzyme assay with 400 μM -substrate (UDP-*N*-acetylglucosamine), 5 μM -polyoxin D gave 50% inhibition.

Young excised stalks of the toadstools, with or without their caps, elongated *in vitro* when kept in a moist atmosphere overnight. The extent of the elongation was predictable and depended on the original size, with the larger stalks elongating as much as they would have done *in vivo*, and the smaller ones progressively less. When the cap was left on, normal morphogenesis occurred, with cap expansion, autolysis and release of viable spores. The active nature of the elongation and subsequent development was confirmed, as they could be prevented by dipping the basal cut ends of the stalks into solutions of metabolic inhibitors in water (e.g. NaCN, NaN₃, cycloheximide, 2,4-dinitrophenol, all at 50 $\mu\text{g}/\text{ml}$). This system has been used *in vitro* to study the effect of polyoxin D on morphogenesis.

The elongation and development of the excised fruit-bodies was totally prevented by 190 μM -polyoxin D and considerably diminished by 19 μM -polyoxin D. This inhibition suggests an intimate involvement of chitin synthetase in these processes.

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.

I thank Dr. S. Suzuki for a gift of polyoxin D, Mr. J. Baker for technical assistance, and the Agricultural Research Council for financial support (Grant no. AG43/54 to Professor J. H. Burnett).

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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.

The authors are grateful to Dr. R. J. Ellis of the University of Warwick for the gift of chloramphenicol isomers. This work was supported by a grant from the Science Research Council to S. B. W.

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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