buffer (v/v), pH 7.4, for 5 min followed by a linear gradient up to 100% methanol over 10 min. An integrator was used for quantitative analysis.

The chromatographic conditions described separated, in order of elution, pyruvate, acetacetate and acetone (Fig. 1a), and gave a linear response over the physiological range of 40–100 μM. Pyruvate (80 μM) gave 6 times the colour yield of acetacetate and 11 times the colour yield of acetone at the same concentration. Alloxan-diabetic rats (Howell & Taylor, 1967) showed the expected increased blood levels of pyruvate, acetacetate and acetone over normal rats. They showed a reduced level of an unidentified keto compound, labelled 'X' in Fig. 1b), compared with normal ones.

The method is effective for the rapid, simultaneous analysis of the major keto compounds in blood and may prove useful in the study of energy metabolism and clinical biochemistry. Results using normal, starved and alloxan-diabetic rats conform to current understanding of these metabolic models (White et al., 1984) and provide evidence that the method is capable of valid measurements of metabolic variables.

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Azurin from Pseudomonas putida: an electron acceptor for p-cresol methyloxydilase

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The enzyme p-cresol methyloxydilase is a flavocytochrome c that has been isolated from a number of species of Pseudomonas (Hopper & Taylor, 1977; Hopper, 1983). It converts p-cresol into p-hydroxybenzyl alcohol by successive dehydrogenation and hydration reactions and can also oxidize the product to p-hydroxybenzaldehyde by subsequent dehydrogenation. The reduced enzyme is not reoxidized by O2 and, when isolated, it requires an added electron acceptor for the reaction to proceed. PMS has been used as an acceptor to link the reaction either to O2 by auto-oxidation of the reduced PMS or to 2,6-dichlorophenolindophenol as the terminal acceptor in spectrophorometric assays. Even when enzyme and substrate were incubated in the presence of membrane particles there was no significant uptake of O2 suggesting that the flavocytochrome does not donate electrons directly to a membrane component, and this has led to a search for the enzyme’s natural electron acceptor.

Cells of Pseudomonas putida NCIB 9869, grown with 3,5-xyleneol, contain a p-cresol methyloxydilase (Keat & Hopper, 1978) and during a purification of this enzyme a blue colour was noticed in some fractions eluted from an ion-exchange column. The protein responsible has been purified to homogeneity as indicated by a single band after isoelectric focusing on polyacrylamide gel, was higher compared with others of the class.

Cells of Pseudomonas putida NCIB 9869, grown with 3,5-xyleneol, contain a p-cresol methyloxydilase (Keat & Hopper, 1978) and during a purification of this enzyme a blue colour was noticed in some fractions eluted from an ion-exchange column. The protein responsible has been purified to homogeneity as indicated by a single band after isoelectric focusing on polyacrylamide gel. The molecular weight of the protein, determined by sedimentation equilibrium in the ultracentrifuge, was 14000 and it contained one copper atom per molecule. These properties are typical of the azurins, a class of low-molecular-weight, copper-containing proteins that have been isolated from several species of bacteria and which function in electron transport. The absorption spectrum (Fig. 1) with a maximum at 620 nm, was also typical of an azurin and the fine structure in the 280 nm region has been previously reported for these proteins (Ugurbil & Bersohn, 1977; Martinkus et al., 1980). The 620 nm peak was bleached on reduction of the protein with dithionite. However, the ratio of A520/A380 of 1.54 was higher than the ratios reported for other azurins although these exhibit wide variability from species to species with values of 0.49 for the A520/A380 of Paracoccus azurin (Martinkus et al., 1980) and 0.57 for the A525/A380 of Pseudomonas aeruginosa azurin (Patt et al., 1976). Also the isoelectric point of 7.3 for the oxidized protein, as measured by isoelectric focusing on thin layers of polyacrylamide gel, was higher than values for other azurins which are generally lower than pH 6.0. However, amino acid analysis does show larger numbers of arginine and lysine residues in this protein when compared with others of the class.

Abbreviations used: PMS, phenazine methosulphate.
proteins. Reduction was measured spectrophotometrically by following the decrease in absorbance at 620 nm. Addition of substrate resulted in a rapid reduction of the azurin. Furthermore, addition of substrate to an incubation mixture containing enzyme, azurin and washed membrane particles donating to a membrane component, and may well be the effect of toxaphene on susceptible seedlings was a chlorosis of chloroplasts from toxaphene-treated seedlings. By this criterion almost all the barley and oat, and about half the rye, varieties tested were susceptible. In contrast, wheat and resistant to toxaphene were precluded because of lack of availability of a sufficient quantity of seed.

Effect of toxaphene on photosynthetic electron flow in susceptible cereals

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Studies of the susceptibility of Avena sp. (oat) to toxaphene (Gardenhire & McDaniell, 1970) suggested that reaction to the chlorinated hydrocarbon insecticide was controlled by a single gene, with susceptibility dominant to resistance. The effect of toxaphene on susceptible seedlings was a chlorosis some 3 days after foliar application, and the damage was localized to those areas in contact with the pesticide.

We have surveyed the susceptibility of cereal varieties from different parts of the world to toxaphene. Preliminary studies had suggested that susceptibility was evidenced by an inhibition of photosynthetic electron flow. In the survey, chloroplasts were isolated from toxaphene-treated seedlings 2 days after spraying with a preparation of technical toxaphene. A variety was classed as susceptible if photoreduction of 2,6-dichlorophenol-indophenol or potassium ferricyanide was inhibited about 50% compared with chloroplasts from untreated seedlings. By this criterion almost all the barley and oat, and about half the rye, varieties tested were susceptible. In contrast, wheat and maize varieties were with few exceptions resistant. Subsequent studies were confined to one variety of susceptible oat, Blyth. Parallel studies on a variety convincingly resistant to toxaphene were precluded because of lack of availability of a sufficient quantity of seed.

Abbreviation: Fd, ferredoxin; [FE-S], bound iron-sulphur centre; PC, plastocyanin; PQ, plastoquinone; PS, photosystem; Q, electron acceptor for PSII, Z, electron donor for PSII.

![Fig. 1. Suggested sites of inhibition by toxaphene in photosynthetic electron flow from H₂O to NADP⁺ in relation to the sites of interaction of the artificial electron donor and acceptor systems]

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