to oxidize iminodiacetate and glycine. The ability to oxidize nitrilotriacetate was inducible; cells grown on acetate showing tenfold less activity.

Cell-free extracts were able to catalyse the degradation of nitrilotriacetate. This activity was assayed by the release of $^{14}\text{CO}_2$ from nitrilotri[1- ^{14}C]acetate and was dependent on the presence of thiamin pyrophosphate, Mg^{2+} and NADH. NADPH was not an effective substitute for NADH. The CO_2 produced in this assay was derived solely from C-1 of nitrilotriacetate, as no release of $^{14}\text{CO}_2$ occurred when nitrilotri[2- ^{14}C]acetate was used. In addition, no $^{14}\text{CO}_2$ was produced from nitrilotri[1- ^{14}C]acetate in incubations under anaerobic conditions. The formation of glyoxalate from nitrilotriacetate was demonstrated in incubation mixtures containing phenylhydrazine, when glyoxalate phenylhydrazone accumulated. The formation of this material was dependent on the presence of NADH. The formation of glycine from nitrilotriacetate was shown in incubation mixtures containing nitrilotri[1- ^{14}C]acetate, NADH and cell-free extract to which non-labelled glycine was added. Under these conditions radiolabel appeared in the glycine.

Cell-free extracts were examined for the presence of enzymes capable of transforming glyoxalate and glycine. When supplemented with thiamin pyrophosphate, Mg^{2+} and NADH, extracts of nitrilotriacetate-grown cells catalysed the anaerobic conversion of glyoxalate into glycerate. In this process 0.5 mol of CO_2 was produced/mol of glyoxalate utilized. When NADH was omitted, a product accumulated whose dinitrophenylhydrazone possessed the spectral properties of the tartronic semialdehyde derivative (Kornberg & Gotto, 1961). This product was metabolized rapidly by extracts in the presence of NADH. Hence the presence of glyoxalate carboligase and tartronic semialdehyde reductase was inferred. The former enzyme was induced by growth on nitrilotriacetate. In addition, nitrilotriacetate-grown cells possessed increased activities of serine hydroxymethyltransferase and hydroxypyruvate reductase, indicating the presence of a metabolic sequence for the conversion of glycine into glycerate. Attempts to demonstrate enzymes capable of the interconversion of glycine and glyoxalate were unsuccessful.

These results indicate a degradatory pathway in which nitrilotriacetate is converted into glyoxalate and glycine. The requirements of NADH and O_2 for this process suggest that a mixed-function oxidase may be involved (Large, 1971). The release of CO_2 from C-1 of nitrilotriacetate and the cofactors required are in accordance with the further metabolism of glyoxalate, by the glyoxalate carboligase reaction, to tartronic semialdehyde and then glycerate. Glycine is apparently also metabolized to glycerate by a separate sequence of reactions.

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Metabolism of Piperonylate by *Pseudomonas fluorescens*

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Methylenedioxyphenyl compounds, important commercially as insecticide synergists, are demethylated by NADPH-dependent mono-oxygenases in rat liver microsomal fraction (Kamiński & Casida, 1970), although cleavage of the methylene bridge was not the major metabolic route of piperonylate.

Cell-free extracts of *Pseudomonas fluorescens* strain PM 3 grown on anisate as the sole carbon source were previously shown to metabolize piperonylate (3,4-methylenedioxybenzoate) as well as the methoxylated compound (Buswell & Mahmood, 1972*a,b*). Oxidative attack to yield protocatechuate and formate involved absorption of 1 mol of O_2 /mol of piperonylate and required NADH as cofactor. Mono-oxygenase activity was present in the soluble fraction (164000g for 3h) and was stable for several months when extracts were stored at -20°C . The data indicate that the methylene carbon is hydroxylated to the unstable hydroxymethylenedioxy derivative, which undergoes hydrolysis to formate and the dihydroxy acid.

Protocatechuate, confirmed as an intermediate on the breakdown pathway by chemical isolation from enzyme-piperonylate incubations and identification by m.p. and by i.r. and u.v. spectrophotometry, was further metabolized via intradiol ring fission to 3-oxoadipate.

Piperonylate mono-oxygenase and 4-O-demethylase activity in extracts of anisate-grown cells was probably catalysed by the same enzyme because: (i) thermal deactivation at 45°C led to a correlated decrease in activity on each substrate; (ii) the presence of inhibitors resulted in equal inhibition of both activities; (iii) addition of either 4-methoxybenzoate or piperonylate to the assay mixture did not increase the rate of reaction when the other substrate was present alone at saturating concentrations; (iv) repression produced by succinate in the growth medium was identical for both activities.

This oxidative attack appears similar to the mono-oxygenase activity towards safrole and dihydro-safrole found by Harrison & Ribbons (1971) in several *Pseudomonas* strains grown on these substrates, although soluble enzyme systems from piperonylate-grown cells were reported not to catalyse the oxygenation of the methylene bridge.

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The Oxidation of Glutathione by a Preparation of Glucose Oxidase from *Aspergillus niger*

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A commercial preparation of glucose oxidase from *Aspergillus niger* (Fermcozyme 653 AM, lot no. 1917; Hughes and Hughes Ltd., Romford, Essex, U.K.) catalyses the reaction:

The catalytic activity is unaffected by dialysis or the presence of 50 mM-EDTA, but is abolished by heating at 90°C for 10 min.

Measurements with an oxygen electrode show that in phosphate-citrate buffer, pH 5.6 (20 mM- Na_2HPO_4), containing 1.6% (v/v) of Fermcozyme, 50 mM-EDTA and 0.005% (w/v) of catalase, 0.25 mol of O_2 is consumed/mol of GSH added. The concomitant disappearance of free thiol can be demonstrated by using 5,5'-dithiobis-(2-nitrobenzoate) (Roberts & Agar, 1971). All the added GSH is recoverable as GSSG, as assayed by a modification of the method of Srivastava & Beutler (1968). The evidence for H_2O_2 as another reaction product is that in the presence of Fermcozyme and peroxidase GSH oxidizes gum guaiacum to a blue compound, the maximum extinction at 610 nm being 40% of that given by an equimolar amount of glucose. These findings are consistent with the equation given above.

The apparent Michaelis-Menten parameters of Fermcozyme for GSH, estimated by the procedure of Wilkinson (1961) from initial rates of O_2 uptake in the presence of excess of catalase and 50 mM-EDTA at pH 5.6, 37°C and about 230 μM - O_2 , are: $K_m = 0.97 \pm 0.04$ (10 mM; $V = 16.3 \pm 0.3$ (10) μmol of O_2 /min per ml of Fermcozyme. In these conditions, the rate of O_2 uptake with 5 mM-cysteine is less than 10% of that with an equimolar concentration of GSH.

When Fermcozyme is saturated with glucose (110 mM) or GSH (5 mM), the ratio v (glucose)/ v (GSH) is 11. The corresponding ratio for a purer preparation of glucose oxidase from *Aspergillus niger* (Glucose Oxidase 250, lot no. 6X; Hughes and

Hughes Ltd.) is 440. Therefore Fermcozyme may contain an enzyme that oxidizes GSH and is not glucose oxidase.

One implication of our finding that may be of practical importance is that, when glucose in the diffusate from a typical haemolysate is measured by using Fermcozyme-peroxidase-gum guaiacum, the GSH also present raises the estimate by about 5%.

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Differences in Triacylglycerols from Acoustic Tissues and Posterior Cranial Blubber of the Narwhal (*Monodon monoceros*)

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Acoustic tissues of the porpoises *Phocoena phocoena* (Varanasi & Malins, 1970b) and *Tursiops gilli* (Varanasi & Malins, 1970a), as well as the Beluga whale (*Delphinapterus leucas*) (Litchfield *et al.*, 1971), contain large amounts of isovalerate (3-methylbutyrate) and long-chain saturated iso acids. However, cranial blubber of *T. gilli*, having no acoustic function, comprises primarily acids characteristic of normal mammalian adipose tissues (Varanasi & Malins, 1971). Moreover, in *T. gilli* large amounts of di-isovaleroyl glycerides in acoustic tissues appear to play a vital role in sound transmission (Varanasi & Malins, 1972).

We analysed in the narwhal (*Monodon monoceros*), an unusual Arctic whale, the triacylglycerols from acoustic tissues at the attachment point of the mandibular waveguide to the bulla. Comparisons were made with triacylglycerols from non-acoustic posterior cranial blubber.

The acoustic tissues and blubber each contained 94.5% of lipid comprised of 82.2% and 85.5% of triacylglycerols respectively. The triacylglycerols were separated and purified, and component acids were analysed by g.l.c. (Varanasi & Malins, 1970b). Triacylglycerols from the acoustic tissues comprised 47.8 mol of isovalerate/100 mol and 18.7 mol of long-chain iso acids (isopentadecanoate, 11.4 mol/100 mol, was the major component)/100 mol. Moreover, straight-chain monounsaturated acids ($\text{C}_{16:1}$ acid, 10.4 mol/100 mol; $\text{C}_{18:1}$ acid, 2.2 mol/100 mol) were