such preparations (J. R. Lagnado & C. Lyons, unpublished work). The subcellular distribution of colchicine-binding activity was therefore further investigated by differential and sucrose-gradient centrifugation of rat cerebral-cortex homogenates prepared in 0.32M-sucrose containing 1mM-phosphate-Mg²⁺ buffer, pH 6.5. Colchicine-binding activity was assayed in the various fractions by the filter-disc method of Weisenberg, Borisy & Taylor (1968) as described by Wilson (1970) with [³H]colchicine (specific radioactivity 1.7Ci/mmol).

It was found that about 45% of the recovered radioactivity is present in the crude nuclear (approx. 32%) and mitochondrial (approx. 13%) fractions. The relative specific radioactivities (% of radioactivity recovered/% of protein recovered) for these fractions ranged between 0.6 and 0.8, in contrast with the values found for the microsomal (1.6–2.4) and soluble fractions (1.5–2.8), which accounted for approx. 8 and 47% of the total recovered radioactivity. Most of the radioactivity present in crude nuclear preparations was due to non-nuclear contamination. Nerve-ending particles ('synaptosomes') purified from washed mitochondrial preparations by the procedure of Gray & Whittaker (1962) accounted for nearly 50% of the radioactivity present in the primary fraction and exhibited relative specific radioactivities several times those of purified mitochondria. Further, it was found that subfractionation of water-lysed nerve-ending-particle preparations by the procedure of Whittaker & Sheridan (1965) released about 15% of colchicine-binding activity in soluble form, while about 10, 30 and 40% of the remaining activity was associated with the vesicle ('D₁'), small-membrane ('D₂') and residue ('J') fractions. The highest relative specific radioactivities (>2) were shown by fractions D₁ and D₂.

The possible relation of membrane-bound and soluble colchicine-binding protein(s) to microtubule protein will be discussed. Some of the results obtained are comparable with those reported for mouse brain by Feit & Barondes (1970).

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The Bacterial Oxidation of Picolinamide

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Picolinamide (pyridine-2-carboxamide) has been shown (Slade & Smith, 1967) to be a photodegradation product of the herbicide Diquat (6,7-dihydrodipyrido[2,2-a:2',1'-c]pyrazinedium ion). A Gram-negative rod, capable of utilizing picolinamide as a sole carbon and nitrogen source, has been isolated from soil.

Washed suspensions of picolinamide-grown cells were capable of immediately oxidizing picolinate, 6-hydroxypicolinate, maleamate and maleate without lag; glucose-grown cells would not oxidize any of the compounds immediately. None of the mono- or di-hydroxypyridines, 3- or 5-hydroxypicolinate or fumaramate was oxidized.

2,5-Dihydroxypyridine and picolinate were identified in the supernatant of cultures oxidizing picolinamide under conditions of restricted aeration, mildly alkaline pH and high substrate concentration. If the pH was allowed to rise to 8 or higher, a green pigment appeared in the culture supernatant; this was shown to arise from 2,5-dihydroxypyridine. 6-Hydroxypicolinate accumulated in cultures inhibited with 5mM-sodium arsenite, and pyruvate in those with 1mM-sodium arsenite.

Crude cell-free extracts prepared ultrasonically or with a Biox X-press were capable of oxidizing picolinamide and picolinate, with a stoichiometric yield of 6-hydroxypicolinate. Picolinamide deamidase remained in the supernatant after centrifugation at 100 000g for 45 min; picolinate hydroxylase in X-press prepared extracts was sedimented at 38 000g, but was solubilized ultrasonically. The soluble hydroxylase required NAD⁺ for activity, and was active anaerobically in the presence of Methylene Blue. One mol of picolinate required 0.5 mol of oxygen and 1 mol of NAD⁺ for complete conversion into 6-hydroxypicolinate.

Cell-free extracts centrifuged at 38 000g for 20 min oxidized 2,5-dihydroxypyridine in the presence of Fe²⁺, GSH and oxygen. 2,5-Dihydroxypyridine oxygenase was precipitated between

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30 and 50% saturation with ammonium sulphate. One mol of 2,5-dihydropyridine required 1 mol of oxygen for conversion into 1 mol of maleamate and 1 mol of formate.

The supernatant fraction of cell-free extracts after centrifugation at 38000g for 30 min oxidized maleamate with the liberation of ammonia and maleate. In the presence of 10 mM-*meso*-tartrate both compounds were converted stoichiometrically into fumarate. No cofactors were found to be required for either reaction.

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The Bacterial Oxidation of *N*-Methylisonicotinate

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N-Methylisonicotinate, a photodegradation product of Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) (Slade, 1966), has been shown to be degraded aerobically by *Achromobacter* D (Wright & Cain, 1970). The pathway involves the partial reduction of the ring and ring-fission leads to the production of methylamine, succinate and CO₂. We now report evidence supporting the existence of a second pathway, in a Gram-positive rod, isolated from soil, capable of utilizing *N*-methylisonicotinate as sole carbon source.

Whole cells grown on *N*-methylisonicotinate were capable of the immediate oxidation of 2-hydroxy-*N*-methylisonicotinate. Crude cell-free extracts oxidized neither *N*-methylisonicotinate nor its 2-hydroxy derivative, though 2-hydroxyisonicotinate was oxidized rapidly with the uptake of 0.5 mol of oxygen/mol. The activity was associated with a particulate fraction sedimenting at 100000g. On the cessation of oxygen uptake, a compound with $\lambda_{\max}$ 338 nm (at pH 7.0) was present. A scaled-up reaction mixture was used to isolate sufficient material to identify this compound, by u.v. and i.r. spectra, chromatography and melting point as 2,6-dihydroxyisonicotinate. The reaction could occur anaerobically in the presence of Methylene Blue.

The 38000g supernatant fraction of cell-free

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extracts deamidated maleamate, and converted maleate into fumarate. In the presence of 10 mM-*meso*-tartrate, 1 mol of maleamate was converted into 1 mol each of fumarate and ammonia. Fumarate and methylamine were not oxidized by either whole cells or cell-free extracts.

Whole cells incubated with *N*-[¹⁴C]-methylisonicotinate released more than 50% of the added radioactivity as [¹⁴C]formaldehyde, and no [¹⁴C]-methylamine could be detected chromatographically. When whole cells were incubated with *N*-methyl[carboxyl-¹⁴C]isonicotinate, 95% of the added radioactivity was released as ¹⁴CO₂.

These data suggest that the first step in the pathway is a hydroxylation to yield 2-hydroxy-*N*-methylisonicotinate, which is demethylated to 2-hydroxyisonicotinate with the release of the methyl group as formaldehyde. The 2-hydroxyisonicotinate is hydroxylated to 2,6-dihydroxyisonicotinate. No information has been obtained on either the ring-fission substrate or product, but it seems that the carboxyl group of *N*-methylisonicotinate is not incorporated into maleamate, or more ¹⁴C would be incorporated via fumarate into cellular constituents from *N*-methyl[carboxyl-¹⁴C]isonicotinate.

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Biosynthesis of Ergotamine

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Numerous investigations (for reviews see Agurell, 1966; Ramstad, 1968) of the biosynthesis of the ergoline nucleus suggests that the primary precursors are tryptophan, mevalonate and the methyl group of methionine. There have been few reports on the biosynthesis of the side chain of the tripeptide alkaloids, for example, ergotamine.

The ¹⁴C-labelled amino acids phenylalanine and alanine were found to be incorporated into ergotamine (Paul, 1957; Vining & Taber, 1963), but the exact distribution of radioactivity in the alkaloid was not determined. Further studies (Minghetti & Arcamone, 1969; Mayer, Keybal & Komersora, 1967), using L-[U-¹⁴C]alanine, have shown that relatively more label entered the phenylalanyl, prolyl and lysergyl moieties than the α -hydroxy- α -