polated to the whole body content was: serum, 3.4; skin, 0.4; muscle, 29.6; bone 1.5; marrow, 0.5.

The percentage of the radioactivity in each tissue was distributed between peaks iii (cholecalciferol), iv and v-vii respectively as follows: serum, 67, 28, 5; skin, 24, 59, 17; muscle, 76, 14, 10; bone 67, 15, 18; marrow, 12, 39, 49. Thus muscle would appear to constitute a store of vitamin D₃ largely in the unmetabolized form.

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The Metabolism of 4-Hydroxypyridine by *Agrobacterium* sp.: a New Ring Cleavage of the Pyridine Nucleus

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In a previous communication Houghton, Wright & Cain (1968) reported the identification of pyridine-3,4-diol as an intermediate in the metabolism of 4-hydroxypyridine by a bacterium, now tentatively identified as *Agrobacterium* sp. Extracts of 4-hydroxypyridine-grown cells, clarified by centrifugation (120 000g for 60 min.) and supplemented with an NADH-generating system, oxidized 4-hydroxypyridine with the consumption of 2 moles of O₂/mole of substrate. In the absence of NADH there was no oxidation of 4-hydroxypyridine, but pyridine-3,4-diol was still rapidly oxidized (1 mole of O₂/mole of substrate). The 4-hydroxypyridine hydroxylase and the 3,4-diol oxygenase could be resolved by standing at 4° for 24 hr. and by Sephadex G-200 chromatography, the very labile oxygenase being inactivated by this treatment.

The hydroxylase was extremely specific for 4-hydroxypyridine (*K_m* 0.24 mM) but showed product inhibition. The product of the enzymic reaction was identified as pyridine-3,4-diol by isolation from a millimolar-scale preparation and comparison with the synthetic 3,4-diol (mixed m.p. of diacetyl derivatives, u.v. and i.r. spectra, chromatography, FeCl₃ reaction and activity with the oxygenase). In crude extracts NADH was some fivefold more effective than NADPH as cofactor, but response to NADPH further diminished during hydroxylase purification. The *K_m* for NADH (tris-HCl, pH 8.5) was 0.04 mM. The flavin component of the hydroxylase was resolved by acid (NH₄)₂SO₄ treatment (0.6–0.8 saturation, pH 5.6,

for 3 hr.). Addition of FAD (*K_m* 0.23 μM), but not FMN or riboflavin, completely restored the activity.

Pyridine-3,4-diol was metabolized by an oxygenase that lost some 10% of its original activity/hr. (pH 7.0–8.5 at 0–4°). The consumption of 1 mole of O₂/mole of 3,4-diol occurred at pH 8.5 with the release of only one titratable acid group and the formation of an uncharacterized compound (compound I) absorbing at 284 nm. at pH 7–10 and at 260 nm. at pH 3–4. Compound I was altered by such acid treatment, since on readjustment to pH 7.0 the absorption peak was displaced to 279 nm.

At pH 7.0 compound I did not accumulate and the final products of pyridine-3,4-diol metabolism by extracts were identified as pyruvate, formate and NH₃. The nature of these products, the titration value and the tautomerism of compound I strongly suggests a 'meta' cleavage of pyridine-3,4-diol. Formate was further indicated by readdition of the particle fraction to extracts, when a further uptake of 0.5 mole of O₂/mole of substrate was observed.

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The Regulation of Enzymes of Aromatic-Ring Fission in Fungi: Organisms using both Catechol and Protocatechuate Pathways

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The fungal metabolism of aromatic compounds differs from that of bacteria in several ways, among which are the multiplicity of interconversions of intact benzenoid compounds and in particular the distinct metabolic route by which they convert protocatechuate into β-oxoadipate. Although some fungi appear genetically incapable of utilizing the catechol pathway for ring cleavage, most mycelia-forming genera can synthesize the enzymes of both catechol and protocatechuate pathways.

In *Aspergillus niger*, *Penicillium spinulosum*, *Schizophyllum commune* and on unidentified *Penicillium* sp. growth on *p*-hydroxybenzoate induced all the enzymes of the protocatechuate branch (but none of the enzymes of the catechol branch) at amounts 10–80-fold the basal values obtained on glucose. Although these genera grew weakly or not at all on catechol, the enzymes of the catechol pathway, but not the protocatechuate pathway, could be elicited by growing the fungi on mixtures of glucose and catechol. In *Aspergillus niger* exposure to benzoate was accompanied by transient accumulation of *p*-hydroxybenzoate in the medium