The presence of pyruvate carboxylase (total activity 7.8 units/g. wet wt.) and NAD-malate dehydrogenase (total activity 307 units/g. wet wt.) in the superovulated rat ovary suggests that the pyruvate-malate cycle, previously described in liver (Lowenstein, 1961; Shrago & Lardy, 1966; Walter, Paetkau & Lardy, 1966) and in adipose tissue (Kornacker & Ball, 1965; Ballard & Hanson, 1967), may operate in this tissue. This cycle provides a means by which mitochondrial or cytoplasmic NADH may be converted into cytoplasmic NADPH for steroidogenesis.

The effect of glucose on steroidogenesis from acetate could be the result of glycolytic pyruvate priming the pyruvate-malate cycle. The effects of lactate and pyruvate can also be explained in these terms. The intracellular concentration malate is increased in the presence of glucose from 16 to 364 μ M. The effect of isocitrate may be due to its metabolism by NADP-isocitrate dehydrogenase.

Ballard, F. J. & Hanson, R. W. (1967). *J. Lipid Res.* **8**, 73.
 Flint, A. P. F. & Denton, R. M. (1969). *Biochem. J.* **112**, 243.
 Kornacker, M. S. & Ball, E. G. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 899.

Lowenstein, J. M. (1961). *J. biol. Chem.* **236**, 1213.
 Shrago, E. & Lardy, H. A. (1966). *J. biol. Chem.* **241**, 663.
 Walter, P., Paetkau, V. & Lardy, H. A. (1966). *J. biol. Chem.* **241**, 2532.

Absorption and Tissue Uptake of α -Tocopherol in the Rat

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Tracer doses of DL-[5-Me- ^{3} H] α -tocopherol were administered orally to groups of six rats housed individually in metabolic cages with precautions to prevent coprophagy. The net absorption (determined from faecal radioactivity) and tissue contents of radioactivity were measured after 7 days.

The effects of (1) the vitamin status of the animal, (2) the vehicle (Tween 80, alcohol or arachis oil) and (3) decreased serum lipoprotein concentrations on the absorption and tissue distribution of radioactivity were studied.

Rats deficient in vitamin E had the same net absorption as supplemented animals, but significantly more radioactivity in liver ($P < 0.02$) and kidneys ($P < 0.005$).

Net absorption of the vitamin was the same whether given in Tween 80, alcohol or arachis oil. The amounts of radioactivity in the liver but not in kidneys of groups given the vitamin in alcohol or arachis oil were significantly different ($p < 0.05$). When Tween administration was compared with

arachis oil the resulting tissue contents of radioactivity with Tween were very much greater ($P < 0.001$).

Prior lowering of serum lipoprotein concentrations in rats by prolonged dietary orotic acid did not affect net absorption, but radioactivity in adrenals ($P = 0.05$), adipose tissue ($P < 0.01$) and liver ($P < 0.001$) were significantly greater and in heart significantly lower ($P < 0.025$) than in control animals.

It is concluded that, although net absorption remains unchanged, the vitamin E status of the animal, the nature of the vehicle in which α -tocopherol is administered and the serum lipoproteins concentrations affect the tissue distribution of the ingested vitamin.

The Distribution of Vitamin D₃ Metabolites in Human Serum and Tissues

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In an earlier communication Mawer & Stanbury (1968) described the persistence for several months of more-polar metabolites in human serum after the injection of isotopically labelled vitamin D₃ (cholecalciferol).

Methods have now been developed that allow the separation of the 'polar metabolite fraction' into its several components (Mawer & Backhouse, 1969).

In the present communication the changes with time within the polar metabolite fraction of human serum will be described. Initially the principal component was peak ivb, which is eluted with diethyl ether and co-chromatographs in our silicic acid system with authentic 25-hydroxycholecalciferol (Blunt & DeLuca, 1969). This peak possesses biological activity in the rat line test approximately equal to that of cholecalciferol. Three other peaks of radioactivity were observed, none of which possessed biological activity and only one, peak vi, eluted by methanol-ether (1:1, v/v), increased in concentration with time and continued to increase after peak ivb had started to decrease.

The concentration of peak ivb and its rate of appearance in serum may be related to the previous vitamin D status of the subject. When the serum vitamin D activity, as assayed in the rat line test, was low, peak ivb was produced more rapidly and represented a higher proportion of the injected dose.

Results will also be presented describing the metabolite pattern in the tissues of a leg amputated 4 days after the subject had received intravenous [4- ^{14} C]cholecalciferol. The percentage of the injected dose present in each tissue when extra-

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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Blunt, J. W. & DeLuca, H. F. (1969). *Biochemistry*, **8**, 671.
Mawer, E. B. & Backhouse, J. (1969). *Biochem. J.* **112**, 255.
Mawer, E. B. & Stanbury, S. W. (1968). *Biochem. J.* **110**, 53P.

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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Houghton, C., Wright, K. A. & Cain, R. B. (1968). *Biochem. J.* **106**, 51P.

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