hydroxybenzoic acids partly free and partly conjugated with glycine and *p*-anisic acid is conjugated with glycine and glucuronic acid (Quick, 1932).

No adverse effects were noted when young guinea pigs were injected intraperitoneally with neutralized homoanisic acid (0.1, 0.2, 0.5 and 1g/kg in normal saline) as a single dose or when homoanisic acid was taken orally by the authors (0.1mmol/kg, 1.08g for S. E. O.). Urine was collected for 24h from the guinea pigs, together with that passed from a control group that received normal saline. Before dosing, phenolic acid intake was restricted in the adult subject, and urine samples were collected at intervals for 24h after the dose. Aromatic acid metabolites in the urine were examined by t.l.c. (Ersser, Oakley & Seakins, 1970) and by g.l.c. (Coward & Smith, 1969), which was used for quantitative determination.

It was found that guinea pigs metabolized homoanisic acid by demethylation to *p*-hydroxyphenylacetic acid, and by conjugation with glycine (and glucuronide) and also excreted the unchanged compound.

In man, ingestion of homoanisic acid resulted in the excretion of the free acid (1%), *p*-hydroxyphenylacetic acid (12%), and four other substances that had the chromatographic properties of the glutamine (3%) and the glucuronide (19%) conjugates and their demethylation products (0.5 and 3% respectively). The rate of excretion showed two maxima, at 2½h, about 7h. Homoanisuric acid was not detected.

The metabolism of homoanisic acid in man is thus similar to that of phenylacetic acid in that it forms a glutamine conjugate, and also to the lower homologue anisic acid in that it conjugates with glucuronic acid. It differs from the halogenophenylacetic acids and anisic acid in not forming a glycine conjugate and, unlike anisic acid, homoanisic acid and its conjugates undergo demethylation. This work was the B.Sc. dissertation of S. E. O. (Oakley, 1970).

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End-Product Inhibition of Aspartate Carbamoyltransferase from Wheat Germ

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Aspartate carbamoyltransferase (EC 2.1.3.2) is regulated by pyrimidine nucleotides in certain organisms including lettuce seedlings (Bethell & Jones, 1969; Neumann & Jones, 1962, 1964). However, the detailed kinetics and structural basis of aspartate transcarbamoylase regulation have not previously been investigated in higher plants as extensively as they have in *Escherichia coli* (Changeux & Gerhart, 1968).

Aspartate transcarbamoylase has been purified 20-fold from commercial wheat germ. The enzyme was strongly inhibited by UMP. In the presence of saturating concentrations of both substrates (1.0mm-L-aspartate and 1.0mm-carbamoyl phosphate) at pH 9.5, 85% inhibition was produced by 0.2mm-UMP. Under the same conditions 2.5mm-UDP produced 35% inhibition, but 2.5mm-UTP, -CTP, -CDP, -CMP, -GTP, -GMP, -ATP and -AMP, tested separately, neither inhibited nor activated the enzyme. Also under the same conditions, a plot of initial rate against UMP concentration was distinctly sigmoidal, but became hyperbolic when the carbamoyl phosphate concentration was decreased to 0.1mm.

Zonal gel filtration of 20-fold purified aspartate transcarbamoylase on Sephadex G-200 with 50mm-phosphate buffer, pH 7.6 at 5°C, resulted in the elution of aspartate transcarbamoylase activity in two peaks with 10–20% overall recovery. Peak A, containing 10% of the recovered activity, emerged in the void volume, suggesting a molecular weight greater than 800000. The remaining 90% of the recovered activity emerged in a single symmetrical peak (peak B) whose elution volume suggested a Stokes radius of 3.9nm (Ackers, 1964) or a molecular weight of 100000 (Andrews, 1965). The aspartate transcarbamoylase species in peak B therefore resembles, in molecular size and weight, the catalytic subunit of the *E. coli* enzyme (Bethell & Jones, 1969; Gerhart & Schachman, 1965).

Pooled fractions of peak B were also inhibited by UMP at pH 9.5. This suggests a different structural basis of inhibition from that of the *E. coli* enzyme. In this end-product inhibition is a property of the native enzyme (mol.wt. 310000) but not of its isolated catalytic and regulatory subunits (mol.wt. 96000 and 30000 respectively) (Gerhart & Schachman, 1965).

Further regulatory kinetics and the possibility of reversible aggregation of wheat germ aspartate transcarbamoylase are under investigation.

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Prenol Phosphates and Mannosyltransferases

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The role of undecaprenol phosphate-sugars as lipid-soluble intermediates in the biosynthesis of bacterial wall polymers is well established (Osborn, 1969). Evidence implying that prenol phosphate-sugars have a similar role in the biosynthesis of polysaccharides of plant cell walls and also of mammalian glycoproteins has been reported (Villemez & Clark, 1969; Kauss, 1969; Caccam, Jackson & Eylar, 1969). Strong evidence that dolichol phosphate-glucose is an intermediate in the transfer of glucose from UDP-glucose to glycoprotein in pig liver has been published (Behrens & Leloir, 1970). We have studied the response to different prenol phosphates of mannosyltransferase activities, from GDP-mannose, in mammalian, plant and fungal systems and report some of the results here.

Monophosphates of pig liver dolichols-17 to -21 (Butterworth & Hemming, 1968), of betulaprenols-6 to -9 (Wellburn & Hemming, 1966), of solanesol, of ficaprenols-10 to -13 (Stone, Wellburn, Hemming & Pennock, 1967), of farnesol and of cetyl alcohol were prepared chemically (Behrens & Leloir, 1970) and purified by chromatography on DEAE-cellulose (Lahav, Chiu & Lennarz, 1969).

The incorporation of [^{14}C]mannose from GDP-[U- ^{14}C]mannose into lipid-soluble products was studied by using the endoplasmic reticulum of pig liver. The isolation of the cell fraction and incubation conditions were essentially as described by Caccam *et al.* (1969) for studies on the incorporation of mannose into glycoproteins of rabbit liver. In a typical experiment, without addition of prenol phosphate, 10% of the added ^{14}C was recovered as lipid. At a concentration of $178\text{ }\mu\text{M}$,

the phosphates of the pig liver dolichols and of the betulaprenols produced fourfold increases in this figure, solanesyl phosphate threefold, the phosphates of the ficaprenols twofold and those of farnesol and of cetyl alcohol no increase. With the dolichyl phosphates and betulaprenyl phosphates the effect was maximal at 5 min. This was followed by a gradual decrease over the next 2h accompanied by a steady rise in the ^{14}C associated with protein.

Whereas in the control only one ^{14}C -labelled lipid could be detected, in the presence of phosphates of the dolichols, betulaprenols, solanesol and ficaprenols a second ^{14}C -labelled lipid was also formed. The relative ^{14}C content of the two lipids varied with time. Acid (pH 2, 100°C , 10min) liberated completely the [^{14}C]mannose from both lipids.

A particulate fraction from mung-bean seedlings that is capable of transferring mannose from GDP-mannose to cell-wall polysaccharides (Villemez & Clark, 1969) was also studied. In a typical experiment the incorporation of ^{14}C into lipid from GDP-[U- ^{14}C]mannose was 4.5% and this increased threefold in the presence of the dolichols and betulaprenols but did not increase in the presence of the other compounds (all at $264\text{ }\mu\text{M}$). Two ^{14}C -labelled lipids were produced in the presence of the dolichols and betulaprenols both of which liberated [^{14}C]mannose on treatment with dilute acid.

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