formation in barley at the serine $\rightarrow$ pyruvate stage in addition to the earlier step(s) as in maize, indicating differences in strength of binding of pyridoxal phosphate to the enzymes in the two plant species studied.

These results are in agreement with an earlier observation (Shah & Rogers, 1969) that, whereas isonicotinyl hydrazide inhibited the incorporation of radioactivity from [2- 14 C]glyoxylate, [2- 14 C]glycine and [14 C]formate into β -carotene of illuminated etiolated maize seedlings, it did not affect incorporation from [U- 14 C]serine. In a further series of experiments related to the latter observations we have shown that incorporation of radioactivity from L-[3- 14 C]serine into β -carotene of illuminated etiolated maize seedlings was extremely low but could be considerably enhanced by supplying the plants at the same time with glycine and formate or with isonicotinyl hydrazide and glycine. The studies indicate that serine supplied to intact plants may be subject to considerable interconversion into glycine before incorporation into pigments. Radioactivity at C-3 of the serine may be diluted out in this process by unlabelled C_1 fragments present *in situ*. Inhibition of this interconversion by the presence of isonicotinyl hydrazide resulted in a 40-fold enhancement of incorporation of C-3 of the added serine into carotenoids, whereas there was little effect on incorporation of radioactivity into sterols.

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A New Type of Bile Salt

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Bile of the very large freshwater teleost *Arapaima gigas* of the family Osteoglossidae was kindly given to us by Dr P. H. Greenwood. The bile salts showed on t.l.c. at least four major components, one corresponding in R_f to taurocholate. Dioxan-

trichloroacetic acid cleavage and separation on Celite gave two crystalline bile alcohols, 'arapaimol-A' and 'arapaimol-B', both showing an intense purple response in the Hammarsten (HCl) test. I.r. and n.m.r. spectroscopy showed that they had the same steroid nucleus; mass spectra indicated formulae $C_{27}H_{48}O_5$ and $C_{27}H_{48}O_6$ and a steroid nucleus containing four hydroxyl groups. Arapaimol-A with lead tetra-acetate consumed about 3g-atoms of oxygen/mol; it did not give an acetonide. N.m.r. spectra suggested $-\overset{25}{CH}(CH_3)\cdot CH_2\cdot OH$ and $-\overset{25}{CH}(CH_2\cdot OH)_2$ for the terminal side-chain group of the molecules of arapaimol-A and -B respectively, and indicated that, if hydroxyl groups were at C-3, C-7 and C-12, the fourth hydroxyl group might be at C-1, C-2 or C-6. All these results pointed to the partial synthesis of 2 β ,3 α ,7 α ,12 α -tetrahydroxycholestan-24-oic acids.

Methyl 7 α ,12 α -diacetoxo-4 β -bromo-3-oxo-5 β -cholestan-24-oate with sodium acetate-acetic acid gave a mixture containing methyl 2 β ,7 α ,12 α -triacetoxo-3-oxo-5 β -cholestan-24-oate (I), as expected from the work of Satoh, Takahashi, Aoki & Hagitani (1969). Reduction of compound (I) with sodium borohydride, followed by hydrolysis, readily gave 2 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β -cholestan-24-oic acid (II), whose i.r. spectrum showed all the bands between 9.0 and 15.0 μ m of the arapaimols. The n.m.r. spectra of (II) showed the same chemical shifts for the C-18 and C-19 protons as found for the arapaimols. We conclude that arapaimols-A and -B are 5 β ,25 ξ -cholestane-2 β ,3 α ,7 α ,12 α ,26-pentol and 5 β -cholestane-2 β ,3 α ,7 α ,12 α ,26,27-hexol respectively.

After alkaline hydrolysis of the bile salts we obtained 'arapaimic acid', believed from i.r., n.m.r. and mass-spectral observations to be 2 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β ,25 ξ -cholestan-26-oic acid, and Dr I. G. Anderson, by g.l.c., confirmed the presence of cholic acid.

2 β -Hydroxylation has not previously been observed in any bile salt, but is a feature of insect steroid hormones.

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Regulation of Enzymes Converting L-Mandelate into Benzoate in Bacterium N.C.I.B. 8250

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The conversion of L-mandelate into benzoate in bacterium N.C.I.B. 8250 involves the enzymes L-mandelate dehydrogenase, benzoylformate

carboxy-lyase (EC 4.1.1.7) and a heat-stable benzaldehyde dehydrogenase. Preliminary evidence, largely obtained from an examination of the enzyme complements of cells grown into the penultimate generation on various intermediates of the pathway, suggested that the three enzymes are co-ordinately induced (Kennedy & Fewson, 1968).

Confirmation of this suggestion has now been obtained by measuring the differential rates of enzyme synthesis (Monod, Pappenheimer & Cohen-Bazire, 1952) in cells growing on glutamate and (a) induced with intermediates of the mandelate pathway, (b) gratuitously induced with thiophenoxyacetate, or (c) induced with mandelate but partially repressed by subsequent intermediates of the pathway. There is a significant correlation (correlation coefficient >0.97 for any pair of enzymes) among the differential rates of synthesis of the three enzymes under all these conditions.

Further supporting evidence has come from a study of mutants unable to convert L-mandelate into benzoate. By using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as mutagen, three classes of blocked mutants have been obtained: those that lack L-mandelate dehydrogenase, those that lack benzoylformate carboxy-lyase and those that lack L-mandelate dehydrogenase, benzoylformate carboxy-lyase and the heat-stable benzaldehyde dehydrogenase. Both L-mandelate and benzoylformate induce the other two enzymes in mutants lacking benzoylformate carboxy-lyase. Benzoylformate is probably the primary inducer of this regulon because it induces benzoylformate carboxy-lyase and benzaldehyde dehydrogenase in mutants lacking L-mandelate dehydrogenase; L-mandelate on the other hand has no inductive capacity in these organisms.

There are clearly considerable differences in the enzymology and regulation of the pathways converting mandelate into benzoate in the organisms that have so far been studied. The system in bacterium N.C.I.B. 8250 is unlike that in *Pseudomonas putida* (Hegeman, 1966), *Pseudomonas aeruginosa* (Rosenberg & Hegeman, 1969) or *Aspergillus niger* (Jamaluddin, Subba Rao & Vaidyanathan, 1970), and these in turn differ from each other.

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The Purification and Properties of a Bacterial Trimethylamine Dehydrogenase

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Bacterium 4B6, a non-motile Gram-negative rod isolated from river-water, is capable of growth on trimethylamine, dimethylamine or methylamine as sole source of carbon and energy; trimethylamine *N*-oxide will not support such growth. Washed suspensions of trimethylamine- and dimethylamine-grown organisms both oxidize trimethylamine and dimethylamine, but not methylamine. Cell-free extracts prepared from trimethylamine-grown organisms have been shown to contain (1) a trimethylamine dehydrogenase that uses phenazine methosulphate as primary hydrogen acceptor, (2) a glutathione-independent NAD-linked aldehyde dehydrogenase and (3) an NAD(P)H-linked dimethylamine mono-oxygenase (cf. Eady & Large, 1969). No methylamine-oxidizing activity has so far been found in cell-free extracts of trimethylamine-grown organisms, although methylamine does not accumulate during growth on trimethylamine.

The trimethylamine dehydrogenase has been purified 30-fold, and this preparation contains a single protein as judged by analytical polyacrylamide-gel electrophoresis. The enzyme has a molecular weight of about 160 000 as determined by gel filtration (Andrews, 1965). Polyacrylamide-gel electrophoresis after exposure to 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol (Shapiro, Viñuela & Maizel, 1967) shows the protein to be composed of two polypeptide chains of molecular weights approx. 70 000 and 80 000. The enzyme has an absorption maximum at 445 nm that disappears on reduction with dithionite.

The purified trimethylamine dehydrogenase catalyses the conversion of trimethylamine into dimethylamine and formaldehyde. Trimethylamine and dimethylamine were determined by g.l.c. (Sze, Borke & Ottenstein, 1963); formaldehyde was determined by a colorimetric method (based on Schryver, 1910) and oxygen uptake by Warburg monometry. The stoichiometry found agrees with the following equations:

where PMS is phenazine methosulphate.

The kinetics of the reaction were studied by using an anaerobic spectrophotometric assay in