as little as 0.01% for dimethylamine. Nitrosamines are also degraded to secondary amines and nitrite, but the enzyme is of low activity; it is located in the cell cytoplasm.

Gut bacteria degrade lecithin to choline, which is then *N*-dealkylated to release dimethylamine and ethanolamine (which were characterized by chromatography of the DNP-amines; Asatoor & Kerr, 1961). This breakdown is carried out by strains of clostridia, bacteroides and bifidobacteria. By using a strain of *Clostridium bifermentans* it was demonstrated that optimum production of dimethylamine from choline occurred at pH 5.5–6.5. Another urinary amine, pyrrolidine, is produced by the bacterial decarboxylation of proline.

There are two sites of the body where bacteria might produce nitrosamines, (a) in the gut from ingested nitrate and amines produced from lecithin or proline, or (b) in the urine of people with urinary-tract infections (90% of which are due to *E. coli*, the nitrosating organism). We have not detected nitrosamine formation in broth cultures at nitrate concentration below 12 $\mu\text{mol/ml}$, and although the concentration of amines is adequate the mean nitrate concentration in normal urine is only 1.5 $\mu\text{mol/ml}$. However, in areas with a high nitrate content in their drinking water the urinary nitrate concentration is higher and approaches that necessary for the production of nitrosamines *in vitro*.

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Effect of Glucagon on Spheroplasts of *Escherichia coli*

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Previously published results (R-Candela & García, 1969, 1970) show that actinomycin D inhibits glucose uptake by spheroplasts of *Escherichia coli*. This inhibition is completely reversed by glucagon, which enhances the glucose uptake. The present work was undertaken in order to throw further light on this effect.

Glucagon produces a stimulatory effect on the activity of β -galactosidase and enhances the incorporation into trichloroacetic acid-insoluble protein of [$1\text{-}^3\text{H}$]leucine, [$5\text{-}^3\text{H}$]uridine and [$2\text{-}^{14}\text{C}$]uracil in spheroplasts of *E. coli*. This effect has not been observed with other proteins (albumin,

insulin, secretin and calcitonin) or after addition of cyclic AMP (10 mM).

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A Catalytic Mechanism for Pig Plasma Benzylamine Oxidase

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Pig plasma benzylamine oxidase (EC 1.4.3.4) has been purified to homogeneity and crystallized (Buffoni & Blaschko, 1964). Although substrate specificity and some K_m values have been studied (Buffoni & Blaschko, 1964; Buffoni, 1966), a detailed steady-state kinetic investigation of the enzymic mechanism has not been made. A detailed kinetic study of the related enzyme bovine plasma amine oxidase (Oi, Inamasu & Yasunobu, 1970) suggests that this enzyme operates through a Ping Pong mechanism (Cleland, 1967) with aldehyde as the first product to be released; the detection of aldehyde under anaerobic conditions (Reed & Swindell, 1969) supports this mechanism.

In the present investigation, initial-velocity and product-inhibition studies on crystalline benzylamine oxidase from pig plasma have been made. The enzyme reaction was monitored by following benzaldehyde production spectrophotometrically at 250 nm; product-inhibition studies where benzaldehyde was added were followed by measuring oxygen disappearance (Estabrook, 1967).

Initial-velocity studies at different fixed oxygen concentrations with different benzylamine concentrations indicated a Ping Pong mechanism. Product-inhibition studies under non-saturating and saturating conditions of each substrate support a Ping Pong mechanism of the type benzylamine on, ammonia off, oxygen on, hydrogen peroxide off, benzaldehyde off. Failure to detect benzaldehyde under anaerobic conditions and the ability of benzyl alcohol to act as a substrate provide further evidence in favour of this mechanism.

It is suggested that fundamental differences in mechanism between apparently related amine oxidases parallel differences in substrate specificity.

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Kinetic Studies of the N-Demethylation of Ethylmorphine by a Cytochrome P-450-Dependent Enzyme System in Human Liver Microsomes

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Oxidation is an important pathway for the inactivation of many lipid-soluble drugs and other foreign chemicals. Studies with liver microsomes from different species of laboratory animals have established a good inverse correlation between the duration of action of many lipid-soluble drugs *in vivo* and the activity of the drug-oxidizing enzymes of the different species (Quinn, Axelrod & Brodie, 1958). However, there have only been a few studies of human liver microsomal enzymes and in many cases the liver samples were obtained either at post mortem or were kept for periods of 1–3 h before use (Ackerman, 1970; Alvares *et al.* 1970). In view of the important role these enzymes may play in determining individual differences in response to various drugs in man (Davies, 1969) it was important to make kinetic studies of the oxidation of drugs with fresh samples of human liver.

Samples of liver were taken during abdominal surgery with the informed consent of the patient. Isolation of the microsomal fraction was commenced within 5–10 min of the samples being taken.

Concentrations of microsomal protein per g of liver ranged from 9.5 to 23 mg in the ten samples analysed. The concentration of cytochrome P-450 ranged from 0.67 to 1.02 nmol/mg of microsomal protein. These values are within the range of concentrations of cytochrome P-450 previously found in different species of laboratory animals (Davies, Gigon & Gillette, 1969). The maximal rates ($V_{\max}$) of N-demethylation of ethylmorphine showed a greater than fourfold range (2.0–8.8 nmol/min per mg), the highest value being similar to that found in the adult male rat. The apparent Michaelis constant (K_m) for the N-demethylation of ethylmorphine ranged from 0.8 to 4.0 mM, indicating

that the substrate has a lower affinity for human liver enzymes than for rat enzymes (K_m 0.32 mM). In addition the K_m values increased with $V_{\max}$, as would be the case in a diffusion-limited system (Davies, Gigon & Gillette, 1968).

The rate of reduction of cytochrome c varied from 176 to 282 nmol/min per mg, the equivalent value for the rat being 158 ± 24.3 . The rate of reduction of cytochrome P-450 ranged from 2.76 to 4.29 nmol/min per mg, which is less than in the rat. Reduction of cytochrome P-450, which is thought to be rate-limiting in the oxidation of drugs (Davies, 1969), correlated with $V_{\max}$ values and was increased after the addition of 2 mM-ethylmorphine. However, as has been previously reported (Gigon, Gram & Gillette, 1969), 0.5 mM-aniline decreased the initial rate of reduction of the cytochrome.

These observations demonstrate that there are individual differences in the rates of oxidation of ethylmorphine by cytochrome P-450-dependent enzymes in human liver microsomes. These differences are apparently not due to variations in the concentration of cytochrome P-450, but rather to differing rates of reduction of the cytochrome.

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The Electrophoretic Mobilities of Native and Chemically Modified Human Placental Alkaline Phosphatase Isoenzymes

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At least 15 phenotypes of human placental alkaline phosphatase (EC 3.1.3.1) resulting from the action of nine structural genes have been recognized on the basis of the different electrophoretic mobilities of the enzymes at several pH values (Robson & Harris, 1967). However, the enzyme variants are closely similar in other respects (Thomas, 1970). To attempt to infer some of the structural differences that presumably underlie the differences in electrophoretic behaviour, we have compared the electrophoretic mobilities over a range of pH values of three common placental phosphatase