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 [1-³H]leucine, [5-³H]uridine and [2-¹⁴C]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.