protein-deficient purified diet (3% casein), phenobarbitone increases cytochrome *P*-450 only from 6 to 30nmol/g. of liver. This effect is only due to dietary protein, however, and re-feeding experiments indicate that the control of induction in protein-deficient animals may be altered so as to divert available protein away from less essential functions, of which the synthesis of cytochrome *P*-450 seems to be one.

The permissive effect of the factor in diet 41B has not been described before; its nature and possible mode of action will be discussed.

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Gut Bacteria and the Metabolism of Cyclamate in the Rat

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It has been established that cyclohexylamine is a minor metabolite of cyclamate (cyclohexylsulphamate) in the urine in man and animals (see, e.g., Golberg, Parekh, Patti & Soike, 1969; Oser, Carson, Nogin & Sonder, 1968).

We have found that Wistar albino rats kept on a normal diet and water excreted very little cyclohexylamine after a single oral dose of cyclamate, but rats kept for 3 months on the same diet but with water containing 0.5% calcium cyclamate (about 550mg./kg./day) developed the ability to convert cyclamate into cyclohexylamine. The latter rats (22) were given a single oral dose of calcium cyclamate (200mg.) and the urinary output of cyclohexylamine was determined by g.l.c. The urinary excretion of cyclohexylamine in 24hr. ranged from 0.7 to 35% of the dose (seven rats excreted more than 10% and 15 less than 10%). The cyclamate-treated rats (three) were given a single oral dose of [¹⁴C]cyclamate (50mg./kg.) and they excreted 93 (88–97)% of the ¹⁴C in 3 days, 64% in the urine and 27% in the faeces. In the first 24hr. after dosing an average of 58% of the ¹⁴C was excreted in the urine, 33% as unchanged cyclamate, 22% as cyclohexylamine and 3% as probably conjugated cyclohexylhydroxylamine. The faeces contained cyclamate and cyclohexylamine.

Three cyclamate-pretreated rats were given simultaneously [¹⁴C]cyclamate (10mg./kg.) intra-

peritoneally and unlabelled cyclamate (320mg./kg.) orally. The 24hr. urine was examined for cyclohexylamine by g.l.c. and scintillation counting. The conversion of [¹⁴C]cyclamate (given intraperitoneally) into cyclohexylamine was 0.9%, whereas that of unlabelled cyclamate (given orally) was 38%. About 90% of injected cyclamate was excreted in the urine in 24hr. compared with 58% when given orally.

When [¹⁴C]cyclamate was incubated for 1.5hr. with preparations of liver, kidney, spleen or blood from cyclamate-pretreated rats the formation of cyclohexylamine was no different from that of controls, i.e. less than 0.05%. The contents of the hindgut of the rats, however, converted 0.8–3.5% into cyclohexylamine under anaerobic conditions and 0.4–2.5% under aerobic conditions.

When the pretreated rats were taken off the cyclamate-containing diet, the ability to produce cyclohexylamine from cyclamate fell to a low level in 5 days.

These experiments suggest that the conversion of cyclamate into cyclohexylamine in the rat is carried out by the gut flora, which acquire this ability when the diet contains cyclamate. This ability is largely lost on removing cyclamate from the diet.

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The Physical Properties and Mechanism of Action of Deoxyxoglucarate Dehydratase from *Pseudomonas acidovorans*

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Deoxyxoglucarate dehydratase catalyses the conversion of D-4-deoxy-5-oxoglucarate into 2,5-dioxovalerate (2-oxoglutarate semialdehyde), CO₂ and water (Jeffcoat, Hassall & Dagley, 1968).

We report here the following physical properties of the enzyme from *Pseudomonas acidovorans* N.C.I.B. 10013 grown on glucarate: *S*_{20,w}⁰, 7.0s; *D*_{20,w}, 5.82 × 10⁻⁷ cm.²/sec.; $\bar{v}$, from the amino acid composition, 0.74 cm.³/g.; *E*_{cm}^{1%} at 280nm., 8.3; mol.wt. by sedimentation equilibrium, 118000; *S*_{20,w}⁰ in 6M-urea, 2.3s; *S*_{20,w}⁰ in 6M-guanidine

hydrochloride, 2·1s; mol.wt. in 6M-guanidine hydrochloride, 57000.

The enzyme is irreversibly inhibited by NaBH₄ (0·01M) in the presence of substrate (Jeffcoat, 1968). Such inhibition is indicative of the reduction of a Schiff base formed between the carbonyl group of the substrate and the free amino group of a specific lysine residue in the enzyme (Horecker, Pontremoli, Ricci & Cheng, 1961). This has been confirmed by treating deoxyoxo[6-¹⁴C]glucarate and NaBH₄ with enzyme from cells grown in the presence of [4,5(*n*)-³H]lysine. The resulting reduced enzyme-substrate complex was dialysed and chromatographed twice on DEAE-cellulose. Co-chromatography of ¹⁴C and ³H was obtained, with the ratio showing that 2 moles of substrate were bound/mole of enzyme. This [³H]enzyme-[¹⁴C]substrate complex was stable to polyacrylamide-gel electrophoresis.

The complex was hydrolysed with 6M-HCl at 110° and the hydrolysate subjected to paper electrophoresis (80 v/cm.) at pH 1·77 for 2 hr. Two radioactive areas were found. The first of these, lysine (82 cm. towards the cathode), contained only ³H. The second (44 cm. towards the cathode) contained both ³H and ¹⁴C and was therefore judged to be a substrate-lysine derivative. This doubly labelled derivative was stable to further paper electrophoresis (pH 6·5) and to repeated paper chromatography. The ratio of the ³H in this fragment to ³H in the free lysine was 1:28. This, together with the amino acid analysis showing 60 μmoles of lysine/μmole of enzyme, is in agreement with the binding of 2 μmoles of substrate/μmole of enzyme.

The mechanism of deoxyoxoglucarate dehydratase action thus involves the intermediate formation of a Schiff base between the substrate and a lysine residue in the enzyme. In this respect, the enzyme is similar to acetoacetate decarboxylase (EC 4.1.1.4) (Westheimer, 1963) and to a number of aldolases (class I aldolases; Rutter, 1964) that have this type of mechanism.

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Multiple Polymers of Histidine Ammonia-lyase

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Histidine ammonia-lyase (histidase, EC 4.3.1.3) catalyses the formation of urocanate from L-histidine. It is activated by GSH, mercaptoethanol and thioglycollate, and inhibited by EDTA (Mehler & Tabor, 1953).

We have purified histidase from *Pseudomonas testosteroni* grown in a medium containing L-histidine and succinate as sources of nitrogen and carbon. It was obtained by heat treatment of crude extracts followed by (NH₄)₂SO₄ fractionation and chromatography on DEAE-cellulose. When prepared in this way, the enzyme was activated some seven- or eight-fold by 3 mM-GSH.

When untreated enzyme was subjected to electrophoresis on 7½% polyacrylamide gels at pH 9·5 and stained with Amido Black, as many as nine bands were visible. If unstained gels were assayed for histidase, five and sometimes six bands of activity were detected. The assay method was not sufficiently sensitive to resolve the activities of the slower and closer-moving components. Addition of GSH (3 mM) before electrophoresis resulted in only one protein band and one activity band being obtained. These corresponded to the fastest-moving band present when GSH was omitted.

When enzyme without GSH was centrifuged in the analytical ultracentrifuge, five peaks were resolved, sedimenting with coefficients of 9·7, 14·1, 17·1, 19·8 and 21·6s respectively. In the presence of GSH (3 mM), a single but much larger 9·7s peak was obtained.

Mercaptoethanol, thioglycollate and dithiothreitol all had the same effect as GSH; substrate and EDTA did not influence the number of enzyme species obtained. The reversal of the effects of thiol treatment was achieved either by (NH₄)₂SO₄ precipitation at pH 6·0 or by chromatography on DEAE-cellulose.

The molecular weight of the GSH-treated enzyme was found to be 200000 by the sedimentation-equilibrium method of Yphantis (1964); a molecular weight of 33000-36000 was obtained for the enzyme in 6M-guanidine hydrochloride and 0·1M-GSH. These values compare with those of 214000 and 35000 respectively obtained by Rechler (1969) for histidase from an unnamed pseudomonad.

It appears that at least some free thiol groups of histidase are readily oxidized to form disulphide bonds, resulting in decreased enzymic activity. We suggest that these disulphide bonds may be formed either intra- or inter-molecularly, and that when