suspended in 0.32M-sucrose at a concentration of 1-2mg. of protein/ml., were treated at 0° for 1hr. with 0.5% Triton X-100 and M-KCl, pH 6.5 or 7.5. After centrifugation at 80000g for 20min., almost all of the activity was in the supernatant and was 170% of that originally present. The enzyme remained very soluble after the Triton had been almost completely removed by ammonium sulphate fractionation and dialysis, but the enzymic activity fell to values that approximated closely to the activity originally present in the untreated mitochondria. Triton measurements showed that a residual amount of Triton (approx. 1mg./mg. of protein) remained with the protein in the preparation. Attempts to retain the increased activity by repeating the fractionation procedure in media containing constituents expected to provide stabilizing conditions were unsuccessful. In each case the increased activity in the Triton extract was lost on removal of the Triton. The extracts, notwithstanding the loss of 'Triton-elevated' activity, proved to be quite stable on storage for weeks at -20°. The addition of Triton to the Triton-depleted extracts resulted in a slight but variable reactivation of about 15%, considerably less than the activation in the original Triton extract. The results are relevant to suggestions of part-latency of brain mitochondrial hexokinase.

M.F.T. is the holder of a Medical Research Council Studentship for training in Research Methods. The work was supported by the Research Fund of The Bethlem Royal Hospital and the Maudsley Hospital.

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Peptide Hydrolase Activity of *Klebsiella aerogenes*

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During extensive investigations (Barker, Pardoe, Stacey & Hopton, 1963; Barker, Somers, Stacey & Hopton, 1965; Barker, Hopton, Somers & Repas, 1966) into the ability of *Klebsiella aerogenes* (N.C.I.B. 9479) to synthesize inducible glycoside hydrolases it has become apparent that the micro-organism is able to utilize a variety of peptides as sole source of nitrogen. The present report describes an investigation of the evident peptide hydrolase activity of the micro-organism.

Four cultures of *K. aerogenes* were grown in minimal media (Barker *et al.* 1966) differing only in

the identity of the single nitrogen source. The four nitrogen sources were ammonium sulphate, glycine, glycyglycine and leucylglycine. Towards the end of the exponential phase the cells from each culture were harvested, washed and suspended in cold 0.02M-phosphate buffer, pH 7.0, and disrupted by shaking with glass beads for 45 min. Cell debris was removed by centrifugation and, after exhaustive dialysis of the extracts, peptide hydrolase activity was determined at pH 7.0 and 37° against glycyglycine and leucylglycine. Identical peptide hydrolase activity was exhibited by all four extracts. The cell-free culture fluids exhibited no significant activity. Intracellular constitutive activity was thus indicated.

An extract from ammonium sulphate-grown cells was chromatographed on DEAE-Sephadex equilibrated with 0.02M-phosphate buffer, pH 7.0. Peptide hydrolase activity was eluted between 280mm- and 300mm-NaCl. The active fractions were concentrated by ultrafiltration and re-chromatographed on Sephadex G-100 equilibrated with 0.02M-phosphate buffer, pH 7.0, containing 1.0M-NaCl. Peptide hydrolase activity was eluted as a single, included, peak. The hydrolase exhibited maximum activity at pH 7.2.

In addition to glycyglycine and leucylglycine the enzyme hydrolysed glycy-leucine, phenylalanyl-glycine, glycyphenylalanine, seryl-glycine, glycy-serine, lysyl-glycine, glycy-lysine, triglycine, tetra-glycine, leucylglycyglycine and glycyglycine ethyl ester. Peptides not hydrolysed included prolyl-glycine, glycyproline and the benzyloxycarbonyl derivatives of glycyglycine, triglycine and tetra-glycine. The ability of the enzyme to hydrolyse glycy peptides and glycyglycine ethyl ester but not the benzyloxycarbonyl derivatives of glycyglycine, triglycine and tetraglycine suggests aminopeptidase activity.

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A Soluble Aerobic Reduced Nicotinamide-Adenine Dinucleotide (Phosphate) Azoreductase

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When the lipid-soluble fungicide 3-methyl-4-(2-chlorophenylhydrazono)-isoxazol-5-one (otherwise known as drazoxolon) was incubated

aerobically with a rat liver homogenate that had been centrifuged for 10 min. at $10000g_{av.}$, in the presence of $NADPH_2$, *o*-chloroaniline was identified by gas-liquid and thin-layer chromatography as one of the reaction products. Subsequently enzyme activity was found to be located in the soluble fraction of the homogenate (the supernatant after 40 min. at $160000g_{av.}$), the microsomes being devoid of activity. This observation was unexpected, as microsomes contain an $NADPH_2$ -dependent azoreductase (EC 1.6.6.7) active against a wide variety of azo compounds (for review see Gillette, 1966).

The activity of the soluble azoreductase was only slightly decreased (10–20%) when $NADH_2$ was used instead of $NADPH_2$. Moreover, when the two nucleotides were added together at saturating concentrations the two activities were not additive. This suggests the participation of a single enzyme in the reaction. Enzyme activity was inhibited by dicoumarol ($I_{50} \ 7 \times 10^{-5}M$), but FAD ($8 \times 10^{-5}M$) produced stimulation (40%); the addition of GSH ($5 \times 10^{-4}M$), EDTA ($10^{-3}M$) or albumin (0.07%) were without effect. Unlike microsomal azoreductase (Hernandez, Mazel & Gillette, 1967), enzyme activity was stimulated (70%) after pretreatment of rats with 3-methylcholanthrene (20 mg./kg.) but not with phenobarbitone (100 mg./kg.).

The enzyme was partially purified by fractionation with ammonium sulphate, most of the activity being obtained at 55–65% saturation. The enzyme thus obtained retained its ability to utilize both $NADPH_2$ and $NADH_2$ with almost equal efficacy.

The properties of this azoreductase, especially with respect to the inhibitory action of dicoumarol and the ability to utilize both nicotinamide nucleotides, are very similar to those of the flavoprotein, DT-diaphorase (Ernster, Danielson & Ljunngren, 1962), and the response to 3-methylcholanthrene is similar to that of menadione reductase (EC 1.6.99.2) (Huggins & Fukunishi, 1964), which these authors equate with DT-diaphorase.

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The Metabolism of 2-Methoxy[^{14}C]ethylmercury Chloride

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Alkylmercury salts show a marked neurotoxic action that is absent from arylmercury salts (Gage &

Swan, 1961). This may be attributed to metabolic differences; the alkyl compounds are stable and are only slowly excreted, but the aryl ones are degraded to inorganic mercury (Gage, 1964). Alkoxyalkylmercury salts appear to resemble the aryl more than the alkyl compounds, both in the absence of the characteristic neurotoxic effects, and in the distribution and excretion of radioactivity after the administration of a sample labelled with ^{203}Hg (Ulfvarson, 1962). When 2-methoxy[^{14}C]ethylmercury chloride is administered subcutaneously to rats, about 6% of the radioactivity appears as CO_2 in the expired air, and if the air is passed through a pyrolysis tube a further 45% can be recovered in 48 hr. About the same amount can be obtained by passing the air through mercury perchlorate solution, a reagent with a specific affinity for olefins (Keller, 1941); the radioactive component is released on acidification and is identical with ethylene by gas chromatography. Within 5 days of dosing, about one-quarter of the dose appears in urine as total mercury and about the same proportion as radioactivity, but chloroform extraction and the kinetics of excretion indicate that only during the first 24–48 hr. is there any appreciable excretion of organic mercury. Thereafter the urinary mercury is inorganic. During the first day there is an excretion of organic mercury in bile, but all faecal mercury is inorganic. There is a storage of mercury, but no radioactivity in kidneys, and it seems probable that there is a breakdown of the compound in the tissues generally, and the mercury migrates to the kidneys where it is stored and later excreted. There is no evidence of any enzymic *O*-demethylation of the compound by slices of liver or kidney, and it is likely that the breakdown is a non-enzymic reversal of the process of synthesis, which occurs in the presence of cysteine at low pH (Weiner, Levy & Mudge, 1962):

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