rose to about 2.5 at 21 days, which is comparable with the value observed in the adult brain. The development of the compartmentation of glutamate in rat brain therefore coincided approximately with that of the rapid conversion of [^{14}C]glucose into glutamate.

The fate of [^{14}C]leucine in the adult brain was further investigated at 5–120 min. after intraperitoneal administration of [^{14}C]leucine. The maximum specific radioactivity was attained at first by glutamine, then by glutamate and considerably later by aspartate. The curves for the specific radioactivities as a function of time did not intersect; the relative specific radioactivity of glutamine even at 2 hr. after the injection was about 1.5.

The present results are consistent with the entry of [^{14}C]acetyl-CoA derived from [^{14}C]leucine into a metabolic compartment containing a relatively small pool of glutamate, which is connected to a relatively large pool of glutamine in the brain (cf. reviews by Berl & Clarke, 1968; Van den Berg, 1968). Evidence was also obtained that the co-ordination of the energy metabolism develops in parallel with metabolic compartmentation in the rat brain.

Berl, S. (1965). *J. biol. Chem.* **240**, 2047.

Berl, S. & Clarke, D. D. (1968). In *Handbook of Neurochemistry*. Ed. by Lajtha, A. New York: Plenum Publishing Co. (in the Press).

Gaitonde, M. K. & Richter, D. (1966). *J. Neurochem.* **13**, 1309.

Himwich, W. A. (1962). *Int. Rev. Neurobiol.* **4**, 117.

Roberts, S. & Morelos, B. S. (1965). *J. Neurochem.* **12**, 373.

Van den Berg, C. J. (1968). In *Handbook of Neurochemistry*. Ed. by Lajtha, A. New York: Plenum Publishing Co. (in the Press).

The Effect of Thyroid Hormones on the Biochemical Maturation of the Rat Brain

By J. A. COCKS, R. BALÁZS and J. T. EAYRS.
(Medical Research Council Neuropsychiatric Research Unit, Carshalton, Surrey)

The growth and maturation of the brain are associated with changes of metabolic pattern resulting from changes in the activity and the co-ordination of different enzyme systems. The rapid incorporation of glucose into the free amino acids, which is characteristic of the adult brain, has been shown to develop in the rat between the ages of 10 and 21 days (Gaitonde & Richter, 1966). The development of this metabolic pattern was used as an index of maturation in studying the action of thyroid hormones.

Rats of Sprague-Dawley strain (10–19 days old) were killed 20 min. after an injection of 10–20 μC /

100 g. body wt. of [^{14}C]glucose of specific radioactivity 10 $\mu\text{C}/\mu\text{mole}$. The amino acids in the acid-soluble fraction were separated from neutral compounds and carboxylic acids as described by Gaitonde & Richter (1966). In the control 10-day-old rats, about 25% of the acid-soluble ^{14}C was recovered in the amino acids. By the age of 19 days the value had increased to 60%, which is close to the value obtained in the adult.

Tri-iodothyronine treatment (Khamisi & Eayrs, 1966) advanced the onset of rapid incorporation of glucose carbon into amino acids in the rat brain. At 12 days the rate of incorporation in the thyroid-hormone-treated animals was 64% higher than in normal control rats, whereas in neonatal radiothyroidectomized animals the incorporation was decreased: at 10 days it was 72% and at 19 days 54% of the normal control value.

The results give evidence that the biochemical maturation of the brain is retarded in thyroid deficiency. Conversely, tri-iodothyronine treatment results in a premature development of the adult metabolic pattern. There is an apparent correlation between the advancement of innately organized behaviour (e.g. startle response; Khamisi & Eayrs, 1966) and the biochemical maturation of brain: in animals treated with thyroid hormone. The behavioural changes observed after neonatal thyroidectomy are apparently associated with the retardation of the biochemical maturation of the brain and structural changes involving a hypoplasia of the cortical neuropil (Eayrs, 1966).

Eayrs, J. T. (1966). *Rev. sci. Basis Med.* p. 317.

Gaitonde, M. K. & Richter, D. (1966). *J. Neurochem.* **13**, 1309.

Khamisi, F. & Eayrs, J. T. (1966). *Growth*, **30**, 143.

Solubilization of Mitochondrial Hexokinase from Ox Cerebral Cortex with Triton X-100

By M. F. THOMPSON and H. S. BACHELARD.
(Department of Biochemistry, Institute of Psychiatry, British Postgraduate Medical Federation, University of London, London, S.E. 5)

The mitochondrial hexokinase of rat and guinea-pig brain has been solubilized by osmotic shock in the presence of ATP (Bachelard, 1967) or glucose 6-phosphate (Rose & Warms, 1967) or by treatment with Triton X-100 in the presence of high salt concentrations (Teichgräber & Biesold, 1968). The hexokinase of purified mitochondria from ox cerebral cortex, prepared by sucrose-density-gradient centrifugation, was found to be relatively resistant to extraction by the methods involving osmotic shock, but was readily solubilized with Triton X-100, with the apparent activation observed in the previous studies. The mitochondria,

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.

Bachelard, H. S. (1967). *Biochem. J.* **104**, 286.

Rose, I. A. & Warms, J. V. B. (1967). *J. biol. Chem.* **242**, 1635.

Teichgräber, P. & Biesold, D. (1968). *J. Neurochem.* **15**, 979.

Peptide Hydrolase Activity of *Klebsiella aerogenes*

By S. A. BARKER and P. J. SOMERS. (Department of Chemistry, University of Birmingham) and R. EPTON (Department of Applied Science, Wolverhampton College of Technology)

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.

Barker, S. A., Hopton, J. W., Somers, P. J. & Repas, A. (1966). *Carbohydr. Res.* **3**, 230.

Barker, S. A., Pardoe, G. I., Stacey, M. & Hopton, J. W. (1963). *Nature, Lond.*, **197**, 231.

Barker, S. A., Somers, P. J., Stacey, M. & Hopton, J. W. (1965). *Carbohydr. Res.* **1**, 106.

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

By J. W. DANIEL. (Imperial Chemical Industries Ltd., Industrial Hygiene Research Laboratories, Alderley Park, Macclesfield, Cheshire)

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