Effect of Undernutrition in Early Life on Glutamate Decarboxylase Activity in the Adult Brain

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Rats were undernourished during the foetal and suckling periods by restriction of maternal food intake (Adlard & Dobbing, 1972). At weaning (25 days of age) undernourished animals were divided into two groups: (a) rats fed *ad libitum* from weaning (rehabilitated group); (b) rats whose diet was further restricted until 12 weeks of age (chronically undernourished group), when they weighed about 60g (controls weighed about 360g). Those animals of the latter group not killed at 12 weeks were fed *ad libitum* until 44 weeks of age. Animals well-nourished throughout life served as controls.

At 12 weeks of age forebrain and cerebellar acetylcholinesterase activities (Ellman *et al.*, 1961) (per g wet wt.) were significantly ($P < 0.01$) higher both in rehabilitated and in chronically undernourished animals compared with controls. These changes, in support of previous findings (Adlard & Dobbing, 1971a,b), were in the range 10–15%, but were not apparent in 44-week-old animals.

At 44 weeks of age glutamate decarboxylase activity (Tapia & Awapara, 1969) (per g wet wt.) was significantly ($P < 0.05$) increased in each group of previously undernourished animals compared with controls. Results [mean $\pm$ s.d. (n)] for forebrain were as follows: control, 38.1 ± 1.0 (9) $\mu\text{mol/h per g}$; rehabilitated, 39.3 ± 1.3 (8) $\mu\text{mol/h per g}$; chronically undernourished, 40.4 ± 2.4 (9) $\mu\text{mol/h per g}$. Corresponding results for cerebellum were: control, 22.7 ± 1.2 (9) $\mu\text{mol/h per g}$; rehabilitated, 24.6 ± 1.8 (8) $\mu\text{mol/h per g}$; chronically undernourished, 24.7 ± 2.2 (8) $\mu\text{mol/h per g}$. In contrast, total glutamate decarboxylase activity (per region) was not affected by undernutrition.

Histochemical studies have shown that glutamate decarboxylase and the product of its reaction, namely γ -aminobutyrate, which is a putative inhibitory neurotransmitter compound, are concentrated in certain nerve fibres and terminals in the mammalian central nervous system (Fonnum, 1972). Glutamate decarboxylase activity is enriched in the synaptosomal fraction of brain homogenates, and there is kinetic evidence for the existence of a compartment that has metabolic properties compatible with those of a ' γ -aminobutyrate-nerve-terminal compartment'

(Balázs *et al.*, 1972); a high proportion of the total γ -aminobutyrate flux is associated in this compartment with a small proportion of total glutamate. A possible interpretation of the present results is therefore that nerve endings are relatively spared after undernutrition in early life.

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Aminopyrine Demethylase Activity in Normal and Vitamin E-Deficient Male Rats

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Aminopyrine is demethylated by the rat liver microsomal mixed-function oxidase system, first to monomethylaminopyrine, then to 4-aminoantipyrine, with the production of formaldehyde at each step (La Du *et al.*, 1955).

Microsomal fraction was prepared from the livers of rats given an adequate diet for 3–4 months, and also from rats given the vitamin E-deficient diet and the vitamin E-and-Se-deficient diet (Diplock *et al.*, 1971), and another 10%-casein vitamin E-and-Se-deficient diet. Further experiments were carried out with microsomal fraction from deficient rats re-fed with vitamin E for 6 days (3mg of DL- α -tocopherol/day orally in 0.1 ml of olive oil).

The apparent K_m and V_{max} for the overall two-stage reaction were obtained by measuring the formaldehyde production by the method of Nash (1953). Microsomal fraction from the vitamin E-deficient animals exhibited abnormal kinetics: the apparent V_{max} fell 15–20% below normal values (from 4.4 to 3.7 $\mu\text{mol/h per g}$ of protein), whereas the K_m increased threefold from 2.5×10^{-4} to 8.7×10^{-4} M. Similar results were obtained with microsomal fraction from the vitamin E-and-Se-deficient animals.

This lowered activity was not due to a decrease in cytochrome P-450 concentration, which, measured by

the method of Omura & Sato (1964), was 0.7 nmol/mg of protein in microsomal fractions derived from rats given each of the dietary treatments. The lowered activity could not be explained by a defect in the electron-transport path by which the cytochrome is reduced, since the total reduction of the cytochrome by NADPH was found to be the same in microsomal fractions derived from rats given each of the dietary treatments.

When the rate of oxidation of NADPH was examined in the presence of aminopyrine, the nucleotide was oxidized more slowly by the microsomal preparation from vitamin E-deficient rats.

These results suggest that the mixed-function oxidase system exhibits a specific requirement for vitamin E. Carpenter (1967, 1968) has also reported abnormal kinetics in microsomal drug metabolism in vitamin E-deficient animals. However, details of her work are not available for comparison with ours.

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Inhibition of the Alternative Pathways of Chlorpromazine Metabolism *in vitro*

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Chlorpromazine and its four main metabolites, namely monodesmethylchlorpromazine, chlorpromazine sulphoxide, 7-hydroxychlorpromazine and chlorpromazine *N*-oxide, were determined, by a radio-t.l.c. technique, after microsomal oxidation by a guinea-pig hepatic preparation in the presence of various compounds as possible inhibitors of drug metabolism *in vitro*.

Inhibition of chlorpromazine demethylation was found with *N*-ethylmaleimide and *p*-chloromercuribenzoate at 1 mM, whereas these compounds, which react with thiol groups, had no effect on *N*-oxidation at this concentration. Sulphoxidation and ring hydroxylation were moderately affected. Compound SKF 525A (2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride) at 1 mM inhibited all routes of metabolism except *N*-oxidation.

1,10-Phenanthroline strongly inhibited all routes of metabolism except *N*-oxidation. 2,2'-Bipyridyl,

another chelating agent, also inhibited demethylation and ring hydroxylation of chlorpromazine, but had little or no effect on sulphoxidation or *N*-oxidation.

Metyrapone [2-methyl-1,2-bis-(3-pyridyl)propan-1-one] and 4-aminobiphenyl inhibited the formation of chlorpromazine *N*-oxide to a greater extent than they did the other routes of chlorpromazine metabolism, whereas imidazole, another compound that binds with microsomal cytochromes, had no effect on *N*-oxidation of chlorpromazine but strongly inhibited the other routes.

Diphenylamine and the water-soluble free-radical scavenger *NN*-diphenyl-*N'*-2,4-dinitro-6-sulphophenylhydrazyl inhibited all routes of chlorpromazine metabolism, but *N*-oxidation was the least affected.

Whereas propyl gallate produced a large decrease in the demethylation of chlorpromazine but had no effect on the formation of chlorpromazine *N*-oxide, another antioxidant, ascorbic acid, had no effect on any route of chlorpromazine metabolism.

Hg²⁺, Zn²⁺, Fe³⁺ and Cu²⁺ at 1 mM caused inhibition of demethylation of chlorpromazine but not of *N*-oxidation.

Some inhibition by cysteamine of *N*-oxidation of chlorpromazine was found [cf. oxidation of nicotine (Gorrod *et al.*, 1971) and normethadone (Beckett *et al.*, 1971)], but this route was not inhibited by dithiothreitol, unlike the *N*-oxidation of normethadone or nicotine. Little or no inhibition of *C*-oxidation by cysteamine or dithiothreitol was reported with nicotine or normethadone, in agreement with our finding with chlorpromazine.

The results indicate that demethylation, ring hydroxylation and sulphoxidation are probably mediated by different enzyme systems from that which catalyses *N*-oxidation of chlorpromazine, a result in accordance with oxidation mechanisms for other xenobiotics (Beckett, 1971).

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A Study of the Possible Metabolism of Trichlorofluoromethane

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Trichlorofluoromethane (CCl₃F), although structurally similar to CCl₄, is regarded as inert and non-toxic (Jenkins *et al.*, 1970), and as such has been used in combination with other chlorofluoroalkanes