Table 1.

Dietary protein (20%)	Corn oil	Change in body wt (%)	Liver wt (% final body wt)	Cytochrome b_5 (nmoles/liver)	Cytochrome P-450 (nmoles/liver)	Hexobarbitone sleeping time (min)
Casein	Fresh	+55	2.9 ± 0.2	322 ± 48 (6)	43 ± 16 (6)	—
	Aged	+35	2.9 ± 0.1	—	105 ± 21 (11)	24 ± 1.2
Soya	Fresh	−3	4.7 ± 0.4	338 ± 73 (6)	80 ± 14 (6)	—
	Aged	+2	3.9 ± 0.1	—	37 ± 12 (9)	109 ± 15
Gluten	Fresh	+52	3.3 ± 0.9	94 ± 25 (4)	68 ± 14 (4)	—
Zein	Fresh	−11	4.9 ± 0.2	41 ± 4 (4)	30 ± 7 (4)	—
Soya + 1% methionine	Fresh	+39	3.9 ± 0.7	82 ± 29 (4)	64 ± 12 (4)	—

Mean values ± S. E. M. The numbers in parentheses indicate the number of animals on which the mean is based.

casein-based diet but, somewhat unexpectedly, a marked decrease in the 'soya' diet. This was in keeping with the considerably prolonged hexobarbitone sleeping time found in the 'soya' animals (Table 1).

These experiments would therefore appear to indicate (i) that different types of dietary protein result in different hepatic microsomal levels of cytochrome P-450 and of cytochrome b_5 and that these effects are apparently independent of the comparative biological values of the protein (ii) that the inter-relationships between detoxication capacity, dietary protein and oxidized lipid material are more complex than was originally supposed.

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The effects of folate on neurotransmitter uptake into rat cerebral cortex slices

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The observation of the stimulatory activity of folate on the brain [1–3] has led to experiments investigating the mechanisms involved. Davies and Watkins [4] applied folic acid (pteroyl monoglutamate; PGA) and formyl tetrahydrofolic acid (folinic acid; f-THF) to neurons of the cat cerebral cortex and found there was little response with quiescent neurones, but enhanced activity following initial stimulation with glutamate. A possible explanation of these results was provided by quantitative studies on the rat dorsal root ganglion. These showed that PGA inhibits high affinity glial uptake of glutamate, with an apparent K_i of 5.6 ± 10^{-4} M [5]. Conversely receptor competition between PGA and inhibitory transmitters has been proposed. Evidence in support of this has been demonstrated *in vivo* in the rat cuneate nucleus [6]. The following is an experiment in which the effect of folate on the uptake of PGA, γ-aminobutyric acid, glutamate and noradrenaline into slices of rat cerebral cortex were measured.

The uptake studies were carried out in Krebs' balanced salt solution. Pieces of rat cerebral cortex were weighed and then cut into 0.1-mm slices in two directions (at 45° to each other) using a Mickle chopper. The fragments (280–300 mg total) were then suspended in 5 ml of ice-cold Krebs' solution and 200-μl aliquots were placed in stoppered test tubes in a metabolic shaker maintained at 37°. The tubes were gassed with 95% O_2 and 5% CO_2. After 5 min incubation the solution of the isotope-labelled transmitter was added in a total volume of 100 μl. Following a further period of incubation the entire contents of the tube were transferred to a Millipore filter and the brain slices were washed four times with 100 ml of ice-cold saline. The Millipore filter pad with tissue particles was transferred to a glass scintillation counting vial. Two hundred μl of distilled water were added to elute the isotope-labelled transmitter from the tissue. After 20 min, 7.0 ml of PPO–POPOP scintillant (2.5-diphenyloxazole, 5 g; 1,4-di-2(5 phenyloxazolyl)-benzene, 1 g; triton X-100, 300 ml; toluene, 700 ml) were added. The radioactivity in the vials was measured in a Beckman automatic liquid scintillation counter. The labelled substances investigated were

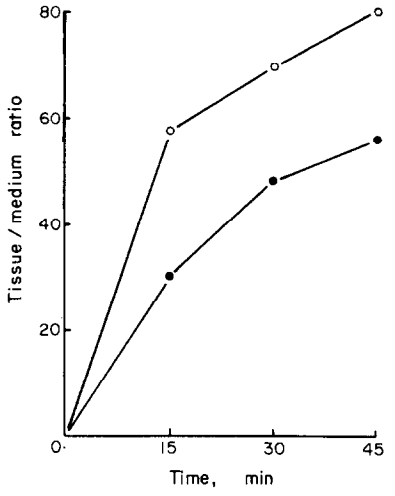

Fig. 1. Tissue/medium ratio of radioactive counts in mouse cerebral cortex slices with no folate added (solid circles) and in the presence of 10^{-4} moles/l f-THF (open circles) at various times after the addition of [^{3}H]glutamic acid.

as follows: glutamic acid-[G-^{3}H]; DL-noradrenaline-[7-^{3}H]; DL-noradrenaline (carbinol [^{14}C]) DL-bitartrate; [^{3}H] γ-aminobutyric acid-3H(G); folic acid-2-[^{14}C], potassium salt.

Over a 60-min period no significant accumulation of [^{14}C] PGA could be measured. The incubations were carried out at two concentrations: 0.5×10^{-4} M and 1 ± 10^{-7} M. Although some radioactivity was detected in the brain particles, this was small in amount and was consistent with diffusion from the medium. The uptake of γ-aminobutyrate, glutamate and noradrenaline were all increased in the presence of 10^{-4}, 10^{-5} and 10^{-6} M f-THF, the tissue/medium ratios increasing by 60–100 per cent (Figs 1, 2, 3.) The same concentrations of PGA increased

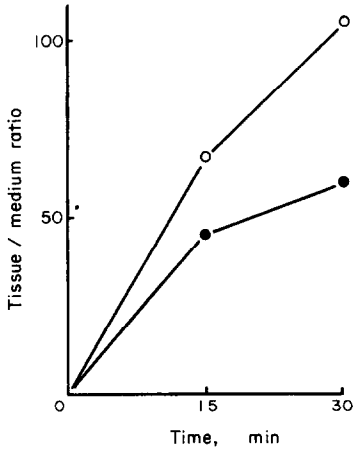

Fig. 2. Mouse cerebral cortex uptake of [^{3}H]GABA in absence of folate (solid circles) and in the presence of 10^{-5} moles/l f-THF (open circles).

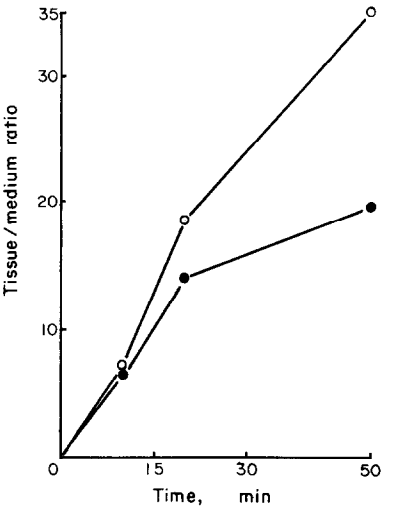

Fig. 3. Uptake of [^{3}H]noradrenaline by mouse cerebral cortex slices in the absence of folate (solid circles) and in the presence of 10^{-4} moles/l f-THF (open circles).

the tissue/medium ratio of tritiated γ-aminobutyric acid by approximately 3–30 per cent. These results do not support the suggestion that folate stimulates the brain by inhibiting the uptake of excitatory neurotransmitters into cells. The experiments show a similar degree of enhancement of uptake of both excitatory (glutamate) and inhibitory (noradrenaline; γ-aminobutyric acid) central transmitter substances. Such a uniform action on different transmitters suggests a non selective action by folate on membrane permeability rather than a highly selective modification of active reuptake mechanisms on a particular type of transmitter.

The uptake studies of labelled folate failed to demonstrate any concentration in brain tissue. Although these results do not rule out the possibility that folate itself is a transmitter, they do indicate that it does not possess a specific reuptake system.

The mechanism of the central stimulating effects of folate remain a mystery, but an action at membrane level appears to be an important possibility.

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