guanine nucleotides under these conditions.) In the presence of ethyl adenosine 5'-carboxylate, there was a smaller decrease in radioactive ATP, total adenine nucleotides and total nucleotides, and a smaller increase in radioactive hypoxanthine and inosine. However, there was a 3.7-fold increase in the accumulation of radioactive adenosine.

These results are similar to those observed when deoxyglucose-induced ATP catabolism was studied in the presence of coformycin [6], a potent and specific inhibitor of adenosine deaminase in these cells [8]. The inhibition of this enzyme led to increased accumulation of adenosine and decreased formation of inosine and hypoxanthine during ATP catabolism; however, considerable inosine and hypoxanthine still were formed by the pathway adenylate → inosinate → inosine [6]. Some of the adenosine, which accumulated when adenosine deaminase was blocked, was rephosphorylated to ATP, whose concentration consequently increased. Inhibition of adenosine kinase or of adenylate deaminase, in the absence of concomitant inhibition of adenosine deaminase, would not produce the same results, as any adenosine that might tend to accumulate would be deaminated [6].

We conclude that ethyl adenosine 5'-carboxylate, like coformycin, may inhibit adenosine deaminase when relatively low concentrations of adenosine are generated intracellularly during nucleotide catabolism. However, because this effect is not observed when cells are incubated with relatively high concentrations of exogenous adenosine, and is not observed in studies with purified enzymes [1, 2], it would seem to be a very weak inhibitor. The relation, if any, of this presumably weak inhibition of adenosine deaminase by ethyl adenosine 5'-carboxylate to its vasodilatory action is not known. No other aspect of purine metabolism was affected by this adenosine analog. It seems likely that the potent physiological effects of this compound are exerted through a mechanism unrelated to inhibition of specific aspects of intracellular purine metabolism.

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Changes in liver microsomal cytochrome P-450 induced by dietary proteins and lipid material

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Nutritional status can influence the activity of the drug-metabolising enzymes located in rat liver microsomes [1, 2]. Protein-deficient diets lower the concentration of cytochrome-P-450 in liver microsomes[3] and reduce the *in vivo* metabolism of drugs [4]. Lipid materials are also necessary for the normal synthesis of microsomal cytochrome P-450 and hydroxylating enzymes [5]. Two types of lipid material (unsaturated fatty acids and oxidized sterols) can fulfil this function, presumably by acting as permissive agents in the induction of microsomal hydroxylation enzymes by substances such as phenobarbitone [5, 6]. The experiments described in this note were designed to extend our knowledge of these inter-relationships and in particular to examine the 'lipid effect' in the presence of different dietary proteins.

Male Wistar rats of initial body weight 60–80 g (CFHB strain, Carworth Europe, Alconbury, Herts) were caged in groups in mesh-floored cages and given a powdered synthetic diet with the following composition (g): Maize starch 700, protein 200, corn oil 50, salt mixture (Glaxo Research Ltd.) 10, choline chloride 0.2, vitamin mixture 0.3; the vitamin mixture had the following composition (mg): riboflavin 160, thiamine hydrochloride 160, pyridoxine 160, calcium pantothenate 400 , cyanocobalamin 0.5, nicotinamide 2000, folic acid 100, biotin 12. All animals received a weekly supplement of 0.5 ml cod liver oil and 0.3 ml of a 1% solution of vitamin K.

The rats received the appropriate diet for 21 days. The proteins used were casein, gluten, soya, zein and soya, with 1% (DL) methionine added, all obtained from BDH Ltd., Poole, Dorset. Rats given the zein and unsupplemented soya diet did not grow during the experimental period. Hexo-barbiturate sleeping time was measured after an intraperitoneal dose of sodium hexabarbiturate (100 mg/kg body wt), liver microsomes were prepared by differential centrifugation; cytochrome P-450 was determined as described by Omura and Sato [7] with an assay concentration of 1–2 mg microsomal protein/cuvette and cytochrome b_5 by the method of Baron and Tephly [8] using a Perkin–Elmer Hitachi 124 double beam spectrophotometer fitted with an external recorder.

Significant increases were found in the total liver cytochrome P-450 in rats given gluten and soya diets when compared with the casein diet; gluten, zein and soya-supplemented diets produced lower microsomal cytochrome b_5 concentrations (Table 1). No obvious explanation exists for the 10-fold difference in cytochrome b_5 concentrations. The presence is implied of an inducer of cytochrome b_5 in the soya and casein diets. Poorer-quality proteins (gluten and soya) unexpectedly perhaps, produced an increase in the total cytochrome P-450.

The substitution of aged (oxidized) corn oil for fresh oil elicited different responses in two diets. Aged oil gave an increase in the microsomal cytochrome P-450 in the

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