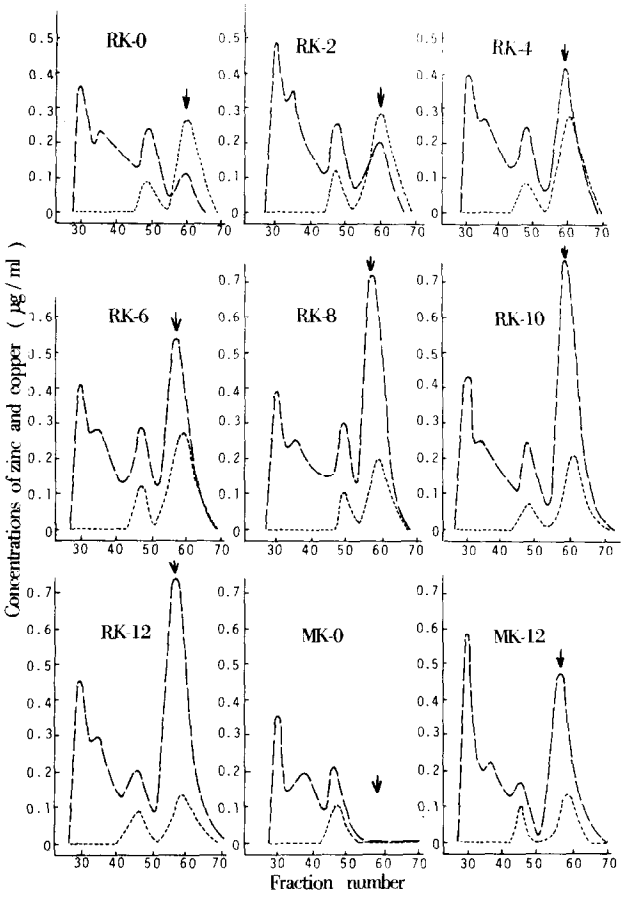

Fig. 2. Sephadex G.75 elution profiles of kidney supernatants of rats and mice injected with zinc. The kidney supernatants (10 ml/column) were applied to a Sephadex G-75 column, eluted with 1 mM Tris buffer solution (pH 8.6), and collected (5 ml/tube). — —, Zn; and – – – –, Cu. The arrow indicates the metallothionein fraction. RK and MK are abbreviated forms of rat kidney and mouse kidney, respectively, and 0–12 indicate injected amounts of zinc.

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Convulsant drug action on GABA and taurine synthesis

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Among convulsant drugs, allylglycine and several pyridoxal phosphate antagonists have been shown to inhibit cerebral glutamic acid decarboxylase activity (L-glutamate carboxy lyase, EC 4.1.1.15, GAD) and reduce brain GABA concentration [1–5]. Taurine has inhibitory actions on neuronal firing [6, 7]; the concentration of taurine is reduced in some forms of focal epilepsy [8] and an antiepileptic action of taurine has been demonstrated experimentally [9, 10]. As a key cerebral enzyme synthesising taurine, cysteine sulphinate decarboxylase (EC 4.1.1.29, CSAD) requires pyridoxal phosphate as a cofactor; it is possible that a reduction in taurine synthesis contributes to the genesis of seizures observed after these convulsant drugs. We have therefore investigated the activity of GAD and CSAD and the concentration of GABA and taurine in mouse brain at the time of seizure onset following the systemic administration of allylglycine

Table 1. *In vivo* effect of convulsants on GAD and CSAD activity measured in mouse brain homogenates

Treatment	Incidence of convulsions	Mean time of killing (min)	GAD activity −PLP	GAD activity +PLP	CSAD activity 5 μM PLP	CSAD activity 500 μM PLP
Control	0/6	—	9.0 ± 0.6	18.3 ± 0.8	18.9 ± 1.8	24.6 ± 0.9
AG	5/6	82 ± 2.5	5.1 ± 0.4†	13.0 ± 1.2†	21.6 ± 4.9	43.2 ± 12.0
4DP	3/6	33 ± 3.0	3.3 ± 0.1†	19.7 ± 0.6	12.7 ± 1.0*	23.7 ± 1.7
INH	4/6	80 ± 6.0	5.0 ± 0.2†	18.5 ± 0.9	18.9 ± 1.5	39.5 ± 5.2*
TSC	2/6	29 ± 2.5	7.0 ± 0.2†	18.6 ± 0.6	12.5 ± 1.1*	26.0 ± 2.1

Mean time of killing is the interval after injection of allylglycine (AG), 4-deoxypyridoxine (4DP), isonicotinic acid hydrazide (INH), thiosemicarbazide (TSC) or saline (control). Brain homogenates were assayed for GAD and CSAD activity in the absence (−PLP) or presence (+PLP) of exogenous PLP, as described in the text. Enzyme activities are presented as mean ± S.E.M. (μmole/g/hr), N = 6. Differences between activity in control and homogenates from drug-treated preparations were compared by Student's *t*-test and are denoted *$P < 0.05$, †$P < 0.01$.

(AG), and three pyridoxal phosphate antagonist convulsant drugs.

Adult Swiss S mice, 20–30 g, were injected i.p. (0·2 ml/mouse) with DL-allylglycine (AG, 200 mg/kg), 4-deoxypyridoxine hydrochloride (4DP, 250 mg/kg), isonicotinic acid hydrazide (INH, 150 mg/kg), thiosemicarbazide (TSC, 100 mg/kg) or saline. The animals were kept at an ambient temperature of 32° and observed for the onset of seizures. After the first convulsion in each drug-treated group, the animals were killed by decapitation (in the same time sequence as they had been injected). Brains were removed, divided mid-sagitally, frozen in liquid nitrogen (within 1 min of killing) and kept at −75° until homogenised.

For the estimation of enzyme activities, frozen right half-brains were weighed and homogenised in 2 ml ice-cold 0.2% (v/v) Triton X-100 containing 2.5 mM AET (2 amino ethyl isothiouroniumbromide hydrobromide). GAD activity was estimated as previously described [11], with 32 mM L-glutamate. CSAD activity was determined using 40 mM L-cysteine sulphinate by a slight modification of the method of Yoneda *et al.* [12]. Brain homogenates (equivalent to 10 mg wet wt of tissue) were prepared immediately before addition to reaction mixtures; samples were incubated for 30 min at 25° together with appropriate tissue blanks. Enzyme activities from saline injected animals (controls) were assayed concurrently with homogenates from drug treated animals. Frozen left half-brains were weighed, homogenised in 0.6 N ice-cold perchloric acid, neutralised and GABA and taurine estimated by previously published methods [13, 14]. DL-[1-^{14}C]Cysteine sulphinic acid (specific radioactivity 11 mCi/mmole) acid was purchased from Centre d'Etudes Nucléares de Saclay and L-[1-^{14}C]glutamate (specific radioactivity 55 mCi/mmole) from The Radiochemical Centre, Amersham, Bucks. All other chemicals were from Sigma Chemical Co.

Table 2. Effect of convulsants on taurine and GABA levels in mouse brain *in vivo*

Treatment	GABA (μmoles/g)	Taurine (μmoles/g)
Control	1.52 ± 0.20	7.85 ± 0.21
AG	1.00 ± 0.09*	8.22 ± 0.56
4DP	0.86 ± 0.06†	8.74 ± 0.20†
INH	1.08 ± 0.11*	8.47 ± 0.68
TSC	1.30 ± 0.15	7.80 ± 0.36

Values are mean ± S.E.M. (N = 6).

Significant differences between control and drug-treated groups are denoted by *$P < 0.05$, †$P < 0.01$ (Student's *t*-test).

In preliminary experiments, linear activity of CSAD over 30 min could be achieved only by incubating at 25°, and with the addition of 5 μM pyridoxal phosphate (PLP). Consequently, GAD was also measured at 25°, giving lower activities than those we have reported previously [15]. Without exogenous PLP a decrease in GAD activity was seen after all four compounds (Table 1) being greatest after 4DP (63 per cent), and least after TSC (23 per cent). With the exception of AG these decreases were not seen in the presence of exogenous PLP (500 μM), in agreement with our previous observations [4, 11].

A decrease in brain GABA concentration was seen (Table 2) after 4DP (43 per cent), AG (34 per cent) and INH (29 per cent) but was not significant after TSC (15 per cent).

Decreases in CSAD activity were observed (Table 1) after 4DP (33 per cent) and TSC (34 per cent). These decreases were not seen when assayed in the presence of higher concentrations of PLP (500 μM). In none of the drug-treated groups was there a decrease in cerebral taurine concentration (Table 2).

These experiments demonstrate that convulsant doses of pyridoxal phosphate antagonist drugs can decrease cerebral CSAD activity. However, this effect appears unlikely to be important in the genesis of convulsions as it is not associated with a reduction in brain taurine concentration. Similarly, we have shown that TSC and 4DP inhibit cerebral L-aromatic amino acid decarboxylase activity (EC 4.1.1.26, 4.1.1.28,) [11]. Such inhibition was not associated with significant decreases in dopamine and 5-hydroxytryptamine concentrations or their apparent turnover rate.

A reduction in the rate of synthesis of GABA remains the most probable explanation for the convulsant effects of AG and 4DP [3, 16].

The relatively small overall decrease in brain GABA content after TSC directs attention to the possibility of subtle regional changes in GABA or in some other transmitter system.

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Phenobarbitone effects on hepatic microsomal enzymes and liver blood flow in the guinea pig*

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Phenobarbitone is a potent inducer of liver microsomal enzymes and is known to increase liver blood flow in the rat [1–3] which is in contrast to other inducing agents such as 3,4-benzpyrene, phenytoin and chlordiazepoxide [1,2]. The increase in liver flow resulting from phenobarbitone pretreatment in the rat is dose-dependent and parallels the increase in liver weight [2]. This compensatory increase in hepatic blood flow is a result of increased portal venous return [1,2] and, together with the enhanced enzyme activity, could have important consequences for the rate of elimination of exogenous compounds [4].

As part of our investigation of the guineapig as a model of enzyme induction in man [5], we have studied the effects of phenobarbitone on hepatic microsomal enzyme activity and liver blood flow in this species.

Enzyme induction. Dunkin Hartley male guinea pigs weighing 320–420 g were housed in groups in rigid plastic cages with sawdust bedding. They were maintained on Oxoid Diet 18 and water (containing 50 mg ascorbic acid/l) *ad lib.* The animals were given intraperitoneal (i.p.) injections of phenobarbitone (40 mg/kg) once daily for 4 days. The phenobarbitone was injected in a volume 1 ml physiological saline/kg body weight (body wt.) and the control group of guinea pigs was injected with 1 ml physiological saline/kg body wt. The animals were randomly allocated to blood flow or enzyme studies.

Measurement of liver blood flow. The radioactive microsphere method was used [6]. Animals were anaesthetised with sodium pentobarbital (40–60 mg/kg i.p.; Sagatal, May & Baker). Artificial ventilation was given through a tracheal cannula. The right femoral artery was cannulated to allow withdrawal of blood at a constant rate (0.6 ml/min) during, and for 70 sec after, injection of the microspheres. Carbonized plastic microspheres of 15 ± 5 μm diameter labelled with ^{141}Ce (3M Co., St. Paul, MN, U.S.A.) were injected into the left ventricle over 20 sec through a cannula passed down the right carotid artery. The microspheres were suspended by ultrasonication in 0.6 ml physiological saline containing 0.02% (v/v) Tween 80. Cardiac output and liver blood flow were calculated as described by Nies *et al.* [1].

Preparation and assay of microsomal components. Animals were killed by cervical dislocation, their livers removed and placed immediately in ice-cold 1.15% KCl. The livers were blotted dry, weighed and roughly chopped over ice

Table 1. Effects of phenobarbitone on liver weight and blood flow in male guinea pigs

	Saline (n = 7)	Phenobarbitone (40 mg/kg/day) (n = 7)
Body weight (g)	373 ± 20	367 ± 10
Cardiac output (ml/min/100 g body wt.)	17.6 ± 0.6	17.5 ± 0.4
Mean arterial pressure (mmHg)	55.8 ± 3.5	56.8 ± 3.3
Liver weight (g/100g body wt.)	3.85 ± 0.23	4.91 ± 0.20**
Liver blood flow (ml/min/100 g body wt.)	5.17 ± 0.41	5.00 ± 0.34
Liver blood flow (ml/min/g liver)	1.36 ± 0.14	1.04 ± 0.11*
% Cardiac output to liver	29.7 ± 1.8	29.0 ± 2.3

Values given as mean ± S.E.M.
n = number of animals in groups.
Statistical significance from saline-treated animals using Student's *t*-test *P < 0.05, ** P < 0.01.