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# Lack of effect of phenobarbitone administered *in vivo* on glutathione synthesis by rat liver supernatants

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Glutathione (GSH) is one of the nucleophilic agents which protect tissues against chemically reactive metabolites formed from foreign compounds by the action, for example, of the microsomal mixed function oxidase (MFO) system [1]. It may react spontaneously with electrophilic agents, or the GSH *S*-transferases may catalyse the conjugations. Depletion of hepatic GSH has been shown to increase the susceptibility of the tissue to reactive metabolites formed from *N*-acetyl-*p*-aminophenol (paracetamol) or bromobenzene [1]. Phenobarbitone administration *in vivo* increases the toxicity of agents such as paracetamol or bromobenzene, presumably by inducing increased MFO activity [1] and marginally increases GSH *S*-transferase activities [2–5]. The availability of GSH rather than its rate of transfer to electrophilic agents may be the major factor in protection against reactive metabolites, although the GSH *S*-transferases may nonetheless be important in the conjugation process [6], and the effect of phenobarbitone on the rate of formation of glutathione is thus of interest.

*Experimental.* Male Wistar rats (150–200 g body wt) were maintained in plastic cages on sawdust bedding and given water and food pellets *ad lib.* Phenobarbitone sodium solution (Macarthy's Ltd, Romford, Essex, U.K.) was injected intraperitoneally (50 mg/kg) once each day for 10 consecutive days and the rats killed by cervical dislocation 24 hr after the last injection. Control animals were injected with 0.9% NaCl and treated similarly. '100,000 *g*' supernatants were prepared from the livers as described previously [2] using ice-cold 1.15% (w/v) KCl containing 0.01 mole/l potassium phosphate, pH 7.4, to make 1:5 (w/v) homogenates of the whole livers and centrifuging the homogenates at 10,000 *g* av for 15 min and the resulting supernatants at 100,000 *g* for 60 min.

The activities of γ-glutamyl-cysteine synthetase and glutathione synthetase, the two enzymes together responsible for GSH formation from free amino acids, were measured by following the incorporation of [U-$^{14}$C]glutamic acid and [U-$^{14}$C]glycine into γ-glutamyl-cysteine and GSH, respectively [7]. For the assay of γ-glutamyl-cysteine synthetase, reaction mixtures contained in a total volume of 0.5 ml pH 7.4:10 μmoles each of ATP and $MgCl_2$; 5 μmoles each of cysteine and dithiothreitol; 50 μmoles of potassium phosphate; 5 μmoles of [U-$^{14}$C]glutamic acid (0.05 μCi); and 100,000 *g* supernatant equivalent to 60 mg of wet liver. For the assay of GSH synthetase, reaction mixtures were similar except that the [U-$^{14}$C]glutamic acid was replaced by unlabelled glutamic acid (5 μmoles) and [U-$^{14}$C]glycine 5 μmoles (0.05 μCi). All reaction mixtures were incubated for 30 min at 37°, it having been established that both enzymic activities were linear with time for at least 30 min. Then the reactions were terminated by the addition of 1.5 ml of 0.4 mole/l trichloroacetic acid (TCA) containing imidazole (70 mmoles/l). The reaction tubes were centrifuged at high speed after standing at 0° for 5 min and 1.5 ml of the supernatants were transferred to conical centrifuge tubes containing 0.1 ml of 0.2 mole/l GSH. A 0.1 ml portion of 0.75 mole/l $CdSO_4$ solution was added to every one of these tubes, followed by 0.1 ml of a mixture of equal parts of bromcresol green and bromcresol purple (each 40 mg/100 ml$H_2O$). The cadmium mercaptides were precipitated by adding 0.5 mole/l NaOH to the end-point and then washed free of residual [U-$^{14}$C]glutamic acid or [U-$^{14}$C]glycine by centrifugation and resuspending the precipitates in water three times. The precipitates were dissolved in 1.0 ml 0.4 mole/l TCA which was transferred to 10 ml of Bray's fluid for radioactivity measurement by liquid scintillation spectrometry (Packard Tricarb 3375 20% gain 25–1000 window). Counting efficiency was checked by adding known amounts of [$^{14}$C]benzoic acid of standard specific activity to each vial. Protein concentration was measured by the biuret method using bovine serum albumin as standard.

Table 1. The effect of phenobarbitone administered *in vivo* on the incorporation of [U-$^{14}$C]glutamic acid or [U-$^{14}$C]glycine *in vitro* intro γ-glutamylcysteine or glutathione catalysed by 100,000 *g* supernatants prepared from rat liver*

| | [U-$^{14}$C]Glutamic acid | [U-$^{14}$C]Glycine |
|---|---|---|
| nmoles·min$^{-1}$ per mg supernatant protein | | |
| Control | 1.46 ± 0.10 | 0.70 ± 0.08 |
| Phenobarbitone | 1.31 ± 0.20 | 0.67 ± 0.07 |
| | NS | NS |
| nmoles·min$^{-1}$ per g wet liver equivalent | | |
| Control | 185 ± 13 | 88 ± 11 |
| Phenobarbitone | 157 ± 19 | 81 ± 9 |
| | NS | NS |
| μmoles·min$^{-1}$ per whole liver equivalent | | |
| Control | 1.88 ± 0.24 | 0.86 ± 0.09 |
| Phenobarbitone | 2.04 ± 0.22 | 1.07 ± 0.12 |
| | NS | NS |

* Data are expressed as means ± S.E.M.
† NS = not statistically significantly different.

*Results and discussion.* Table 1 shows that phenobarbitone treatment *in vivo* caused no change in the activities of the two enzymes involved in the biosynthesis of GSH from free amino acids. This is clear if activities are expressed as nmoles of amino acid incorporated/min/mg of supernatant protein, as nmoles incorporated/min/g wet liver equivalent, or as μmoles incorporated/min/whole liver equivalent. The ratio

$$\frac{\text{moles incorporated from [U-}^{14}\text{C]glutamic acid}}{\text{moles incorporated from [U}^{14}\text{C]glycine}}$$

is also unchanged, being 2.2 ± 0.3 for supernatants from phenobarbitone-treated animals and 2.0 ± 0.2 for those from control animals (mean ± S.E.M.; NS). There has thus been no relative change in the activities of the two enzymes. The phenobarbitone-treatment produced a significant increase in liver weight from 10.4 ± 0.5 to 13.2 ± 0.6 g (mean ± S.E.M.; $P = 0.005$).

The activity found for γ-glutamyl cysteine synthetase is slightly higher than that reported by Wirth and Thorgeirsson [8] but that for GSH synthetase is a quarter of that which they found using Sprague–Dawley rats. Using the values in Table 1, GSH synthesis proceeds at the rate of some 50 μmoles/hr/whole liver which would allow the efflux of the 12 nmoles/min/g wet liver found by Sies *et al.* [9]. The GSH content in normal adult rat liver is approximately 5–8 mmoles/kg [8, 10–12]. Buttar *et al.* [10] suggest that the maximum depletion of rat liver GSH occurs 3 hr after acute dosage of *N*-acetyl-*p*-aminophenol, and that the liver content returns to normal within 12 hr with a low dose. High doses of *N*-acetyl-*p*-aminophenol cause the GSH content to rise above normal 24–48 hr after administration; however, the content is still below normal at 18 hr. From the data presented here, the rate of biosynthesis of GSH is sufficient to restore the liver content to normal within 1 hr of depletion, and it may be that the liver damage caused by paracetamol results in greatly reduced rates of biosynthesis. If paracetamol stimulated the rate of breakdown of GSH, a reduction in GSH content would also occur. Should phenobarbitone stimulate GSH degradation and GSH biosynthesis simultaneously, then it might not be possible to detect the actual increase in biosynthesis. This possibility is currently being investigated. Jones *et al.* [13] have reported that pretreatment of rats with phenobarbitone does increase the hepatic degradation of oxidized glutathione GSSG to its constituent amino acids.

*Note added in proof*—After this communication had been submitted for publication a paper by N. Kaplowitz, J. Kuhlenkamp, L. Goldstein and J. Reeve (*J. Pharmac. exp. Ther.* **212**, 240) was published which described work also showing that phenobarbitone has no effect on hepatic GSH biosynthesis.

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