Biochemical Pharmacology, Vol. 32, No. 23, pp. 3619–3625, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00
© 1983 Pergamon Press Ltd.

DRUG PROTEIN CONJUGATES—VI. ROLE OF GLUTATHIONE IN THE METABOLISM OF CAPTOPRIL AND CAPTOPRIL PLASMA PROTEIN CONJUGATES

JOHN H. K. YEUNG, ALASDAIR M. BRECKENRIDGE and B. KEVIN PARK*
Department of Pharmacology and Therapeutics, Liverpool University, Liverpool L69 3BX, U.K.

(*Received* 3 *May* 1983; *accepted* 11 *July* 1983)

Abstract—Previous metabolic studies of captopril suggest that the rapid dissociation of captopril-plasma protein conjugates *in vivo* is dependent upon endogenous thiols such as glutathione and cysteine. Consistent with this hypothesis, we have found that cysteine (0.06–3 mM) and glutathione (0.02–1 mM) cleave ^{14}C-captopril-plasma protein conjugates *in vitro*. Dissociation of the drug-protein conjugate was accompanied by formation of the corresponding mixed disulphide which indicates that the reaction proceeds via a spontaneous thiol-disulphide interchange. Administration of high doses (50–300 mg/kg) of CP produced a time-dependent and dose-dependent decrease in hepatic glutathione concentrations in the mouse and the rat. The depletion of glutathione observed was similar to that produced by equimolar doses of D-penicillamine and paracetamol. Acute and chronic (7 days) administration of captopril (100 mg/kg) produces the same (11–12%) depletion of hepatic glutathione. However, changes in liver function as determined by elevation of serum glutamic-pyruvic transaminase was only observed at doses of 200 and 300 mg/kg. Thus, although thiol-disulphide interactions between captopril and plasma proteins may contribute to the perturbation of hepatic glutathione concentrations, it is unlikely that this process will be of toxicological significance during therapeutic administration of captopril.

Captopril (CP), D-3-mercapto-2-methyl-propanoyl-L-proline, is an orally active angiotensin-converting enzyme inhibitor used in the treatment of hypertension and congestive heart failure [1, 2]. CP is excreted largely unchanged into urine in man and other species [3–6, 8]. The major metabolites of CP are the mixed disulphides with cysteine and glutathione (GSH), with CP-cysteine being the major urinary metabolite [3, 5, 6]. CP may also become covalently bound to plasma proteins, via a disulphide linkage [7]. Other metabolites of CP reported include CP disulphide, *S*-methyl-CP and its sulfoxide [5, 8]. The formation of mixed disulphides may involve both an enzymatic process catalysed by thioltransferases [9] and a non-enzymatic process which involves a spontaneous thiol-disulphide exchange, a characteristic reaction of sulphydryl compounds [10]. It has been suggested previously that CP–cysteine excreted in urine was derived from CP–GSH formed in the liver [6, 11]. However, our previous study [8] showed that CP-plasma protein (CP–PP) conjugates readily dissociate *in vivo* and it was suggested that the reaction involves a spontaneous thiol-disulphide interaction with endogenous thiols.

In the present study, we have determined the concentrations of cysteine and GSH required to effect the dissociation of [^{14}C]CP–PP conjugates *in vitro*. Because of the requirements of endogenous thiols such as cysteine and GSH in the metabolism of CP and CP–PP conjugates we have also investigated the effects of high doses of CP on hepatic GSH. For comparative purposes, we also studied D-penicillamine, a chemically related thiol compound, and paracetamol, a GSH-depleting drug at high doses [12]. As CP contains a free sulphydryl group, a specific enzymatic method (glyoxalase I) [13, 14] for the determination of GSH was used in our study.

MATERIALS AND METHODS

[^{14}C] Captopril (4.66 μCi/mg) labelled at the amide carbonyl group, and unlabelled CP used in these studies were gifts from Dr. B. H. Migdalof of the Squibb Institute, New Brunswick, NJ. Silica gel thin-layer chromatographic plates (20 × 20 × 0.2 cm) were obtained from British Drug Houses, Poole, U.K. and scintillant (NE 265) from Nuclear Enterprises. The radioactive content of all samples were determined with an Intertechnique SL30 liquid scintillation spectrometer; counting efficiency was determined with automatic external standardisation and previously determined quench curves. Glyoxalase I and methylglyoxal were obtained from Sigma Chemical Company (London), U.K. Other general reagents and drugs were obtained from British Drug Houses and Sigma Chemical Company. All solvents were redistilled before use. SGPT enzymatic kit was purchased from British Drug Houses.

Reaction of [^{14}C] *captopril–plasma protein conjugates* ([^{14}C] *CP–PP) with cysteine, glutathione (GSH) and ascorbic acid* in vitro. A [^{14}C]CP–PP conjugate

* Author for correspondence.

Abbreviations: [^{14}C] CP, ^{14}C-radiolabelled captopril; [^{14}C] CP-GSH, ^{14}C-radiolabelled captopril-glutathione mixed disulphide; [^{14}C] CP-Cysteine, ^{14}C-captopril–cysteine mixed disulphide; [^{14}C] CP-PP, ^{14}C-captopril–plasma protein conjugate; PP, plasma proteins; GSH, reduced glutathione; SGPT, serum glutamic-pyruvic transaminase.

was prepared from rat plasma as previously described [8]. The conjugate was washed with 0.9% saline and concentrated with an Amicon B15 protein concentrator four times. The [^{14}C]CP–PP conjugate (0.05 μCi/ml; 30 mg/ml protein) was then incubated with GSH (0.02–1 mM), cysteine (0.06–3 mM) or ascorbate (0.25–5 mM) in sodium phosphate buffer (0.1 M, pH 7.4; final incubation volume 0.5 ml) for 60 min at 37°. Control incubations contained [^{14}C]CP–PP conjugate and buffer only. When incubation was completed, plasma proteins were precipitated by three volumes of methanol and washed for a further two times with methanol. [^{14}C]CP covalently bound to plasma proteins and [^{14}C]CP metabolites in the methanolic extracts were determined by SDS-equilibrium dialysis and thin-layer radiochromatography respectively, as previously described [8].

Effect of CP, D-*penicillamine and paracetamol on hepatic reduced glutathione (GSH) in mice.* Male CBA/ca mice (25–30 g) with free access to food and water, were given various doses of CP in saline (50–300 mg/kg i.p. dose volume 0.3 ml). Control animals received saline only. The animals were killed after 5 hr. The liver samples were excised, rinsed in ice-cold saline and immediately frozen in liquid nitrogen. All samples were assayed for GSH on the same day. The experiment was repeated with D-penicillamine (43–257 mg/kg i.p.) and paracetamol (70–500 mg/kg).

Time course study of the effect of CP on hepatic GSH in the mouse and the rat. Male Wistar rats (250–300 g) and male CBA/ca mice (25–30 g) were given CP (300 mg/kg i.p.). The test rats and the corresponding control rats were killed at 0.5, 2, 3 and 5 hr. The test mice and the corresponding control mice were killed at 3, 6, 9, 14 and 24 hr. The liver samples were frozen immediately in liquid nitrogen and GSH concentrations were determined on the same day.

The effect of chronic CP treatment on hepatic GSH in mice. Male CBA/ca mice (25–30 g) were divided into 3 groups; a control group was given saline (i.p.) for 7 days; the second group received saline for the first six days and 100 mg/kg CP (i.p.) on the final day. The third group received 100 mg/kg CP (i.p.) for seven days. All injections were given at 9.00 a.m. each day. On the final day, the animals were killed 5 hr after dosing, and the hepatic GSH concentration was determined.

Effect of CP and paracetamol on hepatic GSH and SGPT (serum glutamic-pyruvic transaminase) activity in mice. Male CBA/ca mice (25–30 g) were divided into groups and given CP (50, 100, 200, 250 and 300 mg/kg; i.p.); paracetamol (300 mg/kg); 250 mg/kg CP followed by 300 mg/kg paracetamol or 300 mg/kg paracetamol followed by 250 mg/kg CP. After 24 hr the animals were killed and serum was obtained for SGPT assay and hepatic GSH concentrations were also determined.

Determination of hepatic GSH. The frozen liver was pulverised and an aliquot (0.25–0.5 g) was homogenised with ice-cold 1 M percholic acid (3 ml) containing EDTA (2 mM). The homogenate was then centrifuged (2000 *g*) at 4° for 15 min to obtain a clear supernatant. An aliquot (0.2 ml) of the acidic supernatant was neutralised with 0.1 ml of 2 M potassium hydroxide containing 0.3 M MOPS (morpholino propanesulfonic acid). The mixture was centrifuged for 30 sec at 2000 *g*. The sample was assayed for GSH immediately after neutralisation. The glyoxalase I method [13, 14] was used to determine the concentration of GSH in this study as CP interferes with the colorimetric assay of Ellman [15]. The reaction mixture in the GSH assay consisted of 1.87 ml of 0.05 M potassium phosphate buffer (pH 6.6), 0.1 ml of neutralised sample and 0.01 ml of glyoxalase I. The reaction mixture was kept at 25°. 0.02 ml of methylglyoxal (100 mM) was added to initiate the reaction. The final volume of reaction mixture was 2.0 ml.

The formation of *S*-lactoyl GSH was monitored at 240 nm in a Pye-Unicam dual wavelength u.v. spectrophotometer. When the reaction was completed, methylglyoxal was again added for determination of methylglyoxal blank absorbance (important for samples with low GSH concentrations).

Determination of SGPT activity. Serum was obtained from clotted whole blood centrifuged at 2000 *g* for 15 min. The SGPT activity was determined with a diagnostic kit from British Drug Houses, using the method of Reitman and Frankel [16].

Statistical analysis. All results are reported as the mean ± S.D. Differences between means were determined using the Student's *t*-test.

RESULTS

Reaction of [^{14}C]*CP–PP with cysteine, GSH and ascorbic acid* in vitro. The amount of [^{14}C]CP covalently bound to plasma proteins was decreased by GSH and cysteine after 60 min incubation, as shown in Figs. 1 and 2, respectively. The EC_{50} values for dissociation of the [^{14}C]CP–PP conjugate were 0.47 and 0.74 mM for GSH and cysteine respectively. In each case there was a corresponding increase in the appropriate mixed disulphide. Ascorbic acid (5 mM) did not alter the amount of [^{14}C]CP covalently bound to protein. The dissociation of the [^{14}C]CP–PP conjugate by 1 mM GSH was time dependent (Fig. 3), with a corresponding increase of [^{14}C]CP–GSH with time.

Time course study of the effect of CP on hepatic GSH in the rat and mouse. Depletion of hepatic GSH by CP (300 mg/kg) was time-dependent in both the mouse (Fig. 4) and the rat (Fig. 5). In the rat, hepatic GSH was decreased from 6.28 ± 0.62 to 4.25 ± 1.2 μmoles/g liver at 5 hr, giving a 32.3% depletion (Fig. 5). In the mouse, depletion of hepatic GSH by CP (300 mg/kg) was time-dependent up to 9 hr, after which time there was a large variation in GSH concentrations at both 14 and 24 hr (Fig. 4). There was a significant decrease ($P \leq 0.05$) in hepatic GSH from 3 hr after dosing.

Effect of chronic CP treatment on hepatic GSH in the mouse. As shown in Fig. 6 single and chronic dose of 100 mg/kg i.p. of CP decreased hepatic GSH at 5 hr by 11 ± 7.1% and 13 ± 6.0%, respectively, on the final day of dosing. In this study, depletion of GSH was therefore not affected by chronic treatment with CP.

Dose-dependent depletion of hepatic GSH by CP, D-*penicillamine and paracetamol.* After 5 hr, CP pro-

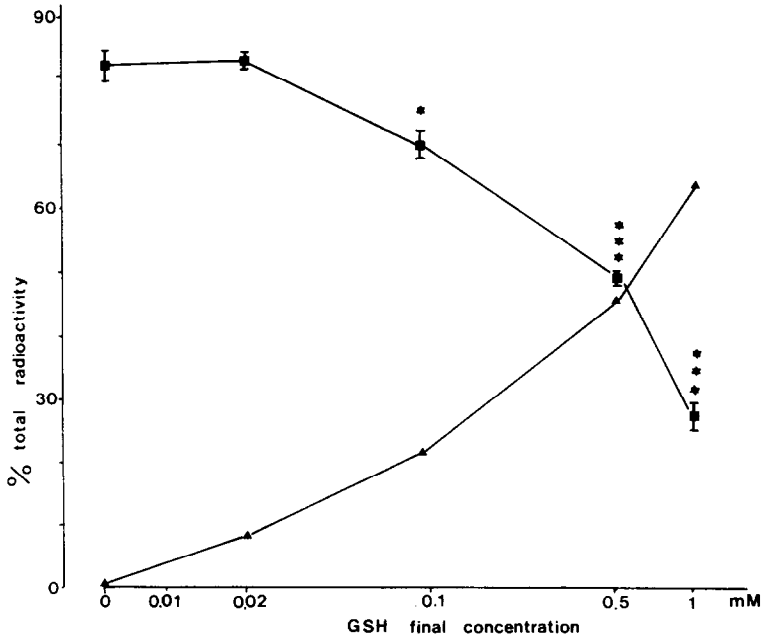

Fig. 1. Dissociation of [^{14}C]CP–PP conjugates (■) by glutathione (GSH), with formation of [^{14}C]CP–GSH mixed disulphide (▲) *in vitro*. Results are mean ± S.D. of three separate incubations. *$P \leq 0.05$, ***$P \leq 0.001$.

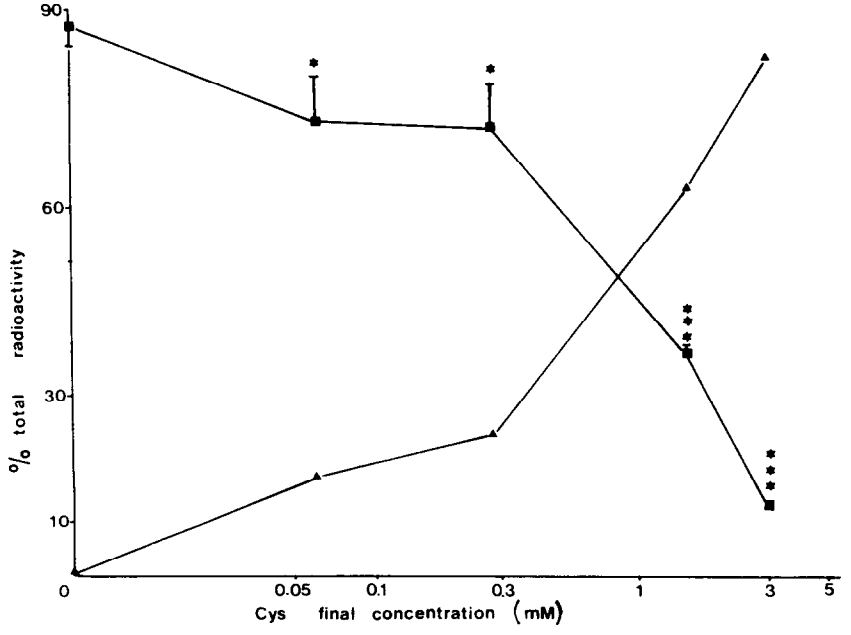

Fig. 2. Dissociation of [^{14}C]CP–PP conjugates (■) by cysteine with the formation of [^{14}C]CP–cysteine mixed disulphide (▲) *in vitro*. Results are mean ± S.D. of three separate incubations. *$P \leq 0.05$, ***$P \leq 0.001$

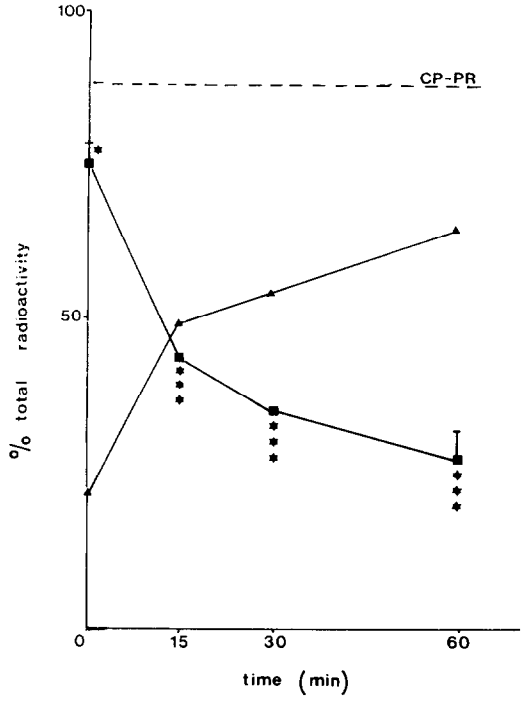

Fig. 3. Time-dependent dissociation of [^{14}C]CP–PP conjugate (■) by 1 mM GSH *in vitro*, with the formation of [^{14}C]CP–GSH mixed disulphide (▲). Results are mean ± S.D. of three incubations. *P ≤ 0.05, ***P ≤ 0.001

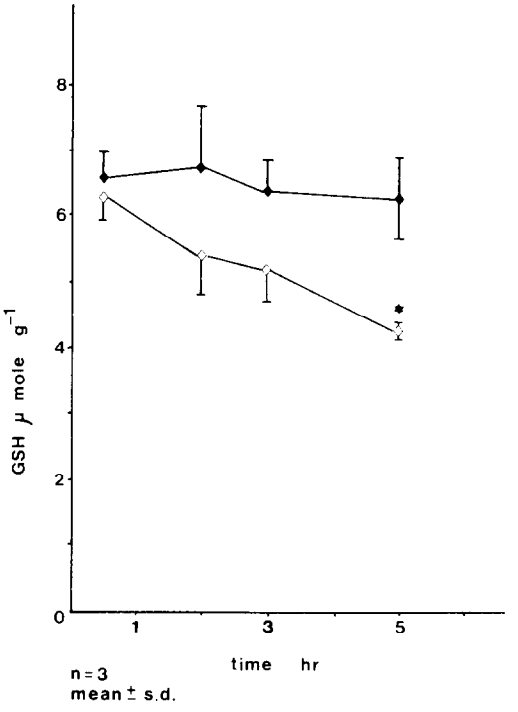

Fig. 5. Effect of 300 mg/kg CP (◇) on hepatic GSH level in rats at various time after i.p. administration. Controls were given saline (◆). Results are mean ± S.D. for 3 animals.* P ≤ 0.05.

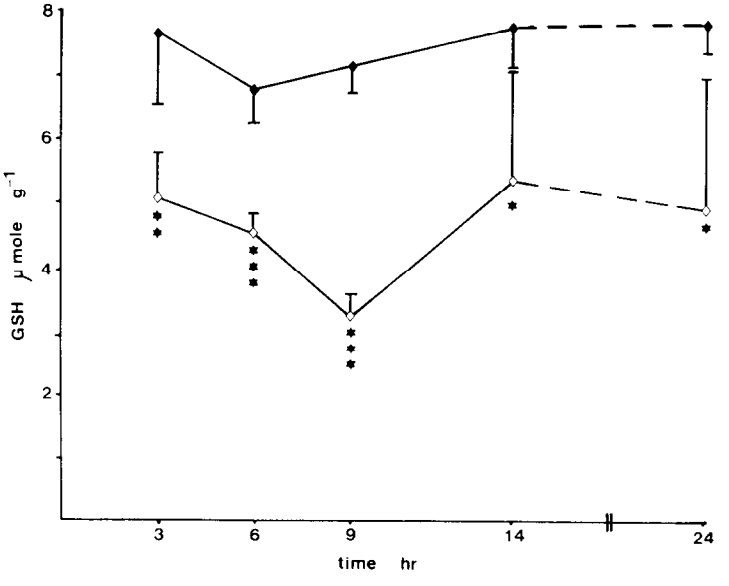

Fig. 4. Effect of 300 mg/kg of CP (◇) on hepatic GSH level in mice at various time after i.p. administration. Controls (◆) were given saline only. Results are mean ± S.D. for six animals. P ≤ 0.001 at 3, 6 and 9 hr, P ≤ 0.05 at 14 and 24 hr compared to controls.

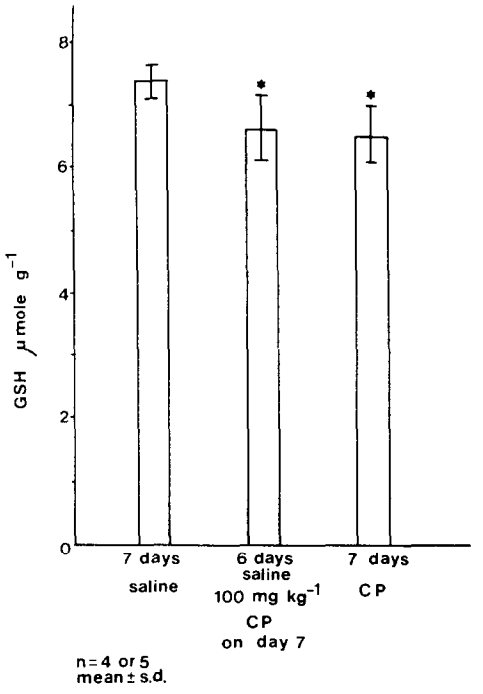

Fig. 6. Effect of chronic and acute i.p. administration of 100 mg/kg CP on hepatic GSH level in mice at 5 hr after i.p. administration on the final day of dosing. Results are mean ± S.D. of 4 to 5 animals. *$P \leq 0.05$.

duced a dose-dependent depletion (12–42%) of hepatic GSH, as did D-penicillamine (10–31%) and paracetamol (15–49%) (Fig. 7). The minimum doses which produced a statistically significant ($P \leq 0.05$) decrease in hepatic glutathione with respect to control animals were CP (50 mg/kg), D-penicillamine (43 mg/kg) and paracetamol (70 mg/kg).

Effect of CP and paracetamol on hepatic GSH and SGPT activity in the mouse. The dose-dependent depletion of GSH by CP (50–300 mg/kg i.p.) after 5 and 24 hr is shown in Table 1. After 24 hr, there was a much larger interanimal variation in the depletion of hepatic GSH and consequently only the groups given 200 and 300 mg/kg showed a statistically significant ($P \leq 0.05$) decrease in GSH. There was a dose-dependent increase of serum glutamic-pyruvic transaminase activity above control in mice 24 hr after treatment with CP. The increase in SGPT activity above control was statistically significant ($P \leq 0.001$) only at high doses (200–300 mg/kg). When CP (250 mg/kg) was given, either 2 hr before or 2 hr after paracetamol (300 mg/kg), there was a significant decrease ($P \leq 0.001$) in plasma SGPT activity compared to when paracetamol (300 mg/kg) was given alone.

Hepatic GSH returned to normal concentrations 24 hr after administration of paracetamol (300 mg/kg). However, when paracetamol (300 mg/kg) was given in combination with CP (250 mg/kg), GSH concentrations were still significantly depressed at 24 hr (Table 1).

DISCUSSION

The reduction of the disulphide and mixed disulphides of CP is thought to involve both enzymatic and non-enzymatic processes: the enzymatic reaction involves glutathione reductase and co-factors such as NADPH or NADH [17–19], the non-enzymatic process involves a thiol-disulphide exchange which is a spontaneous SN_2 reaction characteristic of sulphydryl compounds [10]. Recent *in vitro* work [20] has shown that CP–disulphide is reduced to form CP, CP–cysteine and CP–GSH mixed disulphides in the cytosol fractions of tissues. Our previous study with [^{14}C]CP–PP conjugates *in vivo* [8] also showed

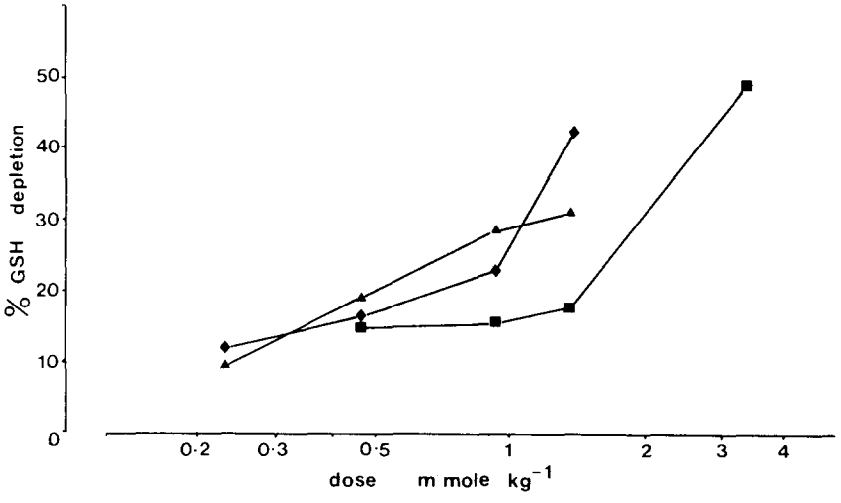

Fig. 7 Dose-response curves of captopril (◆), D-penicillamine (▲) and paracetamol (■) on hepatic glutathione depletion in mice at 5 hr after intraperitoneal administration. Results are mean of six animals. The minimum doses which produced a statistically significant ($P \leq 0.05$) decrease in hepatic GSH with respect to control animals were CP (0.23 mmoles/kg), D-penicillamine (0.23 mmoles/kg) and paracetamol (0.46 mmoles/kg).

Table 1. Effect of either captopril or paracetamol on hepatic gluathione levels (at 5 or 24 hr) and serum glutamic-pyruvic transaminase (SGPT) activity (at 24 hr) in mice after i.p. injections

Treatment	Hepatic glutathione (GSH) (μmoles/g) (5 hr)	(24 hr)	SGPT activity (%increase above control)
Control	5.64 ± 0.37	8.42 ± 0.62	—
Captopril 50 mg/kg	4.94 ± 0.13**	8.04 ± 1.03	17.3 ± 47.3
(CP) 100 mg/kg	4.72 ± 0.37**	7.46 ± 1.19	50.6 ± 70.1
200 mg/kg	4.33 ± 0.44***	6.41 ± 1.16**	97.5 ± 37.4***
250 mg/kg	n.d.	5.26 ± 1.64*	109.2 ± 16.1***
300 mg/kg	3.28 ± 0.75***	5.65 ± 2.85*	160.7 ± 31.2***
Paracetamol 300 mg/kg	n.d.	8.40 ± 0.82	222.8 ± 19.1
CP (250 mg/kg) 2 hr before paracetamol (300 mg/kg)	n.d.	4.49 ± 1.13***††	180.7 ± 2.6***††
CP (250 mg/kg) 2 hr after paracetamol	n.d.	6.04 ± 1.74*†	140.0 ± 25.0***††

Results are means ± S.D. of 6–8 animals. *$P \leq 0.05$, **$P \leq 0.005$ and ***$P \leq 0.001$ compared to controls using Student's non-paired *t*-test.

†$P \leq 0.01$, ††$P \leq 0.001$ compared to paracetamol (300 mg/kg) alone. n.d. Not determined.

that the disulphide linkage between CP and PP dissociates to give CP and other metabolites. In the present study, further evidence of thiol-disulphide interchange involving CP and its metabolites was obtained when [^{14}C]CP–PP conjugates were dissociated *in vitro* with cysteine and GSH, forming the respective [^{14}C]CP–cysteine and [^{14}C]CP–GSH mixed disulphides. Furthermore, the [^{14}C]CP–PP conjugates were unaffected by ascorbic acid concentrations up to 5 mM. Thus, the dissociation of [^{14}C]CP–PP by thiols is unlikely to be a simple redox reaction. GSH (EC_{50} 470 μM) was found to be more potent than cysteine (EC_{50} 740 μM) in dissociating the CP–PP conjugate *in vitro*: the plasma concentrations of GSH and cysteine are 5 and 180 μM in the rat, [21, 22] although approximately 50% of the thiols are covalently bound to albumin. Plasma protein conjugates of CP remain in the plasma compartment after intravenous administration [8]. It is therefore probable that the ready dissociation of CP–PP conjugates which occurs *in vivo* in plasma is dependent upon endogenous thiols derived from extravascular sources (e.g. the liver), thus affecting the regulation and turnover of endogenous thiols in such tissues.

There are several mechanisms by which CP may interact with hepatic GSH. Firstly, CP may be oxidised by a hepatic thioltransferase to give CP–GSH mixed disulphide directly [6]. Secondly, hepatic GSH may be utilised in spontaneous thiol-disulphide reactions with CP–PP conjugates in plasma to give CP–GSH mixed disulphide as outlined above. In this context it is important to note that oxidation of CP to mixed disulphides is more important in plasma, than in tissues such as kidney, lung and liver [30]. In addition, it is possible that CP may inhibit glutathione synthesis or glutathione reductase.

It was therefore of interest to determine the effect of CP administration on hepatic GSH concentrations. We also investigated the effects of D-penicillamine, which like CP possesses a free sulphydryl group and paracetamol, a drug known to deplete hepatic GSH [12]. The hepatic GSH levels obtained for the control animals using the glyoxalase I method are similar to previously published data [13, 14].

Intraperitoneal administration of CP (300 mg/kg) produced a time-dependent depletion of hepatic GSH in both the rat (Fig. 5) and the mouse (Fig. 4). The degree of depletion of GSH appeared to be similar in both species. A more detailed time–course study was undertaken in the mouse where it was seen that GSH concentrations began to recover after 9 hr, although this was partially obscured by wide inter-animal variation. A clearer picture of the recovery of hepatic GSH concentrations was obtained by measuring GSH at 5 and 24 hr (Table 1) after administration of CP at various doses (50–300 mg/kg). Hepatic GSH was depressed significantly at 5 hr after administration of only 50 mg/kg CP whereas a higher dose (200 mg/kg) was necessary to deplete GSH for 24 hr. Accordingly, chronic and acute administration of an intermediate dose (100 mg/kg) produced the same degree of hepatic GSH depletion (Fig. 6).

CP, D-penicillamine and paracetamol all produced dose-dependent GSH depletion (Fig. 7). The effect of CP was similar to that of D-penicillamine, a drug which also possesses a free sulphydryl group and forms mixed disulphides *in vivo* [31], but somewhat greater than paracetamol which must be oxidised to an iminoquinone [12] before reacting with GSH. The oxidation of paracetamol to the chemically reactive metabolite represents a minor metabolic pathway for the drug [23].

Hepatic GSH has many functions [9, 24–27], among them conjugation with and detoxification of electrophilic species. It has been suggested recently [28] that GSH forms a major defence against injury to cells from free radicals and active metabolites. In the present work, high doses of CP (200 and 300 mg/kg) elevated SGPT activity, which reflects the importance of GSH for normal cellular integrity. However, the elevation of SGPT by CP may be of a different mechanism to that which occurs with paracetamol. Paracetamol suppresses GSH synthesis

at high doses [29] and the toxicity of the drug involves a reactive iminoquinone intermediate.

In this study, high doses of CP have been used to study the interactions of GSH with CP in dissociating CP–PP conjugates. The minimum effective dose of CP which increased SGPT above control was over 100 mg/kg. The normal maximum daily therapeutic dose of CP is 0.9–6.4 mg/kg. It is therefore unlikely that CP at therapeutic doses would produce a significant depletion of hepatic GSH. Furthermore, chronic treatment of CP(100 mg/kg) did not show any cumulative effect in terms of hepatic GSH depletion.

Finally, we considered the possibility that CP might interact, with drugs such as paracetamol which produce hepatotoxicity after depleting hepatic GSH [12, 23]. However, we found that CP *diminished* the increase in SGPT activity (a measure of hepatoxicity) produced by a high dose of paracetamol (Table 1). These data suggest that CP may act as an alternative sulphydryl nucleophile to GSH and thus prevent arylation of essential cellular macromolecules by the iminoquinone metabolite of paracetamol.

Acknowledgements—This work was supported by E. R. Squibb & Sons Ltd and the Wellcome Trust. We also thank Mrs. P. Williams for typing the manuscript.

REFERENCES

1. A. B. Atkinson and J. I. S. Robertson, *Lancet* **11**, 836 (1979).
2. H. Gavras, D. P. Faxon, J. Berkoben, H. R. Brunner and T. J. Ryan, *Circulation* **58**, 770 (1980).
3. K. J. Kripalani, D. N. McKinstry, S. M. Singhvi, D. A. Willard, R. A. Vukovich and B. H. Migdalof, *Clin. Pharmac. Ther.* **27**, 636 (1980).
4. K. L. Duchin, S. M. Singhvi, D. A. Willard, B. H. Migdalof and D. N. McKinstry, *Clin. Pharmac. Ther.* **31**, 452 (1982).
5. T. Ikeda, T. Komai, K. Kawai and H. Shindo, *Chem. Pharm. Bull.* **29**, 1416 (1981).
6. H. Shindo, T. Komai, T. Ikeda, K. Kawai, W. Kawamata and E. Kameyama, *J. Pharmacobio-dynam.* **3**, s-9 (1980).
7. K. K. Wong, S. J. Lan and B. H. Migdalof, *Biochem. Pharmac.* **30**, 2643 (1981).
8. B. K. Park, P. S. Grabowski, J. H. K. Yeung and A. M. Breckenridge, *Biochem. Pharmac.* **31**, 1755 (1982).
9. W. B. Jakoby, *Functions of Glutathione in Liver and Kidney*, pp. 157–163. Springer, Berlin (1978).
10. P. C. Jocelyn, *Biochemistry of the SH Group* pp. 121, 164 and 240. Academic Press, New York, (1972).
11. T. Komai, T. Ikeda, K. Kawai, E. Kameyama and H. Shindo, *J. Pharmacobio-dynam.* **4**, 677 (1981).
12. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. ther.* **187**, 211 (1973).
13. E. Racker, *J. biol. Chem.* **190**, 685 (1951).
14. T. P. M. Akerboom and H. Sies, *Meth. Enzym.* **77**, 373 (1981).
15. G. L. Ellman, *Archs. Biochem. Biophys.* **82**, 70 (1959).
16. S. Reitman and S. Frankel, *Am. J. clin. Path.* **28**, 56 (1957).
17. T. W. Rall and A. L. Lehninger, *J. biol. Chem.* **194**, 119, (1952).
18. L. W. Mapson and D. R. Goddard, *Biochem. J.* **49** 592 (1952).
19. E. Racker, *J. biol. Chem.* **217**, 855 (1955).
20. S. J. Lan, S. H. Weinstein and B. H. Migdalof, *Drug metab. Disposition* **10**, 306 (1982).
21. M. H. Malloy, D. K. Rassin and G. E. Gaull, *Analyt. Biochem.* **113**, 407 (1981).
22. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
23. J. R. Gillette, *Archs. int. Med.* **141**, 375 (1981).
24. A. Meister, *Metabolic Pathways* (Ed. D. M. Greenberg), 3rd edn, p. 101. Academic Press, New York, (1975).
25. I. M. Arias and W. B. Jakoby, *Glutathione: Metabolism and Function.* Raven, New York, (1976).
26. N. S. Kosower and E. M. Kosower, *Int. Rev. Cytol.* **54** 109, (1978).
27. D. J. Reed and P. W. Beatty, *Rev. Biochem. Toxicol.* **2**, 213 (1980).
28. M. A. Trush, E. G. Mimnaugh and T. E. Gram *Biochem Pharmac.* **31**, 3335 (1982).
29. B. H. Lauterburg and J. R. Mitchell, *Hepatology* **2**, 8 (1982).
30. O. H. Drummer, P. J. Worland and B. Jarrott, *Biochem. Pharmac.* **32**, 1563 (1983).
31. K. Kazuaki, K. Inove, T. Otake, K. Noda and M. Ohzeki, *Yakugaku Zasshi* **104**, 322 (1982).