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IN VITRO BIOTRANSFORMATIONS OF [^{14}C]CAPTOPRIL IN THE BLOOD OF RATS, DOGS AND HUMANS*

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Abstract—The biotransformations of captopril (CP), an orally effective antihypertensive agent, were studied *in vitro* in whole blood, plasma, and washed blood cells of rats, dogs and humans, using ^{14}C-labeled CP. Thin-layer chromatographic profiles of the radioactivity extracted into methanol showed that, in plasma and whole blood, [^{14}C]CP was biotransformed to its disulfide dimer, [^{14}C]CP-CP, and to two other compounds identified as the [^{14}C]CP-glutathione and [^{14}C]CP-L-cysteine mixed disulfides. After incubation with either washed blood cells or a saline control, most of the [^{14}C]CP remained unchanged. Since not all of the radioactivity in the biological samples could be extracted, the nature of the unextracted radioactivity was investigated. Results obtained using methanol extraction, ultrafiltration and acid precipitation techniques indicated that [^{14}C]CP was extensively and covalently bound to plasma proteins. In human plasma, [^{14}C]CP was shown to be bound primarily to albumin through covalent disulfide bonds. In the plasma of all three species, these covalent disulfide bonds could be cleaved by glutathione or L-cysteine, resulting in the formation of mixed disulfides, or by dithiothreitol, resulting in the release of [^{14}C]CP. The behavior of CP *in vitro* in blood was qualitatively similar to that reported for other endogenous and xenobiotic sulfhydryl compounds and was consistent with the results obtained from *in vivo* studies with CP.

Captopril (CP)§, [1-(D-3-mercapto-2-methylpropanoyl)-L-proline(S,S)] (Fig 1), is a potent and specific inhibitor of angiotensin I-converting enzyme [1–4] and, in clinical testing as an oral antihypertensive agent, CP has proven effective in reducing arterial blood pressure in most forms of hypertension [5–8]. Drugs containing sulfhydryl groups are rare and, because of their unusual biotransformations involving conjugation with endogenous sulfhydryl-containing compounds to form mixed disulfides, they are particularly interesting compounds for biotransformation studies. For this reason, and because *in vitro* studies frequently provide useful information relating to processes that occur *in vivo*, studies of the *in vitro* biotransformation of ^{14}C-labeled CP were undertaken.

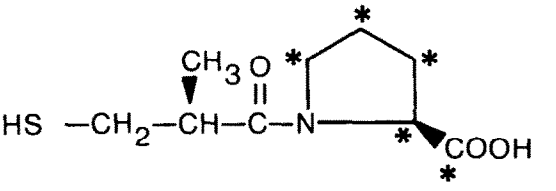

Fig. 1. Structure of [^{14}C]captopril. An asterisk denotes ^{14}C.

This paper presents the results of our initial *in vitro* studies, and describes the reversible covalent binding of CP to plasma proteins of rats, dogs and humans, and the thin-layer chromatographic biotransformation profiles obtained from methanol extracts after incubation of [^{14}C]CP with whole blood, washed blood cells, and plasma of these three species.

MATERIALS AND METHODS

Materials. Captopril (SQ 14,225), uniformly labeled with ^{14}C in the proline moiety, was synthesized in our laboratories with a specific activity of 7.72 μCi/mg and with a radiochemical purity of 98.2 per cent. Nonradiolabeled captopril (CP), the disulfide dimer of CP (CP-CP), and the captopril-L-cysteine (CP-CYS)and captopril-glutathione (CP-GSH) mixed disulfides were synthesized at The Squibb Institute for Medical Research (Princeton, NJ). Chemicals were obtained commercially as follows: glutathione (GSH), ICN Pharmaceuticals, Inc. (Cleveland, OH); L-cysteine (CYS), Sigma Chemical Co. (St. Louis, MO); dithiothreitol (DTT), Calbi-

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§ Abbreviations: CP, captopril; CP-CP, the disulfide dimer of captopril; CP-CYS, captopril-L-cysteine mixed disulfide; CP- GSH, captopril-glutathione mixed disulfide; CYS, L-cysteine; GSH, glutathione; DTT, dithiothreitol; TCA, trichloroacetic acid; NEM, *N*-ethylmaleimide; CP-NEM, the derivative of captopril and *N*-ethylmaleimide; NADH, the reduced form of nicotinamide adenine dinucleotide; and NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate.

ochem (San Diego, CA); human serum albumin and γ-globulin (Fraction II), Calbiochem-Behring Corp. (La Jolla, CA); Soluene-350, Packard Instrument Co. (Downers Grove, IL); and *N*-ethylmaleimide (NEM), Eastman Kodak Co. (Rochester, NY). The scintillation mixture of Anderson and McClure [9] was purchased from National Diagnostics (Somerville, NJ). All other chemicals were of reagent grade and were purchased from the Fisher Scientific Co. (Springfield, NJ). Ultrafiltration membrane cones (Centriflo) were obtained from the Amicon Corp. (Lexington, MA). Thin-layer chromatographic plates were purchased from Analtech, Inc. (Newark, DE). Vacutainer syringes were obtained from Becton-Dickinson (Rutherford, NJ).

Blood sampling and preparation of plasma and washed blood cells. Charles River CD outbred albino rats (200–300 g) (Charles River Breeding Laboratories, Inc., Wilmington, MA), purebred beagle dogs (10–12 kg) (Hazleton Research Animals, Vienna, VA), and healthy human volunteers were studied. Blood was drawn into heparinized syringes from the abdominal aortae of ether-anesthetized rats and into Vacutainers from the jugular veins of dogs and the antecubital veins of humans. For dogs and humans, experiments were carried out on blood samples from individual animals and volunteers respectively. Samples from groups of three to four male or female rats were pooled. A portion of each whole blood sample was stored in ice prior to use. The remainder was centrifuged at 2000 *g* at 5° for 15 min. The plasma was separated and kept in ice prior to incubation. The buffy coat was carefully removed and discarded. Prior to incubation, packed blood cells were washed three times at 0–5° with 3 vol. of 0.9% saline, and the washed blood cells were suspended in an equal volume of saline.

Incubation. Unless otherwise specified, each experiment was carried out using blood from four dogs (two males, two females), four human volunteers (two males, two females), and four groups of rats (two groups of male and female rats; four rats in each group). [^{14}C]CP was present at an initial concentration of 20 μg/ml (0.092 mM) in plasma, washed blood cells, whole blood, and 0.9% saline. All incubations were carried out aerobically in a shaking water bath at 37°.

Protein binding and biotransformation studies. Immediately after mixing, and after 40, 80 and 120 min of incubation, 0.5-ml aliquots of each incubation mixture were taken for determination of the metabolic profiles, and, for plasma, an additional 1 ml was taken for determination of protein binding by the ultrafiltration method. Immediately after sampling, an equal volume of 1% aqueous NEM solution was added to each sample to derivatize and thereby stabilize [10] the unchanged drug.

Studies of the effects of other sulfhydryl compounds on the binding of [^{14}C]CP to plasma proteins. A 400-μg/ml (1.84 mM) solution of [^{14}C]CP and 60 mM solutions of GSH, CYS and DTT in potassium phosphate buffer (0.28 M, pH 7.4) were prepared immediately prior to use. The plasma was freshly prepared and kept at 0–5° prior to incubation. A 9.5-ml portion of each plasma sample was mixed with 0.5 ml of [^{14}C]CP solution in a 25-ml Erlenmeyer flask. The initial concentration of the drug in the incubation mixture was 20 μg/ml (0.092 mM). Duplicate samples (0.2 ml) were taken immediately after mixing for the determination of protein-bound radioactivity. After 120 min, samples were again taken for the determination of protein-bound radioactivity, and 1.9-ml aliquots of the incubation mixture were transferred to four separate 10-ml Erlenmeyer flasks. To attain an initial 3 mM concentration in the incubation mixtures, 0.1 ml of the GSH-, CYS- or DTT-solution was added to each flask, and the incubation was continued. A control, to which 0.1 ml of phosphate buffer was added, was also prepared. Thirty and 60 min after the addition of the sulfhydryl compounds, duplicate 0.2-ml aliquots were taken for the determination of protein-bound radioactivity. The incubation was terminated 60 min after the addition of the sulfhydryl compounds by adding an equal volume of the 1% NEM solution. The samples were refrigerated until they were analyzed for unbound radioactivity.

Extraction. Samples were extracted three times with 3 vol. of methanol. The samples were centrifuged after each extraction and the supernatant fluids were recovered and pooled. The volumes of the pooled extracts were recorded and duplicate aliquots (0.1 ml) were counted. The total radioactivity present in each methanolic extract was calculated, and the total amount extracted from each sample was calculated using the total radioactivity present in the saline control as 100 per cent. The volume of each pooled methanolic extract was reduced to approximately 0.5 ml in a rotary evaporator at 50° under reduced pressure. The concentrated solutions were then subjected to thin-layer chromatography. The fraction of radioactivity in plasma that was not

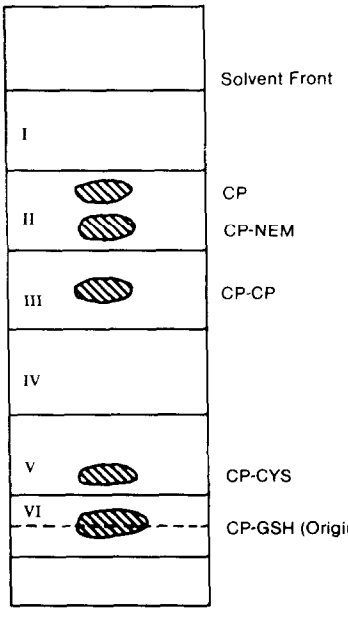

Fig. 2. Thin-layer chromatogram of CP, CP-NEM, CP-CP, CP-CYS and CP- GSH. Plate: Analtech silica gel GF. Solvent system: chloroform/ethyl acetate/glacial acetic acid (4:5:3, v/v).

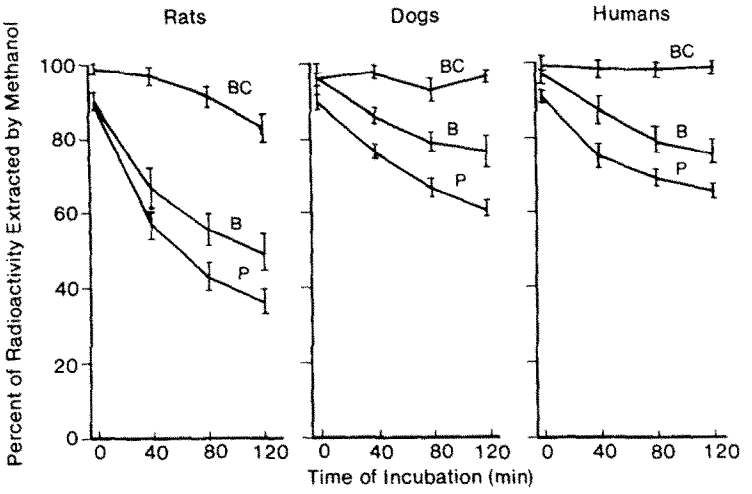

Fig. 3. Percentages of radioactivity extracted into methanol after incubation of [^{14}C]CP with plasma (P), washed blood cells (BC) and whole blood (B) of rats, dogs and humans. Each value shown is the mean ± S.E.M. of four experiments (2 males, 2 females).

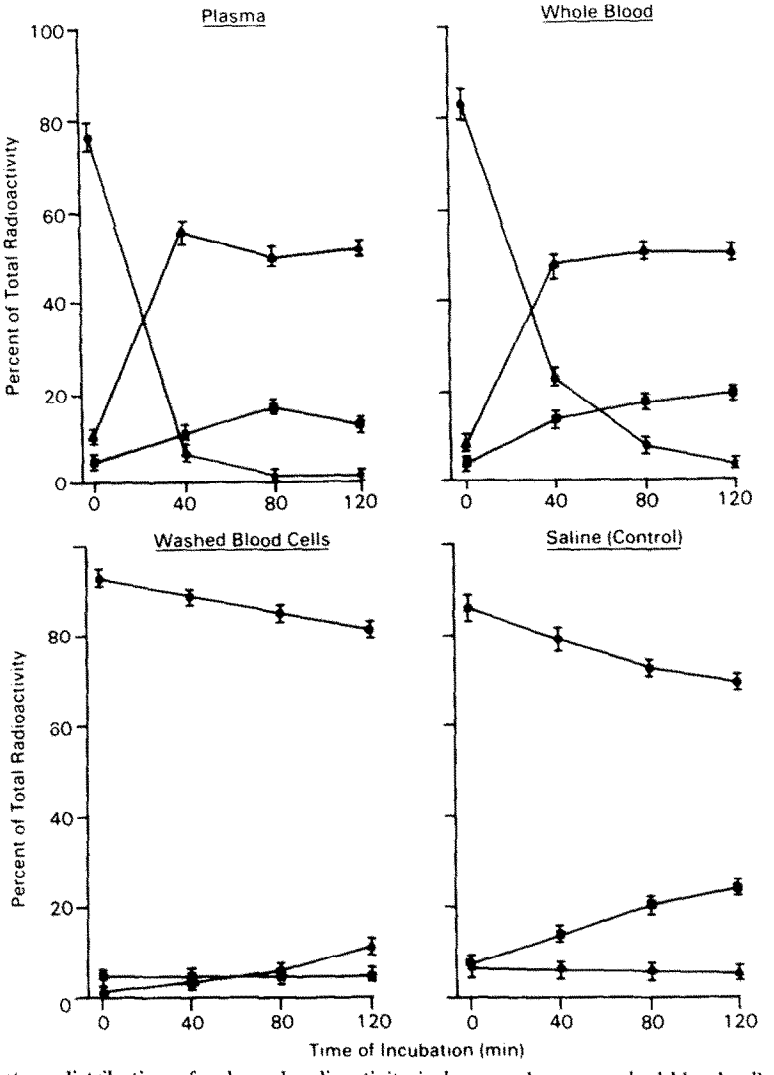

Fig 4. Percentage distribution of unbound radioactivity in human plasma, washed blood cells, whole blood and the saline control after incubation with [^{14}C]CP. Each value shown is the mean ± S.E.M. of four experiments (two males, two females). Key: (●) captopril; (■) disulfide dimer; and (▲) other metabolites or oxidation products.

extracted into methanol was considered to be covalently bound to plasma proteins.

Thin-layer radiochromatography (t.l.r.c.). Thin-layer radiochromatography was carried out using Analtech silica gel GF (5 × 20 cm) thin-layer chromatographic plates in chloroform/ethyl acetate/glacial acetic acid (4:5:3, v/v). In selected cases, CP, CP-NEM, CP-CP, CP-CYS, and CP-GSH were all used as reference compounds. For the generation of the zonal profiles of extracted radioactivity, 20 μl of the concentrated methanolic extract and 50 μg each of CP-NEM and CP-CP (as references) in 5 μl of distilled water or methanol were applied to each plate. After development of the plates, the reference compounds were visualized by exposing the plates to iodine vapor. The silica gel on each plate was divided into six zones (I to VI), according to the scheme shown in Fig. 2, and the radioactivity in each zone was determined by liquid scintillation counting. The percentage of radioactivity in each zone was calculated using the total radioactivity on each plate as 100 per cent.

Scintillation counting. Samples of plasma, protein-free filtrate, and methanol extracts were mixed with 15 ml of the scintillation mixture of Anderson and McClure [9]. Samples containing silica gel were first mixed with 2 ml of distilled water, followed by 15 ml of scintillation mixture. All samples were counted in a Packard Tri-Carb model 2425 liquid scintillation spectrometer. Counting efficiency was determined with automatic external standardization.

Determination of protein-bound radioactivity. The total binding (covalent and noncovalent) of [^{14}C]CP to plasma proteins was determined by ultrafiltration according to Singhvi *et al.* [11]. In the study of the effect of other sulfhydryl compounds on the binding of [^{14}C]CP to plasma proteins, the amount of protein-bound radioactivity was determined by precipitation with trichloroacetic acid (TCA). The proteins in each 0.2-ml portion of plasma were precipitated with an equal volume of 10% TCA. The denatured proteins were separated by centrifugation and washed twice with 0.4 ml of 5% TCA. The washed proteins were then digested with 1 ml of Soluene-350, neutralized with 0.2 ml of PGM [a mixture that contained a saturated solution of sodium pyruvate in methanol, glacial acetic acid, and methanol in the ratio of 4:3:1 (v/v)], mixed with 15 ml of the scintillation mixture and counted. The super natant fluid and the washings were combined, mixed with 1 ml of Soluene-350 and 15 ml of scintillation mixture and counted. The percentage of protein-bound radioactivity was calculated using the total radioactivity present in the supernatant liquid and the acid-precipitated proteins as 100 per cent.

Polyacrylamide gel electrophoresis and fluorography. Human plasma was incubated with [^{14}C]CP (200 μg/ml) at 37° for 3 hr. After incubation, plasma proteins were precipitated and washed with acetone (0°). The washed proteins were then subjected to sodium dodecylsulfate—polyacrylamide gel electrophoresis according to the method of Laemmli [12] except that β-mercaptoethanol was omitted from the sample buffer. Human serum albumin and γ-globulin were used as references. Radioactivity on the acrylamide gel was determined by fluorography according to the method of Bonner and Laskey [13].

RESULTS

Extraction of radioactivity from plasma, blood cells, and whole blood. The percentages of radioactivity extracted into methanol after incubation of [^{14}C]CP with plasma, washed blood cells, and whole blood of the three species studied are shown in Fig. 3. Radioactivity in the saline control was completely recovered in methanol, whereas only a fraction of the radioactivity in whole blood and plasma was extracted into methanol. Generally, similar results were found among the three animal species studied, although quantitative differences existed with regard to the amount of radioactivity that could be extracted into methanol. The percentage of nonextractable radioactivity in the plasma of rats was found to be greater than that in the plasma of dogs or humans. In addition, the percentage of methanol-extractable radioactivity in plasma and whole blood of the three species decreased as a function of time. Most of the radioactivity in washed blood cells, however, could be extracted into methanol even after 120 min of incubation. After 120 min of incubation, substantial amounts of [^{14}C]CP, its metabolites, or both were associated with plasma proteins, but not with the washed blood cells. The percentage of radioactivity that could be extracted into methanol from whole blood was found to be greater than from plasma and less than that from washed blood cells.

Metabolic profiles of unbound radioactivity of [^{14}C]CP. Figure 4 shows the percentage distribution of unbound radioactivity in human plasma, washed blood cells, whole blood, and the saline control. In plasma and whole blood, CP disappeared rapidly during incubation. Concomitant with the disappearance of the drug, the amounts of CP-CP and other metabolites (essentially the metabolites located at Zones V and VI of Fig. 2) increased. In the washed blood cells, CP was relatively stable. More than 80 per cent of the unbound radioactivity (79 per cent of the added drug) remained as CP after 120 min of incubation. In the saline control, CP was slowly oxidized to CP-CP. After 120 min of incubation, 70 per cent of the added drug was unchanged. The metabolic profiles of the unbound radioactivity obtained after incubation of [^{14}C]CP with whole blood, plasma, and washed blood cells of rats and dogs were qualitatively the same as those for the corresponding human samples. Quantitative differences were observed. Figure 5 shows the biotransformation profiles of radioactivity extracted by methanol after 120 min of incubation of [^{14}C]CP with whole blood and plasma of rats, dogs, and humans.

Binding of [^{14}C]CP to plasma proteins. Incubation of [^{14}C]CP with plasma of rats, dogs or humans resulted in extensive binding of radioactivity to plasma proteins. The protein-bound radioactivity could be recovered in proteins precipitated by TCA. In subsequent experiments, the binding of [^{14}C]CP to plasma proteins was studied by ultrafiltration and methanol extraction. Ultrafiltration provided a means of determining the total amount of drug that

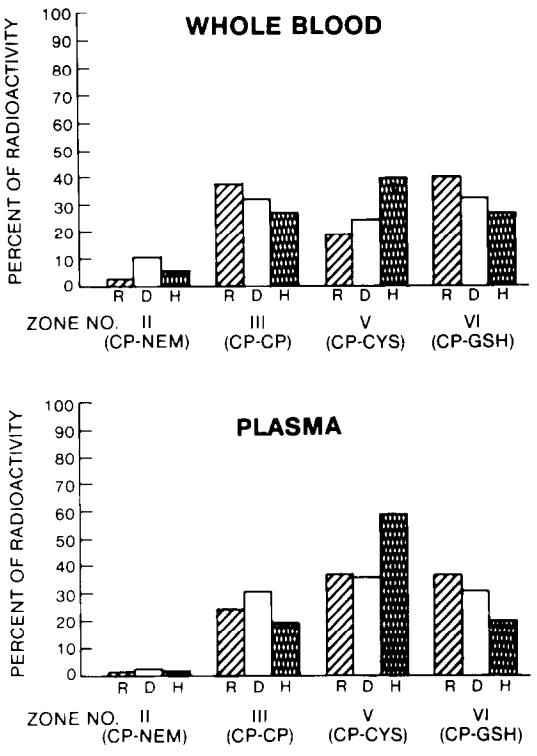

Fig. 5. Biotransformation profiles of radioactivity extracted in methanol after 120-min incubations of [^{14}C]CP with whole blood and plasma of rats (R), dogs (D) and humans (H).

was bound to plasma proteins (covalently and non-covalently), whereas methanol extraction provided a means of determining the amount of drug covalently bound to plasma proteins.

In the plasma, CP was extensively bound to proteins. Since, in all three species, essentially the same extent of binding to plasma proteins was found whether determined by ultrafiltration or by methanol extraction, it appears that all [^{14}C]CP binding to plasma proteins was covalent binding. This point was further demonstrated by the direct measurement of radioactivity in TCA-precipitated plasma proteins. The covalent binding was presumed to result from disulfide bond formation between [^{14}C]CP and plasma proteins. As expected for disulfide linkages, the binding of [^{14}C]CP to plasma proteins could be easily reversed by the addition of other sulfhydryl compounds. The results of polyacrylamide gel electrophoresis and fluorography of plasma proteins obtained after incubation of human plasma with [^{14}C]CP indicated that the majority of the bound radioactivity was associated with the albumin fraction. Incubation of purified human serum albumin with [^{14}C]CP resulted in the extensive covalent binding of [^{14}C]CP to albumin. This binding could also be reversed by the addition of other sulfhydryl compounds to the incubation.

Figure 6 shows the percentages of radioactivity that were bound to plasma proteins after incubation of [^{14}C]CP with the plasma of rats, dogs, and humans. The percentages of protein-bound radioactivity determined by both methods were in good agreement. In the plasma of the three species studied, the percentages of protein-bound radioactivity increased as a function of time. [^{14}C]CP was bound to the greatest extent to the proteins of rat plasma. After 120 min of incubation, the percentages of radioactivity bound to plasma proteins decreased in the order rats > dogs ≥ humans, and essentially the same results were obtained by the methanol extraction and ultrafiltration methods.

Effects of other sulfhydryl compounds on protein-bound CP. The disulfide linkages between [^{14}C]CP and plasma proteins could be cleaved by the addition of other sulfhydryl compounds such as GSH, CYS and DTT to the incubation. At 30 min after the addition of 3 mM GSH, CYS or DTT to human plasma preincubated with [^{14}C]CP, the average percentage (±S.E.M.) of radioactivity bound to plasma proteins decreased to 6.4 ± 0.2, 12.1 ± 0.5 and 8.2 ± 0.5 per cent, respectively (Fig. 7). The corresponding value for the control was 36.4 ± 0.7

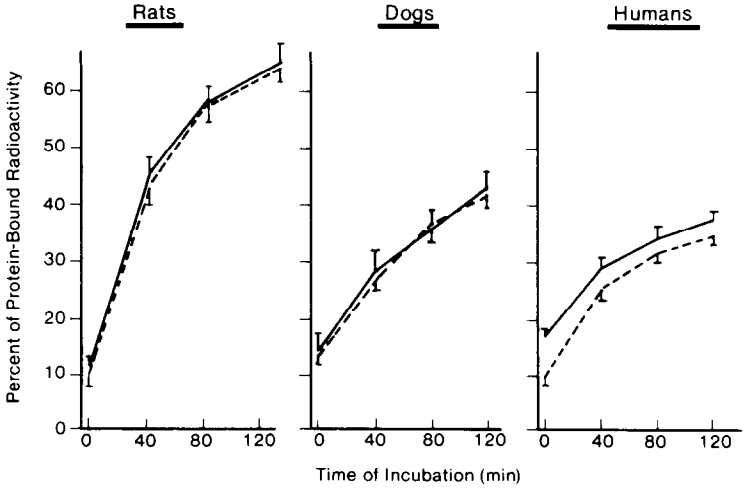

Fig. 6. Percentages of radioactivity bound to proteins after incubation of [^{14}C]CP with plasma of rats, dogs and humans. Each value shown is the mean ± S.E.M. of four experiments (two males, two females). Key: (———) ultrafiltration; and (------) methanol extraction.

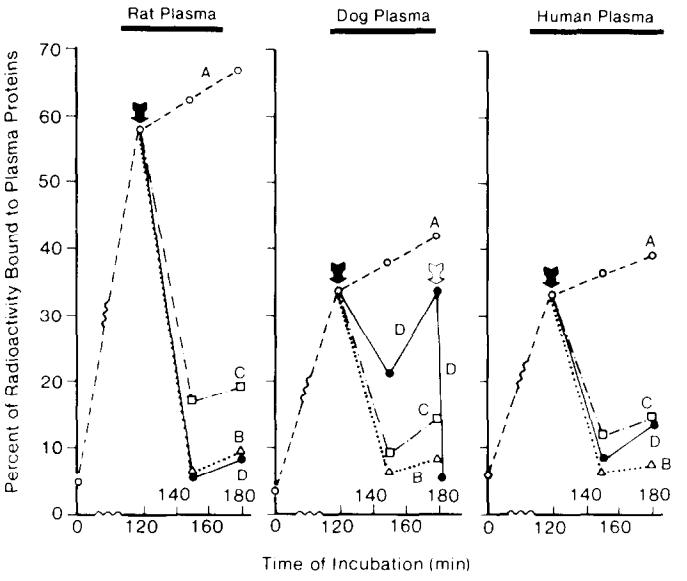

Fig. 7. Effect of sulfhydryl compounds on protein-bound [^{14}C]CP in plasma of rats, dogs and humans. Key: (A) control; (B) GSH added; (C) CYS added; (D) DTT added; (◆), GSH, CYS or DTT added to 3 mM; and (◇) DTT added up to 9 mM.

per cent. These results indicated a reduction of 82, 67 and 77 per cent in the extent of radioactivity bound to plasma proteins in the presence of GSH, CYS and DTT respectively.

A similar decrease of the radioactivity bound to plasma proteins after incubation in the presence of excess GSH, CYS and DTT was also found in the plasma of rats and dogs (Fig. 7). The extent of DTT-induced release of the protein-bound radioactivity in the plasma of dogs, however, was much less than in the plasma of rats and humans. Thus, 30 min after the addition of DTT (to 3 mM) to dog plasma preincubated with [^{14}C]CP, the percentage of protein-bound radioactivity decreased from 37.6 ± 0.9 per cent of the control to 21.3 ± 0.8 per cent, indicating a decrease of only 43 per cent of the protein-bound radioactivity. On the other hand, 30 min after the addition of the same amount of DTT to plasma of rats and humans, the percentage of pretein-bound radioactivity decreased by 91 and 77 per cent respectively. To test whether the lower value for dog plasma might have been due to the relatively rapid oxidation of DTT in those samples, more DTT was added. When additional DTT (to 9 mM) was introduced into the incubation of dog plasma 60 min after the initial addition of DTT (to 3 mM), the percentage of protein-bound radioactivity further decreased to 5.1 ± 0.5 per cent 5 min after the DTT had been added.

Profiles of protein-bound radioactivity after liberation by sulfhydryl compounds. The metabolic profiles of the unbound radioactivity from the 180-min saline control and from the samples obtained 60 min after the addition of GSH, CYS or DTT were determined by t.l.r.c. (Fig. 8). In human plasma, the control sample showed little radioactivity in the area corresponding to CP or the CP-NEM adduct (Zone II), suggesting extensive biotransformation of the unbound radioactivity. There were three radioactive peaks on the chromatogram. One of the radioactive peaks corresponded to CP-CP (Zone III). The other two peaks, located in Zones V and VI, corresponded to CP-CYS and CP-GSH respectively. The chromatogram of the radioactivity extracted from the plasma sample to which GSH was added showed a major radioactive peak located in Zone VI (CP-GSH), and two minor peaks in Zones III (CP-CP) and V (CP-CYS). The chromatograms of the sample to which CYS had been added showed a major radioactive peak in Zone V (CP-CYS), plus two minor radioactive peaks. One of these two minor peaks corresponded to CP-CP (Zone III) and the other peak corresponded to CP-GSH (Zone VI). When DTT was added to plasma that had been preincubated with [^{14}C]CP, the thin-layer chromatogram of extracted radioactivity showed one major radioactive peak corresponding to the CP-NEM adduct (Zone II) and three minor peaks corresponding to CP-CP, CP-CYS and CP-GSH. These results indicated that DTT probably released protein-bound CP by reduction of disulfide bonds, whereas GSH and CYS probably released the protein-bound CP by displacement.

DISCUSSION

General biotransformations of sulfhydryl compounds. Sulfhydryl compounds have a tendency to undergo auto-oxidation to disulfides and, in biological systems, disulfides can be reduced to sulfhydryls either by a sulfhydryl-disulfide interchange or by reduction catalyzed by a reductase requiring NADPH or NADH [14]. The most abundant cellular sulfhydryl and disulfide compounds are CYS, GSH and their corresponding disulfides. Despite a propensity to oxidize spontaneously, CYS and GSH are

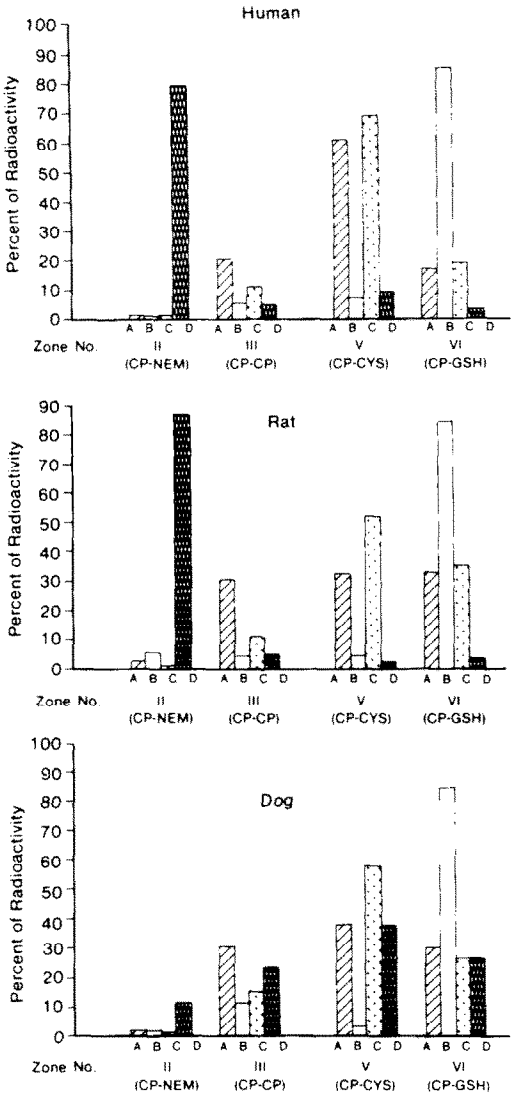

Fig. 8. Biotransformation profiles of unbound radioactivity after 120 min incubations of [^{14}C]CP with human, rat and dog plasma followed by the addition of GSH, CYS and DTT, and further incubation for an additional 60 min. Key: (A) control; (B) GSH added; (C) CYS added; and (D) DTT added.

maintained predominantly in the reduced form intracellularly, whereas the corresponding disulfides are more likely to be found extracellularly. The conversion of CYS to L-cystine presumably proceeds by the auto-oxidation of CYS; the reduction of L-cystine to CYS is believed to involve a sulfhydryl–disulfide interchange with GSH [15,16]. L-Cystine can also be reduced directly by NADPH or NADH and a specific L-cysteine reductase [17]. The level of GSH in cells is controlled by NADPH and GSH-reductase, an enzyme widely distributed in animals [18].

Sulfhydryl and disulfide groups are also commonly found in protein molecules. The sulfhydryl group appears to predominate in intracellular proteins such as hemoglobin [19]. On the other hand, more disulfide than sulfhydryl groups are found extracellularly in albumin and other plasma proteins [20].

The formation of mixed disulfides of CYS with xenobiotic sulfhydryl compounds has been reported; for example, the mixed disulfide of CYS and D-penicillamine was reported to be an urinary metabolite of D-penicillamine in patients with cystinuria [21, 22].*

Biotransformation profiles of extracts. Because CP contains a free sulfhydryl group, it was anticipated that it might also exhibit interesting conjugation reactions with endogenous sulfhydryl compounds. The *in vitro* studies carried out confirmed that such reactions occurred. A concentration of 20 μg/ml for CP was selected for the *in vitro* incubation studies since this approximates the maximum blood concentrations attained after oral administration of CP to rats (50 mg/kg) and dogs (25 mg/kg).† After 40 min of incubation with plasma of rats, dogs or humans, little unchanged [^{14}C]CP remained. Three radioactive biotransformation products were isolated and tentatively identified as CP-CP, CP-CYS and CP-GSH. These results are consistent with *in vivo* data obtained in human subjects and laboratory animals; CP-CYS was the major urinary metabolite in humans, whereas CP-CP was excreted in the urine of both humans [23] and other animals [24–26]. When [^{14}C]CP was incubated with washed blood cells or saline, most of the added [^{14}C]CP remained unchanged. Results of incubations with whole blood were intermediate between plasma (extensive biotransformation) and washed blood cells (little biotransformation).

Binding to plasma protein. The results obtained suggested that the formation of disulfide linkages between CP and albumin, and the cleavage of disulfide bonds induced by exogenous sulfhydryl compounds, were—at least in part—nonenzymatic and spontaneous. Covalent binding to proteins by the formation of disulfide bonds has also been reported for other sulfhydryl compounds such as D-penicillamine [27, 28] and 2-mercaptopropionylglycine [29]. Disulfiram and aminopentylaminoethylphosphorothioate (WR2823) are converted *in vivo* to compounds containing free sulfhydryl groups, which are then covalently bound to proteins [30, 31]. Reversible covalent binding of [^{14}C]CP to plasma proteins also has been observed in our *in vivo* studies in man, monkey, dog, rat and mouse [10, 23, 24].

Profiles of protein-bound radioactivity after liberation by sulfhydryl compounds. The distributions of unbound radioactivity after incubation of [^{14}C]CP with plasma from rats and dogs, with and without the addition of GSH, CYS and DTT, were qualitatively similar to those obtained for human plasma (Fig. 8). Samples of rat and human plasma to which DTT was added showed essentially only one major radioactive peak, which corresponded to the CP-NEM adduct (Zone II), and three very minor peaks. The biotransformation profile of the sample of dog plasma to which DTT had been added, however, contained a much smaller amount of the CP-NEM adduct (Zone II)) and the three other radioactive peaks, that corresponded to CP-CP, CP-CYS and

* The disulfide dimer of D-penicillamine was also reported to be a urinary metabolite.

† Unpublished data.

CP-GSH, were larger. These results suggested that, in the dog plasma, DTT probably was oxidized at a rate faster than in the plasma of rats and humans. The more rapid oxidation of DTT in dog plasma, as compared to the plasma of rats and humans, would result in decreased capacities of DTT to release protein-bound CP, to reduce CP-CP, CP-CYS and CP-GSH, and to prevent the oxidation of unbound CP in dog plasma.

In conclusion, the *in vitro* studies conducted on [^{14}C]CP have shown that CP behaves similarly to other sulfhydryl compounds with respect to the formation of covalent disulfide bonds to plasma proteins and to endogenous sulfhydryl compounds such as CYS and GSH. Qualitative similarities have been observed between the biotransformations of CP in these *in vitro* studies and of *in vivo* studies of CP. The covalent bonds between CP and plasma proteins and between CP and other endogenous sulfhydryl compounds appear to form and break *in vitro* and *in vivo* in a dynamic process resulting in an exchange between the mixed disulfides and the free sulfhydryl compounds.

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