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A NEW PATHWAY OF METABOLISM OF CHLORAMPHENICOL WHICH INFLUENCES THE INTERPRETATION OF ITS IRREVERSIBLE BINDING TO PROTEIN *IN VIVO*

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Abstract—The mechanism of the metabolic activation of chloramphenicol (CAP) *in vivo* in the rat has been studied by measuring the irreversible binding to protein of specifically labeled [1, 2-^{14}C]dichloroacetamido and 1-^{3}H-benzylic derivatives of CAP. In every tissue examined, the ^{14}C-label of CAP bound significantly more than the ^{3}H-label, indicating that only part of the structure of CAP was bound. When ^{14}C-labeled plasma protein, from rats given [^{14}C]CAP, was hydrolyzed chemically or enzymatically, glycine and serine were identified by thin-layer chromatography and reverse isotope dilution analysis as the predominant radioactive products. These results indicate that the majority of the irreversible binding of CAP to protein *in vivo* is not due to its metabolic activation into a reactive metabolite as was found *in vitro* in liver microsomes, but instead is due to its metabolism into small molecular fragments, which are incorporated into pools of normal tissue constituents. These small molecules are subsequently biosynthesized into glycine and serine, and these amino acids are then incorporated into the structure of tissue protein. The general importance of these results and possible pathways for the metabolism of CAP into glycine and serine are discussed.

For several years, bone marrow depression [1] has been associated with the use of the clinically important [2] broad spectrum antibiotic, chloramphenicol (CAP). Although this toxicity is usually reversible if CAP treatment is discontinued, approximately 1 in 20,000 patients develops aplastic anemia, a bone marrow disease which is oftem irreversible and fatal [1]. It has been suggested that CAP may produce this blood disorder by inhibiting directly bone marrow mitochondrial protein synthesis [3–5] or DNA synthesis [5]. Other findings suggest that the bone marrow depression is an immunological disease [6–9]. However, this toxicity has not been produced in laboratory animals, and therefore these or other potential mechanisms have not been established unequivocally.

The rare incidence of CAP-induced aplastic anemia suggested to us that a minor reactive metabolite of CAP might be involved in the induction of this toxicity. This possibility seemed reasonable since the toxicity [10–12] and carcinogenicity [10, 13–15] of other drugs and environmental chemicals were known to be related to the formation of electrophilic metabolites that reacted with tissue macromolecules. Consequently, we have attempted to determine whether CAP is metabolized to minor reactive metabolites which potentially might induce aplastic anemia.

Previous studies with CAP labeled with ^{14}C in the 1 and 2 positions of the dichloroacetamido group ([^{14}C]CAP) revealed that this compound is bound irreversibly to rat tissue protein when it is incubated with liver microsomes of rat [16, 17]. The binding appeared to be related to the formation of an unknown reactive metabolite which was produced by a cytochrome P-450 mono-oxygenase. More recently, the mechanism of this *in vitro* process has been studied in greater detail by using specifically labeled ^{14}C-and ^{3}H-derivatives of CAP [18–20]. The results of these investigations indicate that CAP is metabolically activated by an oxidative dechlorination process to produce a reactive oxamyl chloride intermediate. This metabolite appears to acylate microsomal protein [18–20] or hydrolyze to an oxamic acid [18–20].

[^{14}C]Chloramphenicol has also been shown to bind irreversibly to tissue protein in various organs, including the bone marrow, when it is administered to rats [16, 17]. In addition, an unknown metabolite of [^{14}C]CAP has been detected in the plasma of rats [21]. This product was devoid of antimicrobial activity and appeared to be bound irreversibly to plasma protein.

In order to determine whether the *in vivo* binding of CAP results from the same metabolic activation process that was observed *in vitro* with liver microsomes [18–20], in the present investigation we have conducted binding studies *in vivo* with derivatives of CAP labeled specifically with ^{14}C in the dichloroacetamido group and with ^{3}H in the benzylic position. Moreover, we have isolated radiolabeled plasma protein and have determined the structure of two radiolabeled products.

MATERIALS AND METHODS

Chemicals. [1, 2-^{14}C]Dichloroacetic acid, 2.42mCi/mmole, and [^{14}C]chloramphenicol [(1R,2R)-(+)-1-*p*-nitrophenyl - 2 - [1,2-^{14}C]dichloroacetamido - 1,3 - propanediol], 4.87 mCi/m-mole, were purchased from New England Nuclear. [1-^{3}H]Chloramphenicol [(1R,2R)- 1-*p*-nitrophenyl- 2-dichloroacetamido- 1,3- [1–^{3}H] propanediol], 23.4 mCi/m-mole, was synthesized as described previously [19]. These compounds were found to be radiochemically pure (99

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per cent) by thin-layer chromatography (t.l.c.) with the following three solvent systems: chloroform–methanol (100:15); benzene–methanol–acetic acid (45:8:4) and isopropanol–water–ammonium hydroxide 85:15:5). Amino acids, 2,4-dinitrophenyl amino acids and 2,4-dinitrofluorobenzene were purchased from Sigma Biochemicals. Pronase was purchased from CalBiochem. Constant boiling hydrochloric acid (sequanal grade) was purchased from Pierce. A scintillation mixture, consisting of 0.4% BBOT [2,5-bis-(5-*tert*-butyl-benzoxyzoly)thiophene], 0.8% naphthalene, and 40% methylcellulose in toluene] was purchased from Yorktown Research. An Aquasol scintillation mixture was purchased from New England Nuclear. Scintillation counting was performed with a Tri-Carb model 3375 spectrophotometer, using typically 15 ml of the scintillation mixture.

Animals. Male Sprague–Dawley rats (160–200 g), obtained from Hormone Assay Laboratories (Chicago, IL), were allowed free access to water and food (Purina Rat Chow). Some rats were pretreated with phenobarbital (80 mg/kg, in saline, i.p.) 72, 48 and 24 hr prior to each experiment. On the day of the experiment, each rat was transferred to a glass metabolic cage, which permitted the separate collection of urine and feces. In addition, carbon dioxide was collected by passing the expired air through a gas trap containing 150 ml methoxyethanol–ethanolamine (2:1).

Irreversible binding to tissue protein in vivo. [^{14}C]Dichloroacetic acid (DCA) (0.093 m-mole/kg, 226 μCi/kg, in 0.5 ml saline) or a mixture of [^{14}C]-and [1-^{3}H]CAP (0.093 m-mole/kg; 1479 μCi/m-mole ^{3}H; 140 μCi/m-mole ^{14}C; in 0.5 ml propylene glycol) was administered to two control rats and two phenobarbital-pretreated rats. After 24 hr, the animals were decapitated, and samples of plasma, liver, kidney, lung, bone marrow (from femur) and carbon dioxide were collected from each rat. The tissues were homogenized in 3 vol. of water, and trichloroacetic acid (10%, 10 ml) was then added to three aliquots (0.5 ml) of each homogenate and plasma (100 μl) sample in order to precipitate protein. The samples were centrifuged and the resulting pellets were washed in methanol–ether (3:1, 5 ml). The washing was repeated ten times until virtually no radioactivity was detected in the washings. After aspiration of the final wash, the protein was dissolved in 1 N sodium hydroxide (1 ml). Irreversible binding was determined by counting an aliquot (0.5 ml) of the alkaline solution [16]. Protein concentration was determined by the method of Lowry *et al.* [22]. Excretion of [^{14}C]carbon dioxide was determined by counting three aliquots (100 μl) of the methoxyethanol–ethanolamine solution. The specificity of the trapping of [^{14}C]carbon dioxide as a carbamate derivative was confirmed by counting aliquots (100 μl) of the methoxyethanol–ethanolamine solution after acidification with concentrated hydrochloric acid. Approximately 95 per cent of the initial radioactivity was lost after acidification, indicating that the trapped $^{14}CO_2$ was converted into a carbamic acid, which spontaneously decarboxylated to $^{14}CO_2$.

Isolation of radiolabeled plasma protein from rats given [^{14}C]CAP or [^{14}C]DCA. [^{14}C]CAP (0.130 m-mole/kg, 635 μCi/kg) in 0.5 ml propylene glycol or [^{14}C]DCA (0.093 m-mole/kg, 226 μCi/kg) in 0.5 ml saline was administered intraperitoneally to two rats. After 24 hr, the whole blood from each animal was collected in a beaker containing heparin and the plasma was separated from the blood cells by centrifugation. An aliquot of the untreated plasma was chromatographed on a Sephadex G-200 column. Another aliquot of plasma from each group of rats was treated with 10% trichloroacetic acid. The precipitated protein was washed as described previously until virtually no radioactivity was detected in the washings. The washed plasma protein sample was then subjected to enzymatic or chemical hydrolysis.

Enzymatic hydrolysis of washed plasma protein. Washed samples of plasma protein (30 mg) from [^{14}C]CAP- or [^{14}C]DCA-treated rats were incubated with Pronase (2.5 mg, 113 P.U.K.) in 5 ml of 0.033 M phosphate buffer containing 5% ethanol (pH 7.4) at 37° under an atmosphere of nitrogen. After 48 hr, more Pronase (2.5 mg, 113 P.U.K.) was added and the incubation was continued for an additional 48 hr. The reaction mixtures were lyophilized and dissolved in water (2.5 ml). No radioactivity was lost in either sample during lyophilization. The hydrolysates were then fractionated on a Sephadex G-10 column.

Acid hydrolysis of washed plasma protein. Washed samples of plasma protein (15 mg) from [^{14}C]CAP- or [^{14}C]DCA-treated rats were hydrolyzed in constant boiling hydrochloric acid (15 ml) under vacuum in a sealed ampule at 110 ± 2° for 25 hr. The hydrolysates were evaporated under vacuum to give a brown residue. At least 85 per cent of the radioactivity in each hydrolysate was recovered after evaporation.

Extraction anaylsis of the enzymatic and acid hydrolysate of plasma. Samples of the enzymatic (fraction III from the Sephadex G-10 column, Fig. 3) and acidic hydrolysates of plasma protein from [^{14}C]CAP- and [^{14}C]DCA-treated rats were dissolved in 3 N hydrochloric acid and subjected to the following extraction steps. First, the acidic solutions were extracted with ethyl acetate and then made alkaline (pH approximately 12) with 10 N sodium hydroxide. Second, the alkaline solutions were extracted with ethyl acetate. Third, the aqueous solutions were then adjusted to pH 9, with saturated sodium bicarbonate followed by the addition of at least 10-fold excess of dinitrofluorobenzene (DNFB). The reaction mixtures were protected from light and mixed at 40° for 2.5 hr. The reaction mixture were then made more alkaline (pH approximately 12) with sodium hydroxide and extracted with ethyl ether. Fourth, the alkaline solutions were then acidified with concentrated hydrochloric acid and extracted with ethyl ether. Fifth, the radioactivity in each extract was determined by scintillation spectrophotometry and expressed as a percentage of the initial radioactivity present in each hydrolysate.

Thin-layer chromatographic analysis of the enzymatic and acid hydrolysates of plasma protein before and after derivatization with DNFB. Samples of the enzymatic (fraction III from the Sephadex G-10 column, Fig. 3) and acidic hydrolysates of plasma protein, were analyzed by t.l.c. on Analtech silica gel GF, 1000 μm plates before and after derivatization with DNFB. Amino acid standards were applied to the plates containing the underivatized samples; these plates were then eluted with butanol–acetone–diethyl amine–water (30:30:6:15, 18.5 cm). 2,4-Dinitrophenyl (DNP) derivatives of amino acids were applied

Table 1. Irreversible binding of [^{14}C]- and [1-^{3}H]CAP to protein in various tissues 24 hr after intraperitoneal administration (0.093 m-mole/kg, 30 mg/kg) to normal and phenobarbital (PB)-pretreated rats *

		Irreversible binding† (pmoles/mg protein)		
				[^{14}C]CAP
Tissue	Pretreatment	[1-^{3}H]CAP	[^{14}C]CAP	[1-^{3}H]CAP
Liver	Normal	54 ± 12	530 ± 113	10
	PB	53 ± 8	193 ± 15	4
Kidney	Normal	17 ± 1	110 ± 7	7
	PB	18 ± 1	83 ± 3	5
Lung	Normal	15 ± 2	105 ± 10	7
	PB	16 ± 1	71 ± 5	4
Plasma	Normal	8 ± 2	409 ± 31	51
	PB	14 ± 1	156 ± 5	11
Bone marrow	Normal	7 ± 2	123 ± 15	18
	PB	10 ± 1	92 ± 4	9
Percentage of administered dose excreted as $^{14}CO_2$†				
	Normal		4.0 ± 0.4	
	PB		2.2 ± 0.2	

* Irreversible binding of ^{14}C- and 1-^{3}H-labels to protein was measured as outlined under Materials and Methods.

† Results are expressed as means ± S.E. of determinations on two animals in each group. Determinations were performed in triplicate on each animal.

as standards to the plates containing the DNP derivatives; these plates were eluted with chloroform–t-butanol–acetic acid (70:30:3, 14 cm). Each of the plates was then sectioned into 1 cm fractions and each fraction was transferred to a scintillation vial containing Aquasol (15 ml) or BBOT (20 ml) and counted in the scintillation spectrophotometer.

Reverse isotope dilution of the acid hydrolysates of plasma protein with glycine and serine. Glycine (100 mg) and serine (100 mg) were added to samples (16,700 cpm) of the acid hydrolysates of plasma protein from [^{14}C]CAP- or [^{14}C]DCA-treated rats, and recrystallized from 95% ethanol to constant specific activity.

RESULTS

Irreversible binding of CAP and DCA to protein in vivo. Twenty-four hr after the intraperitoneal administration of a mixture of [^{14}C]- and [1-^{3}H]CAP (0.093 m-mole/kg) to normal and phenobarbital-pretreated rats considerably more ^{14}C- than 1-^{3}H-label was bound to protein of liver, kidney, lung, plasma and bone marrow (Table 1). Pretreatment of rats with phenobarbital led to a decrease in the binding of the ^{14}C-label, without appreciably affecting the binding of the 1-^{3}H-label (Table 1).

When [^{14}C]DCA was administered i.p. (0.093 m-mole/kg) to normal and phenobarbital-pretreated rats

Table 2. Irreversible binding of [^{14}C]DCA to protein in various tissues 24 hr after intraperitoneal administration (0.093 m-mole/kg, 12 mg/kg) to normal and phenobarbital (PB)-pretreated rats *

Tissue	Pretreatment	Irreversible binding† (pmoles/mg protein) [^{14}C]DCA
Liver	Normal	880 ± 33
	PB	624 ± 27
Kidney	Normal	357 ± 10
	PB	240 ± 10
Lung	Normal	287 ± 13
	PB	252 ± 9
Plasma	Normal	1084 ± 43
	PB	664 ± 33
Bone marrow	Normal	465 ± 13
	PB	305 ± 24
Percentage of administered dose excreted as CO_2†		
	Normal	29 ± 1
	PB	15 ± 1

* Irreversible binding of the ^{14}C-label to protein was measured as outlined under Materials and Methods.

† Results are expressed as means ± S.E. of determinations on two animals in each group. Determinations were performed in triplicate on each animal.

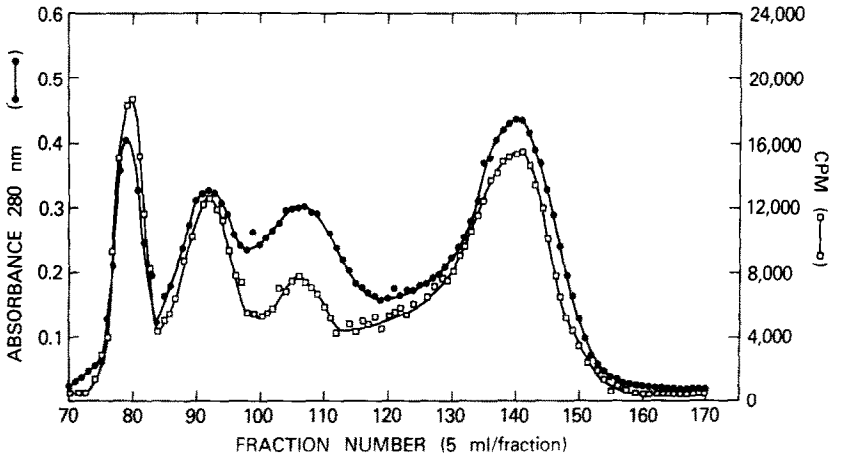

Fig. 1. Fractionation on a Sephadex G-200 column of plasma obtained from two rats 24 hr after the administration of [^{14}C]CAP (0.130 m-mole/kg, 635 μCi/kg). An aliquot (2.3 ml) of pooled plasma was applied to a Sephadex G-200 column (4 cm × 100 cm, void volume = 425 ml) which was previously equilibrated with 0.05 M phosphate buffer containing 0.1 M NaCl, pH 6.8. Elution was performed at approximately 4° with the NaCl phosphate buffer (37 ml/hr, 5-ml fraction). The amount of radioactivity and protein (absorption at 280 nm) in each fraction was determined on 1-ml aliquots.

the ^{14}C-label appeared to be bound to tissue protein to a greater degree than did that of CAP (Table 2). Moreover, as in the case with CAP (Table 1), pretreatment with phenobarbital led to a decrease in the binding (Table 2).

Anaysis of the chemical nature of the bound metabolites. To learn more about the mechanism of the ^{14}C-binding of CAP to tissue protein *in vivo*, the chemical nature of the bound metabolites was investigated. Plasma protein was chosen as the source of bound products because it was relatively easy to isolate and it contained a high level of bound ^{14}C-products relative to the amount of ^{3}H-product, which suggested a limited number of bound metabolites to be identified. The properties of the bound products of [^{14}C]DCA were studied concurrently, because it was felt that at least part of the binding of the ^{14}C-label of CAP *in vivo* might result from its hydrolysis to DCA [23].

Fractionation of plasma on a Sephadex G-200 column. The results of the fractionation on a Sephadex G-200 column of plasma from rats given [^{14}C]CAP or [^{14}C]DCA are presented in Figs. 1 and 2. The elution profiles of plasma protein, as measured by absorption at 280 nm, were nearly identical (Figs. 1 and 2) and corresponded closely to the elution profile of plasma from an untreated rat. The profiles of radioactivity (Figs. 1 and 2) followed the elution pattern of the plasma proteins; thus, the radioactivity appeared to be associated with all the plasma proteins. At least 90 per cent of the radioactivity applied to each column was recovered in the eluted fractions. Moreover, over 93 per cent of ^{14}C-label associated with the eluted plasma protein from both samples was nondialyzable.

Fractionation on a Sephadex G-10 column of enzymatic hydrolysates of plasma protein. Figure 3 shows the elution profiles of the Pronase hydrolysates of

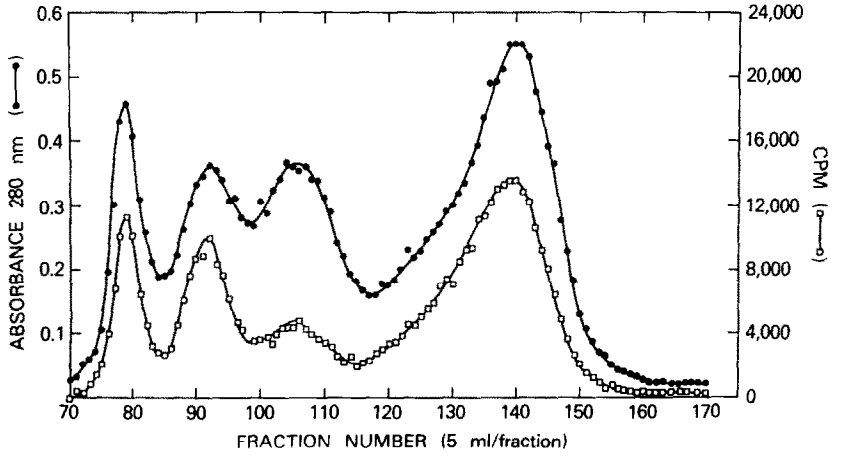

Fig. 2. Fractionation on a Sephadex G-200 column of plasma obtained from two rats 24 hr after the administration of [^{14}C]DCA (0.093 m-mole/kg, 226 μCi/kg). An aliquot (2.3 ml) of pooled plasma was fractionated on a Sephadex G-200 column as described for [^{14}C]CAP (Fig. 1).

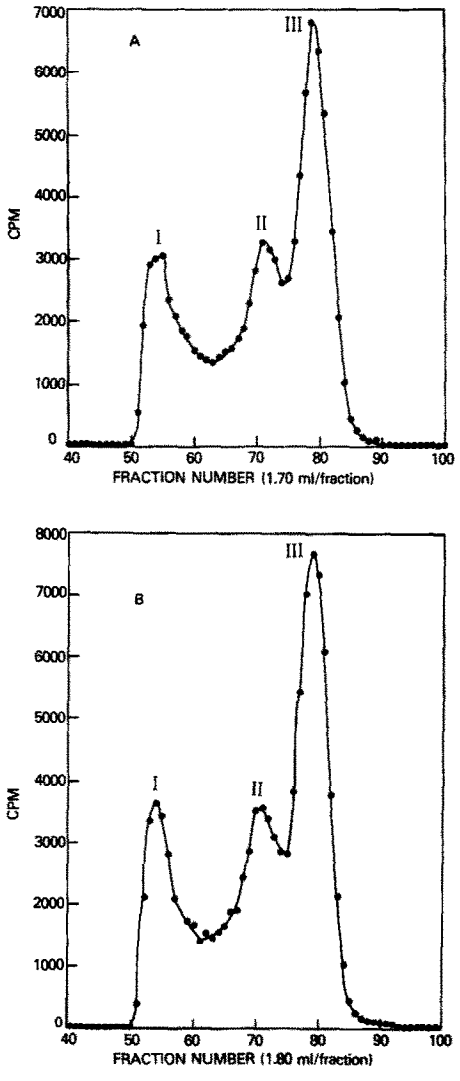

Fig. 3. Fractionation on a Sephadex G-10 column of the Pronase hydrolysate of washed plasma protein from rats given (A) [^{14}C]CAP; or (B) [^{14}C]DCA. The hydrolysates of 30 mg of washed plasma protein were applied to a Sephadex G-10 column (2.5 cm × 50 cm, void volume = 102 ml) which was previously equilibrated with water. Elution was performed with deionized water (30 ml/hr) at approximately 4°. Fractions of 1.70 ml were collected for the elution of the [^{14}C]CAP hydrolysate, whereas 1.80 ml was collected for the [^{14}C]DCA hydrolysate. The plasma samples were collected, washed, and hydrolyzed as described in Materials and Methods.

washed protein from rats given [^{14}C]CAP (Fig. 3A) or [^{14}C]DCA (Fig. 3B). The chromatographs were virtually identical with three distinct fractions. Fraction III eluted with the same retention volume as a mixture of amino acid standards, whereas fraction I eluted in the void volume.

Extraction analysis of the enzymatic and acid hydrolysates of plasma protein. The solubility and chemical properties of the radiolabeled Pronase and acid hydrolysates of plasma from rats given [^{14}C]CAP or [^{14}C]DCA were investigated so that the chemical nature of these products might be understood more clearly. Fraction III (Fig. 3) was utilized as the enzyme hydrolysate since it appeared to represent totally hydrolyzed plasma protein.

The ^{14}C-label from the Pronase and acid hydrolysates of washed plasma protein from [^{14}C]CAP- or [^{14}C]DCA-treated rats was not extracted into ethyl acetate from either acidic or basic solution, nor was it extracted into ether from alkaline solution after the hydrolysates were reacted with DNFB. However, after acidification of these solutions more than 70 per cent of the radioactivity in each DNFB reaction mixture was extracted into ether, indicating that the hydrolyzed products were zwitterions and, therefore, possibly amino acids.

Thin-layer chromatographic analysis of the enzymatic and acid hydrolysates of plasma protein before and after derivatization with DNFB. The results of the thin-layer chromatographic analysis of the acid hydrolsates of washed plasma protein from rats treated with [^{14}C]CAP or [^{14}C]DCA revealed that the radioactive profiles of both chromatographs were virtually identical and contained two major radioactive fractions (Fig. 4). Fraction I had the same R_f (0.43) as glycine and accounted for 44 per cent of the radioactivity in each acid hydrolysate, whereas fraction II had the same R_f (0.59) as serine and accounted for 48 per cent of the radioactivity in each hydroylsate.

The thin-layer chromatographic analysis of the Pronase hydroylsates (Fraction III, Fig. 3) also yielded the same two major fractions. The chromatograms from both samples of hydrolysates were again virtually identical. Fractions I and II accounted for 40 and 28 per cent, respectively, of the radioactivity in each hydrolysate. The lower level of Fraction II in the Pronase hydrolysate than in the chemical hydrolysate may reflect incomplete enzymatic hydrolysis.

After derivatization of the acid and Pronase hydrolysates with DNFB, the radioactive profiles of the thin-layer chromatographic analysis showed the presence of

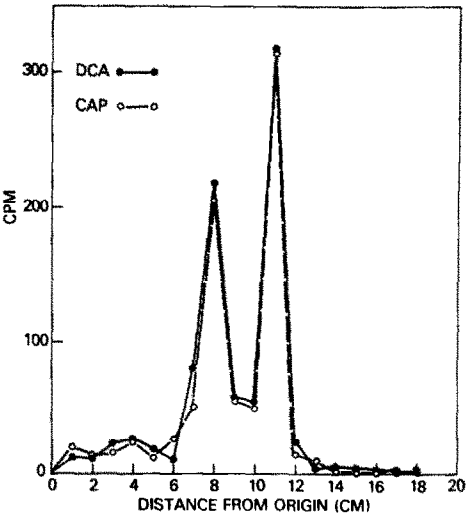

Fig. 4. Thin-layer chromatography of the acid hydrolysates of plasma protein from rats given [^{14}C]CAP or [^{14}C]DCA. The hydrolysis and thin-layer chromatographic analysis were performed as described in Materials and Methods.

two major fractions. These components were present in virtually identical proportions on the chromatograms from the hydrolysates of plasma from the rats treated with [^{14}C]CAP or [^{14}C]DCA. Fraction I had the same R_f (0.25) as the 2-dinitrophenyl (DNP) derivative of serine, whereas Fraction II had the same R_f (0.54) as the DNP derivative of glycine. Fractions I and II represented 41 and 47 per cent, respectively, of the radioactivity from the acid hydrolysates of plasma protein and 24 and 56 per cent of the radioactivity from the Pronase hydrolysates.

Reverse isotope dilution of the acid hydrolysates of plasma protein with glycine and serine. To confirm that glycine and serine were the major ^{14}C-labeled hydrolyzed products of plasma protein, from rats given [^{14}C]CAP or [^{14}C]DCA, reverse isotope dilution analysis of the acid hydrolysates of washed plasma protein was performed with authentic samples of these amino acids. Gycine and serine accounted for 49 and 51 per cent, respectively, of the radioactivity in the hydrolysate from the rats treated with [^{14}C]CAP and 46 and 54 per cent from the rats treated with [^{14}C]DCA.

DISCUSSION

The results of previous *in vitro* studies with various ^{14}C- and ^{3}H-derivatives and analogues of CAP indicate that this compound is metabolically activated in rat liver microsomes by an oxidative dechlorination process to produce an oxamyl chloride intermediate. This metabolite subsequently acylates microsomal protein or undergoes hydrolysis to an oxamic acid [18–20].

In the present investigation, we have found that another mechanism for the covalent binding of radiolabel to tissue protein also occurs *in vivo*. In this process, some of the dichloroacetyl carbons of [^{14}C]CAP are converted to ^{14}C-labeled glycine and serine, which are incorporated subsequently into protein. This pathway accounts for nearly all of the ^{14}C-label in plasma protein found 24 hr after administration of CAP. Since glycine and serine are present in most proteins, this finding explains why all of the protein fractions of plasma eluted from the Sephadex G-200 column were radioactive (Fig. 1). It is also possible that most of the ^{14}C-label of CAP bound to protein in the other tissues may be due to glycine and serine (Table 1). The small amount of the 1-^{3}H-label of CAP bound to protein in these tissues may be due to CAP molecules which have acylated protein after metabolic activation. Because the relative rates of replacement of the two covalently bound radioactive derivatives remain unknown, it is not possible to determine which of these pathways is the dominant pathway in any given tissue.

The ^{14}C-label of DCA also enters the glycine and serine pools of plasma protein. Indeed, the patterns of distribution of radioactivity of plasma before (Figs. 1 and 2) and after hydrolysis with Pronase (Fig. 3) or acid (Fig. 4) were nearly identical for ^{14}C-labeled CAP and DCA. The results thus suggest that CAP is hydrolyzed to DCA, which is then biotransformed into glycine and serine as shown in Fig. 5. In support of this view, CAP is metabolized to DCA *in vitro* by rat liver microsomes [23]. In this proposed mechanism, DCA is initially dechlorinated into glyoxylic acid, possibly by the same enzyme system in the rat liver that hydrolytically dehalogenates dihalomethanes into formaldehyde [24, 25]. In turn, glyoxylic acid undergoes transamination to form glycine [26–29] and glycine is hydroxymethylated into serine [30, 31].

Alternatively, it is possible that CAP is hydrolytically dechlorinated into an aldehyde derivative (Fig. 6). It is possible that this product is hydrolyzed into gloxylic acid, which then could be biotransformed into glycine and serine. The earlier finding of CAP alcohol as an *in vivo* metabolite of CAP in cat and man [32] supports this pathway, since CAP aldehyde would be expected to be reduced, at least in part, to the alcohol.

The proposed intermediary role of glyoxylic acid in the conversion of CAP into glycine and serine is supported by work with the organophosphorus insecticide, dichlorvos (2,2-dichlorovinyl dimethyl phosphate) [33]. When [vinyl-1-^{14}C]dichlorvos was administered to rats, most of the radioactivity retained in the liver protein of treated animals was identified after hydrolysis as [^{14}C]glycine and [^{14}C]serine. Approximately equal amounts of these two radiolabeled amino acids were found as was obtained in the present study. The authors speculated that dichlorvos was metabolized to dichloroacetaldehyde and this product was further dechlorinated into glyoxylic acid, which was then incorporated into biosynthetic pathways.

Glyoxylic acid is also known to be oxidative decarboxylated into formic acid and CO_2 by rat liver [34]. This pathway could be responsible, at least in part, for the formation of $^{14}CO_2$ from [^{14}C]CAP (Table 1) and [^{14}C]DCA (Table 2).

The finding that pretreatment of rats with phenobar-

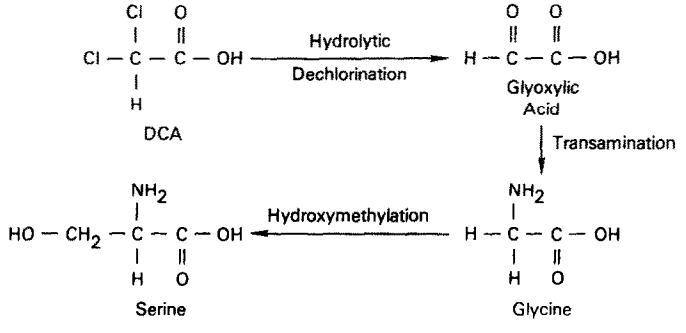

Fig. 5. Potential pathway for the metabolism of DCA into glycine and serine.

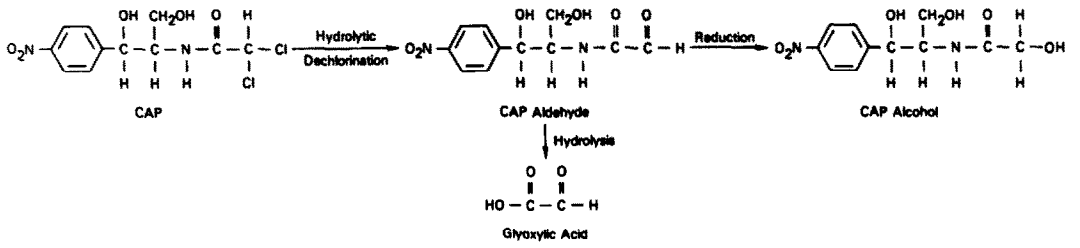

Fig. 6. Potential pathway for the metabolism of CAP into glyoxylic acid and CAP alcohol.

bital decreases the binding and formation of CO_2 after injection of either ^{14}C-labeled CAP (Table 1) or DCA (Table 2) is difficult to understand. It seems possible, however, that the oxidation of glyoxylic acid to oxalic acid [26, 35, 36] may be selectively induced by phenobarbital. If this occurred, less glyoxylic acid would be available for the formation of glycine, serine, formic acid and carbon dioxide. In this regard, phenobarbital has been reported recently to cause the induction of rat liver cytosolic aldehyde dehydrogenase activity [37, 38].

The results of the present investigation illustrate the care that should be taken when interpreting *in vivo* binding data of radiolabeled drugs and environmental chemicals. For instance, the toxicity of various drugs and environmental chemicals has been correlated with the *in vivo* binding of radiolabeled products in the target tissues. The binding is generally assumed to be due to the covalent linkage of a reactive metabolite with tissue molecules. However, until the structure of the bound products is elucidated, such an assumption can lead to erroneous conclusions [39]. The potential magnitude of this problem is illustrated by the results of metabolic studies with other drugs and environmental chemicals such as nihydrazone [40], ronidazole [41], viloxazine [42], and nitroglycerin [43]. In each case, the ^{14}C-labeled derivatives of these compounds were metabolized into small molecular fragments, which were subsequently incorporated into pools of normal tissue constituents.

Although a new pathway for the metabolism of CAP has been found, the pharmacologic and toxicologic relevance of this observation remains to be determined.

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