placed far to the left by Tris. This could be expected to occur if some heavy metal, e.g., Cu, were to be an essential component of the binding site of I.

This interpretation of the effects of AP on ox-kidney UO solutions is not purely speculative. Numerous additional items of evidence have been obtained in our Laboratory, pointing to the presence of a factor such as the postulated component I in ammonia extracts; work is still in progress on this subject, and the results will be communicated in a future paper.

An analogous effect was reported by Pontremoli *et al.*[6] for the action of fluorodinitrobenzene on rabbit-liver fructose-1,6-diphosphatase; the reagent activated the enzyme at low concentrations, and progressively inactivated it at higher ones. These authors attributed the activating effect to a conformational change following dinitrophenylation of a particular thiol group, postulated as being in an allosteric site of the enzyme. Subsequent inactivation at higher concentrations of reagent was ascribed to dinitrophenylation of a component of the catalytic site of the enzyme. While this interpretation of the results may have been applicable to Pontremoli's enzyme preparation, which was crystalline, and apparently homogeneous, it seems more likely that our interpretation would apply to our findings, inasmuch as the activating effect of AP was largely abolished by partial purification of UO.

As regards possible interference by AP with the determination of the urate content of blood and urine by the uricase method, as applied by Watts *et al.*[2], this would not seem to be of any significance, even at a daily dosage level of 1 g. If all of this were to be excreted in the urine, its concentration therein would amount to 5 mM (assuming a 1500-ml 24-hr output). Since urine is diluted 10-fold for determination of its urate content the AP concentration would fall to 0·5 mM, and this value would be lowered still further by addition of the other components of the reaction mixture. It is evident from the curves of Fig. 1 that such concentrations of AP could not significantly affect the activity of UO preparations used for urate determinations.

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Chlorpromazine-*N*-oxide formation by subcellular liver fractions

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CHLORPROMAZINE (CP) has been shown to be metabolized by several species. Urine samples of different species[1–4] show the following metabolites of CP: CP sulfoxide, nor$_1$- and nor$_2$-CP sulfoxides, nor$_1$-CP and nor$_2$-CP, 7-hydroxy CP, nor$_1$- and nor$_2$-7-hydroxy CP, glucuronides of 7-hydroxy CP, and CP-*N*-oxide (CPNO). Ziegler and Pettit[5] have shown the formation of *N*-oxide in the oxidative demethylation of *N*,*N*-dimethylaniline with a liver microsomal system. CP-*N*-oxide has also been

reported as a metabolite from CP with dog and rat liver microsomes.[6,7] Studies on the oxidative demethylation system of liver microsomes by McMahon and Sullivan[8] indicate that an aliphatic tertiary amine such as 1-propoxyphene does not proceed through an *N*-oxide intermediate as reported for dimethylaniline. To clarify the status of the mechanism of demethylation, Machinist *et al.*[9] recently reported that pig liver microsomes could catalyze the dealkylation of only those *N*-oxides in which the nitrogen atom was directly attached to an aromatic ring. In support of this observation, Mao and Tardrew[10] report that the *N*-oxide cannot be an intermediate in oxidative demethylation of erthyromycins by rabbit tissue *in vitro*. The results reported in this paper are consistent with the above findings, since CPNO was not demethylated in the absence of NADPH. Further, CP was more rapidly demethylated than CPNO with liver microsomes in the presence of the NADPH-generating system.

MATERIALS AND METHODS

Cellular fractions were prepared according to the procedure of Gillette *et al.*.[11] A Beckman model L-2 ultracentrifuge was used in these studies. Microsomes were resuspended in 0·2 M glycine buffer (pH 8·8) for sonication treatment. Solubilization of CPN-oxygenase was effected according to the method of Ziegler *et al.*[12]

The assay system used for CPN-oxygenase was as reported by Shuster and Hannam.[13] In this case, microsomes or other cellular fractions equivalent to 500 mg fresh liver were incubated in a shaking incubator at 37° for 30 min under an oxygen atmosphere. After stopping the reaction, the precipitated protein was removed and the supernatant was used for determination of CPNO and formaldehyde.

Separation and identification of CPNO. A 5-ml aliquot of the supernatant was made alkaline by addition of 1·0 ml of 10% sodium hydroxide and then extracted twice with 15-ml portions of chloroform. The chloroform layers were removed with a separatory funnel, pooled, and evaporated to dryness in a rotary evaporator and the residue was dissolved in 1·0 ml of 95% alcohol. Aliquots (100 μl) of the concentrates were spotted on Eastman Kodak TLC sheet (type K301R1, silica gel, without fluorescent indicator) with Lang-Levy pipettes. The chromatograms were developed in a solvent system consisting of ethylacetate:methanol:diethylamine (14:4:5), by an ascending technique.[14] Authentic CPNO was spotted on the same sheet for reference. After development, the chromatogram was sprayed with a color development reagent consisting of H_2SO_4:water: 95% ethanol (1:1:8).[3]

Quantitative determination of CPNO. CPNO produces a pink color on the TLC when sprayed with the sulphuric acid reagent. The chromatogram section containing the spot was cut into small pieces and extracted with 3·5 ml of 50% H_2SO_4[15] and then allowed to stand for 1 hr with occasional shaking. The polythene support was removed and then the sample was centrifuged to remove the silica gel to the bottom of the tube. The absorbancy of the colored acid extracts were determined in a Klett-Summerson colorimeter with a 54 filter (540 mμ) or a Beckman DU spectrophotometer. The control was used as the blank.

In the separation and elution of CPNO from the incubation mixture, 2·0 ml of the concentrate was spotted on the chromatogram sheet in a horizontal line. After development in the above solvent system, part of the chromatogram was sprayed. A corresponding section of the chromatogram containing pure CPNO from the unsprayed portion of the chromatogram was cut into small pieces and extracted with 25·0 ml methanol. The methanol layer was removed after centrifugation, evaporated to dryness in a rotary evaporator, and taken up in 2·0 ml of 95% alcohol. Aliquots of 100 μl were spotted along with authentic CPNO and developed in different solvent systems (see Table Table I) to confirm the identification of CPNO.

Other procedures. The Cary 14 recording spectrophotometer was used to determine the absorption spectra of reference compounds and isolated metabolites. High voltage paper electrophoretic separation of metabolites and reference compounds was conducted on 3 mm Whatman paper, 0·05 M borate, at 2500 V for 2 hr on a Precision Measurements Co. instrument. Formaldehyde was determined by a modification of the Nash method.[16]

RESULTS AND DISCUSSION

From Table 1 it may be seen that reference CPNO and a product from the incubation mixture have the same R_f value in four solvent systems with a TLC technique.

The u.v. absorption spectra of CP was found to be different from reference CPNO and the microsomal metabolite. Reference CPNO and the microsomal metabolite have essentially the same spectra. High voltage electrophoresis of reference and microsomal CPNO shows a migration of 7 and 7·3 cm, respectively, under the conditions described. A CP reference migrated 0 cm under the same conditions. From these data it may be concluded that CP was converted to CPNO by a microsomal preparation.

TABLE 1. CHROMATOGRAPHIC IDENTIFICATION OF CHLORPROMAZINE-*N*-OXIDE

Thin-layer solvent system*	Reference CPNO	Microsomal metabolite
Ethylacetate–methanol–diethylamine (14:4:5)	0·27	0·26
Acetone–diethylamine (9:1)	0·00	0·00
Acetone–isopropanol–1% NH_4OH (9:7:4)	0·81	0·82
n-Butanol–acetic acid–water (50:12:50)†	0·72	0·74

* Eastman Kodak TLC sheet (type K301R2, silica gel on polythene backing).
† Organic phase used.

Table 2 presents data obtained with the same system for the formation of CPNO and formaldehyde. The demethylase reaction apparently proceeds at a slower rate than the CPNO reaction. This would

TABLE 2. CHLORPROMAZINE-*N*-OXIDE (CPNO) AND FORMALDEHYDE FORMATION FROM CHLORPROMAZINE BY MOUSE LIVER FRACTIONS

Mouse fraction	CPNO (μmole/g liver/hr)			Formaldehyde (μmole/g liver/hr)		
	1	2	3*	1	2	3*
Homogenate		0·81	1·49		1·76	1·97
Supernatant (10,000 *g*)		3·20	4·46		1·58	2·02
Microsomes	3·24	4·50	6·48	1·19	0·60	0·76
Microsomal sonication supernatant	3·78	4·70	4·20	0·62	0·21	0·30

* A different species mouse (brown) was used in this experiment.

not exclude CPNO as an intermediate in demethylation, since both compounds are found as metabolic products in the urine. Both enzyme activities observed in these microsomal preparations were solubilized by a sonication treatment. Table 2 shows less *N*-oxide formation by homogenates than by microsomes. Possible explanations for these data are as follows: (1) the CPN-oxide formed by microsomes in the homogenate may have been demethylated (*N*-demethylase was found in the mitochondrial and light mitochondrial fractions); (2) CP-*N*-oxide may be reduced by xanthine oxidase (as a source of oxygen);[17] and (3) possibly some inhibitor may be present in the homogenate.

Table 3 shows the relative activities of CP-*N*-oxygenase and demethylase in the mouse, rat, and dog. The mouse and dog show higher CPN oxygenase activity at the microsomal level, but the dog is the only species reported to excrete CPNO as a urinary product. The liver microsomal demethylase activities of the three species were similar.

The NADPH requirement of the CP-*N*-oxygenase is established by the data in Table 4. CP was not converted to the *N*-oxide and was not demethylated to an appreciable extent in the absence of NADPH.

It was also observed that the demethylation of CP was greater than that of CPNO in the presence of NADPH. This would indicate that CPNO may not be an intermediate in oxidative demethylation of CP.

TABLE 3. CHLORPROMAZINE-*N*-OXIDE (CPNO) and FORMALDEHYDE FORMATION FROM CHLORPROMAZINE WITH LIVER MICROSOMES

Animal	CPNO formed (μmole/g liver/hr)	Formaldehyde formed (μmole/g liver/hr)
Mouse 1	2·70	1·13
Mouse 2	3·24	1·19
Mouse 3	4·50	0·60
Rat 1	1·57	1·19
Rat 2	1·33	0·60
Rat 3	1·26	1·01
Dog 1	4·05	1·49
Dog 2	4·73	0·98

TABLE 4. REQUIREMENTS FOR NADPH OF MOUSE LIVER MICROSOMES* IN THE DEMETHYLATION OF CHLORPROMAZINE (CP) AND CHLORPROMAZINE-*N*-OXIDE (CPNO)

Additions to liver microsomal preparation	CPNO produced (μmole/g liver/hr)	Formaldehyde produced (μmole/g liver/hr)
CP+NADPH-generating system (complete)	2·16	1·85
CP+NADPH-generating system	2·79	1·31
CP	0·00	0·12
CP	0·00	0·14
CPNO+NADPH-generating system		0·79
CPNO+NADPH-generating system		0·44
CPNO		0·28
CPNO		0·19

* Data are representive, although all experiments were not conducted with the same microsomal preparation. CPNO (5 μmole) was used in the last 4 assays in place of CP.

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The sub-cellular partition and metabolism of orally administered 1,1,1-trichloro-2, 2-*bis* (*p*-chlorophenyl) ethane by rat liver cells*

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Abstract—Uptake of orally administered DDT† by rat liver cells has displayed the following characteristics:

1. The pesticide was not exclusively segregated from any one cell fraction.
2. Pesticide was depleted with time from all fractions.
3. A redistribution of pesticide occurred with time. A passive DDT distribution either occurs at some point in time or at low levels of exposure.
4. Non-lipid binding of pesticide appeared to occur.
5. Liver cells appeared tc be detoxifying DDT to DDE and DDD by 16 hr post treatment.

THE ABILITY of DDT to influence specific metabolic processes of cellular constituents has been of interest to investigators for some time. Rat liver has been the most universal material studied.

DDT has been shown to interfere with oxidative metabolism in rat liver mitochondria.[1] The soluble cell fraction contains enzymes which are inhibited by DDT.[2] The important role played by rat liver microsomes in the detoxification of DDT[3] and also the stimulation of microsomal drug-metabolizing enzymes by DDT have been demonstrated.[4]

Since DDT is capable of interactions of a very diverse nature depending upon its cellular location, it is important to characterize the uptake of orally administered DDT and subsequent intracellular involvement. This work involved analysis of the various subcellular components of rat liver cells from dosed and control rats for DDT and its analogs. Blood samples and studies *in vitro* were included to aid in the characterization.

EXPERIMENTAL

University of Arizona strain female rats of Sprague–Dawley origin, weighing 200–300 g and maintained on a Wayne Lab Blox diet, were used as experimental animals. The rats were dosed via a stomach tube with 10 mg of 99·3% *p,p'*-DDT in 1 ml peanut oil. The dosed rats and control rats

* Arizona Agricultural Experiment Station, Technical Paper No. 1219.

† Abbreviations: DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene; DDD, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane.