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DISTRIBUTION OF CHLORPROMAZINE METABOLITES IN SELECTED ORGANS OF PSYCHIATRIC PATIENTS CHRONICALLY DOSED UP TO THE TIME OF DEATH

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Abstract—The major groups of chlorpromazine metabolites were determined qualitatively by TLC and quantitatively by spectrophotometric assay. The groups of nonphenolic and phenolic metabolites were measured in the visceral tissues of 6 patients chronically dosed with chlorpromazine up to the time of death. Chlorpromazine, chlorpromazine sulfoxide and their demethylated derivatives constituted the major group of metabolites present in most tissues. On the average, highest concentrations were seen in lung and liver tissues. Other tissues examined usually contained low levels of the major group of metabolites. Traces of chlorpromazine-*O*-glucuronides were detected in lung, liver, pancreas and kidney. Chlorpromazine-*O*-glucuronides were not detected in other tissues. Most tissues contained lower levels of the 7-hydroxychlorpromazine group of metabolites than of the nonphenolic metabolites. The liver tissue of one patient with chlorpromazine-induced hyperpigmentation of the skin contained the same drug metabolites as liver tissues from patients without this side-effect. However, the concentrations of the nonphenolic and phenolic groups of metabolites were very high in the liver of the hyperpigmented patient. In view of the present data, 7-hydroxychlorpromazine is considered a normal biotransformation and storage product of chlorpromazine metabolism in man.

IN MAN, the metabolism of chlorpromazine (CP)† is known to involve both the nucleus and the sidechain of the molecule, giving rise to a great number of metabolites.[1] The urinary excretion of CP metabolites has been studied by numerous investigators and was recently reviewed.[2–5] However, very little is known of the distribution and storage of CP in the tissues of man and experimental animals.[6–10] In a previous study of CP metabolites in mammalian tissues, CP, chlorpromazine sulfoxide (CPO) and their demethylated derivatives were measured.[6] Recently, hydroxylated and conjugated derivatives of CP were detected in animal tissues.[11] A new assay has therefore been

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† The following abbreviations were used: CP, chlorpromazine; nor_1CP, desmonomethylchlorpromazine; nor_2CP, desdimethylchlorpromazine; CPO, chlorpromazine sulfoxide; nor_1CPO, desmonomethylchlorpromazine sulfoxide; nor_2CPO, desdimethylchlorpromazine sulfoxide; 7-OHCP, 7-hydroxychlorpromazine; $nor_1$7-OHCP, desmonomethyl-7-hydroxychlorpromazine; $nor_2$7-OHCP, desdimethyl-7-hydroxychlorpromazine; 7-OHCPO, 7-hydroxychlorpromazine sulfoxide; $nor_1$7-OHCPO, desmonomethyl-7-hydroxychlorpromazine sulfoxide; $nor_2$7-OHCPO, desdimethyl-7-hydroxychlorpromazine sulfoxide; CPNO, chlorpromazine-*N*-oxide; TLC, thin-layer chromatography; DCM, dichloromethane.

devised to measure the phenolic as well as the nonphenolic metabolites of CP stored in mammalian tissues.

7-Hydroxylchlorpromazine (7-OHCP) has been implicated as a toxic agent in CP-induced hyperpigmentation of exposed skin in chronically dosed patients.[12] 7-OHCP was reported to be the only drug metabolite present in the liver of a hyperpigmented patient. However, data from patients without hyperpigmentation of the skin were not cited for comparison. It was therefore of interest to determine to what extent 7-OHCP is normally stored in human viscera after chronic CP administration. A preliminary report of these studies has been presented.[13]

MATERIALS AND METHODS

Chlorpromazine HCl, desmonomethylchlorpromazine HCl, desdimethylchlorpromazine HCl and their respective sulfoxides were kindly provided by Smith, Kline & French Laboratories. 7-Hydroxychlorpromazine, desmonomethyl-7-hydroxychlorpromazine ($nor_1$7-OHCP), desdimethyl-7-hydroxychlorpromazine ($nor_2$7-OHCP) and their respective sulfoxides (with the exception of the unavailable $nor_2$7-OHCPO) were donated by Drs. D. H. Efron and A. A. Manian of the Psychopharmacology Research Branch, National Institute of Mental Health, Bethesda, Md.

Autopsied tissues from 6 patients were used for chemical analyses. In all cases, autopsies were performed between 24 and 72 hr after death. Tissues were kept frozen until the analyses were performed. Age, weight, dose, lapse of time between death and autopsy, drug regimen and cause of death are summarized in Table 1. The tissue sample from the hyperpigmented patient (VI) was provided by Drs. A. C. Greiner and G. A. Nicolson of Riverview Hospital, Essondale, B.C., Canada.

I. Determination of CP and its demethylated derivatives

Frozen autopsied tissues,* stored in a freezer at temperatures between −10 and −20° for various periods of time, were brought to room temperature, and samples of 1–10 g wet wt. were used per determination. The tissues were homogenized in glass tissue grinders of 50-ml capacity with ground-glass or teflon pestles at their natural pH, with 10 ml distilled water/g of tissue.† Freshly prepared 1-cysteine HCl in water, 100μg/g wet tissue, was added, and the homogenates were adjusted to pH 13 with NaOH, transferred to glass-stoppered Erlenmeyer flasks and shaken with 50 ml heptane containing 1·5% isoamyl alcohol for 35 min on a wrist-action shaker. The mixture was centrifuged for about 5 min at 3600 rpm, the organic phase was set aside and the aqueous phase was extracted again with heptane mixture. The organic extracts were pooled, concentrated on a rotary evaporator at reduced pressure to a volume of approximately 15 ml, transferred to a small stoppered Erlenmeyer flask, and extracted with 2 × 2·5 ml of 0·1 N sulfuric acid by shaking for 7 min and centrifuging for 5 min. The acid extracts were combined and the heptane phases discarded. Exactly 2·5 ml of the acid extract was transferred to a glass-stoppered 5-ml volumetric flask, the flask was immersed in an ice-water bath and 2·5 ml of concentrated sulfuric acid was added. The flask was placed in a boiling water bath for 1 min and then

* The drug content of tissues preserved in formaline was rapidly destroyed; it was, however, stable for periods of at least 1 yr in tissues preserved by freezing, stored at −10 to −20°.

† Use of the Waring–Blendor for homogenization of tissues caused up to 50 per cent losses of CP and 7-OHCP in recovery experiments.

TABLE 1. DESCRIPTION OF AUTOPSY CASES*

Patient	Age (Yr)	Wt. (lb)	Time lapse from death to autopsy (hr)	Medication during last 4 months: Last CP dose (hrs before death)	CP (mg/day)	Other phenothiazine drugs occasionally	Other drugs occasionally and PRN†	Post-mortem diagnosis
I (M)	36	170	24–36	6	1050	None	Biperiden	Chest & lung pathology; bronchial asthma
II (M)	47	148	24–36	11	600	None	Amitriptyline Trihexyphenidyl	Congestive Heart failure; arterio-sclerosis
III (M)	49	161	24–36	1	600	None	Digitalis Sodium amytal Mercuhydrin Reserpine Laxatives Acetylsalicylic acid Antacids	Congestive heart failure; coronary thrombosis
IV (M)	60	170	72	10	400	None	Secobarbital Chloral hydrate Laxatives Antispasmodics	Myocardial infarct; congestive heart failure
V (M)	44	175	24–36	1	200	Promethazine	Pentobarbital Meperidine Hydromorphone Heparin Neomycin SodiumWarfarin Stilbesterol Scopolamine Acetylsalicylic acid Laxatives	Cancer metastases to lung, liver, left adrenal; embolus due to thrombo-phlebitis
VI (F)	32	144	24–36	6 or 18‡	600	Levomépromazine + perphenazine (both discontinued 4 months before death)	Phenobarbitone Phenytoin Benztropine	Acute intes-tinal ob-struction; volvulus of sigmoid colon; pul-monary atelectasis

* Abbreviations used: M, male; F, female; CP, chlorpromazine.

† When required.

‡ Medical records equivocal on this point.

brought to room temperature; 1 drop of 0·1% hydrogen peroxide was added, the solution stood for 15 min and was made up to volume with 50% sulfuric acid. The absorption spectrum of the acid solution was recorded between 400 and 700 mμ.* Absorbance was measured at 530 mμ by a previously described background cancellation

* Absorption spectra were recorded by using a Beckman DB recording spectrophotometer.

technique.[14, 15] A standard calibration curve was prepared from CP-HCl by using spectrophotometric cells of both 1-cm and 4-cm light-path. The absorbance of CP after reaction with 50% sulfuric acid was proportional to concentration between $0{\cdot}5 \times 10^{-5}$ and $2{\cdot}5 \times 10^{-5}$ M for 1-cm light-path, and between 2×10^{-6} and 8×10^{-6} M for 4-cm light-path.* Recovery experiments were carried out with 50 μg CP per g wet control tissues.

II. Determination of 7-OHCP and its demethylated derivatives

The tissue homogenate after extraction with heptane was adjusted to pH 9·2 with dilute sulfuric acid and extracted with 2×50 ml dichloromethane (DCM), shaken and centrifuged as previously described for the corresponding heptane extractions. (Centrifugation time was extended to 10 min for optimum separation of phases.) The combined DCM phases were reduced in volume to approximately 15 ml and extracted with $2 \times 2{\cdot}5$ ml of 0·1 N sulfuric acid. A 2·5-ml aliquot of the combined acid extracts was transferred to a 5-ml volumetric flask, immersed in an ice-water bath and 2·5 ml 50% sulfuric acid containing 75 μg $FeCl_3.6\ H_2O$ per ml was added. The flask was allowed to revert to room temperature and the volume was adjusted to exactly 5 ml with 25% sulfuric acid. The absorption spectrum was recorded between 400 and 700 mμ and absorbance measured at 565 mμ by using the background cancellation technique. A maximum of only 5 min was allowed to elapse between the addition of acid and the start of the spectrophotometric measurement.† A standard calibration curve was prepared from 7-OHCP in 0·01 N sulfuric acid with cells of 1-cm and 4-cm light-path. The absorbance of 7-OHCP after reaction with 25% sulfuric acid containing $FeCl_3$ was proportional up to $2{\cdot}5 \times 10^{-5}$ M concentration for 1-cm light-path and up to $8{\cdot}0 \times 10^{-6}$ M for 4-cm light-path. Recovery experiments were carried out with 20μg 7-OHCP per g control tissue.

III. Determination of chlorpromazine-7-O-glucuronides

Specimens of autopsied tissues after removal of unconjugated chlorpromazine metabolites according to I and II (above) were incubated for 24 hr with 15% by volume of Ketodase‡ at pH 4·5 and 37°. The resulting mixture was assayed for 7-OHCP and its derivatives according to II, described above.

IV. Identification of drug metabolites by TLC

The balances of the 0·1 N sulfuric acid extracts of 2·5 ml each, resulting from the re-extractions of the heptane and DCM phases, according to I and II above, were adjusted to pH 9·2 and re-extracted with 2×15 ml DCM. These organic phases were evaporated to dryness on the rotary evaporator, dissolved in 0·2 ml DCM and spotted on glass plates coated with 250 mμ of silica gel H.§ The plates were developed in two

* The 4-cm light-path cells were obtained from Scientific Cell Co., Forest Hills, N.Y.

† Chromophores derived from pure solutions of 7-OHCP were stable for 2 hr. However, the absorption of biologically derived chromophores of 7-OHCP decreased by 10% on standing for 15 min, and by 20 per cent on standing for 40 min.

‡ Ketodase, a brand of β-glucuronidase, was kindly donated by Warner-Chilcott, Morris Plains, N.J.

§ The plates were air-dried at room temperature and used without further treatment. Occasionally, extracts containing interfering endogenous material (e.g. cholesterol or indole derivatives) required further purification. This was obtained by one or more cycles of extraction of the organic phases into 0·1 N sulfuric acid and re-extraction into DCM at pH 9·2.

solvent systems: i.e. in system A, ethanol (95%)–ethyl acetate–acetic acid–water (17:56:17:10); and in system B, butanol–ethanol (absolute)–diethylamine (12:6:1). CP metabolites were detected by spraying with a mixture of sulfuric acid–water–ethanol (1:1:6). Sulfides appeared immediately, while the sulfoxides required heating for 3 min at 110°.[16] CP, desmonomethylchlorpromazine (nor_1CP) and desdimethylchlorpromazine (nor_2CP) appeared as pink spots, while CPO, desmonomethylchlorpromazine sulfoxide (nor_1CPO) and desdimethylchlorpromazine sulfoxide (nor_2CPO) were a slightly darker pink. All metabolites derived from 7-OHCP or 7-hydroxychlorpromazine sulfoxide (7-OHCPO) were purple. This spray reagent yielded relatively stable color spots, especially after development of the plates in solvent system A. Such plates, wrapped in transparent plastic film and kept in the dark, were suitable for color photography for several weeks after preparation. Other detection sprays containing e.g. sulfuric acid, water, ethanol and ferric ions, produced satisfactory but more rapidly fading color spots.

The aglycones obtained by enzymatic hydrolysis with Ketodase were extracted from the mixture at pH 9·2 with 2 × 50 ml DCM. The organic extract was prepared for TLC as described for the phenolic CP metabolites.

RESULTS

Drug analyses were carried out on the autopsied tissues from 5 male patients (I to V, Tables 1 and 2) chronically dosed with 2·5 to 13·6 mg/kg of CP for periods of 6 months or longer, up to the time of death. All had died from causes unrelated to drug therapy and none had shown any manifestations of drug toxicity during CP therapy. The results for the two major groups of drug metabolites, i.e. unconjugated metabolites derived from CP and 7-OHCP, are summarized in Table 2. Drug analyses were also carried out on a sample of autopsied liver from a hyperpigmented female patient who had received CP for several years prior to death (patient VI, Table 2).

Recovery experiments were performed for each type of tissue (Table 2). CP and 7-OHCP were recovered at the rate of 75–98 per cent, with low values of 50–65 per cent obtained for the recovery of 7-OHCP in mesentery and fat, intestinal tissue, and testes. Recovery experiments of added CPO were carried out in control liver tissue and the per cent recoveries were consistently higher than those for CP. Recovery experiments of added CP and 7-OHCP were also carried out in control brain, spinal cord and keratinous tissues. While CP was recovered at a satisfactory rate, 7-OHCP recovery rates were unsuitable. Accordingly, the present methods were unsatisfactory for fatty, nervous and keratinous tissues. In previous studies, unconjugated nonphenolic CP metabolites were found in small amounts in fatty and nervous tissues, while elevated levels were seen in keratinous tissues.[8] However, a modified procedure is being developed for fatty, nervous and keratinous tissues (unpublished data).

Three groups of CP derivatives were found in tissues: (1) the unconjugated and nonphenolic derivatives of CP and CPO (2) the corresponding 7-OHCP derivatives (3) the conjugated derivatives of 7-OHCP. The results are presented without correction for per cent recoveries and are summarized in Table 2. In all tissues of the 6 patients examined, the unconjugated nonphenolic metabolites (group 1) constituted the major group, except in the case of testes, where the phenolic metabolites (group 2) predominated. However, only 2 specimens of testes were available. In addition, there were 5 instances of higher amounts of phenolic metabolites, i.e. in liver of patient I,

Table 2. Drug content found in selected autopsied tissues*

Tissue	% Recoveries		Patient I (13·6 mg/kg)		II (8·9 mg/kg)		III (8·2 mg/kg)		IV (5·2 mg/kg)		V (2·5 mg/kg)		VI† (9·2 mg/kg)	
	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP
Liver	80	91	22	33	38	6·2	8·1	3·8	2·3	1·5	13	Tr	190	37
	81	88	21	33	37	5·9	8·1	3·7	2·3	1·5	12	Tr	190	36
Lung	80	82	25	18	79	59	29	23	11	5·2	8·5	4·6		
	80	84	25	18	77	58	29	24	11	5·2	8·5	4·5		
Kidney	89	84	7·0	5·6	2·3	2·3	9·1	8·2	0·6	0·7a	0·7	0·4		
	88	81	7·1	5·6	2·3	2·4	8·8	8·1						
Spleen	82	78	6·2	5·1	7·6	2·0	1·3	0·8	1·3	1·7	2·8	1·0		
	81	78	6·0	5·2	7·1	2·0	1·4	0·7	1·3	1·7	2·7	0·9		
Heart	80	78	7·3	4·0	Tr	Tr	1·1	0·4	1·5	Tr	0·5	Tr		
muscle	78	75	7·3	3·7					1·5	Tr				
Pancreas	89	80	21	14	3·3	1·3	8·8	6·2	Tr	0·8	Tr	0·9		
	91	82	21	14	3·0	1·3	8·7	6·0	Tr	0·8	Tr	0·9		
Adrenal	88	82	4·4	Tr	1·7	1·7	1·5	Tr	1·6	0·8				
	87	81	4·4	Tr			1·6	Tr	1·6	0·8				
Thyroid	78	98	1·1	Tr	1·5	1·8	0·9	Tr						
	77	95	1·1	Tr	1·5	1·7	0·8	Tr						
Mesentery	89	50			4·4	Tr	1·6	Tr						
and fat	87	50			4·2	Tr	1·4	Tr						
Intestinal	87	65	4·3	Tr	1·7	Tr	0·8	Tr						
tissue	84	64	3·8	Tr	1·6	Tr	0·9	Tr						
Testes	79	64					1·0	1·6	1·6	2·3				
	81	62							1·6	2·3				

* The drug metabolites derived from chlorpromazine (CP) are expressed in μg of CP-HCl per g wet tissue, and the metabolites derived from 7-hydroxychlorpromazine (7-OHCP) are expressed as μg of 7-OHCP, free base, per g wet tissue; Tr = trace.

† Patient VI had hyperpigmentation of the skin.

‡ Tissue was unavailable.

a Material available for a single determination only.

spleen of patient IV, thyroid of patient II and pancreas of patients IV and V. The amounts of conjugated metabolites (group 3) were not measurable in any of the tissues studied. The hyperpigmented patient VI showed distinctly elevated amounts of CP and 7-OHCP metabolites in the liver, the only tissue available from this patient. Especially the metabolites of group 1, the unconjugated and nonphenolic CP derivatives, were abundantly present.

The components of the 3 groups of metabolites were identified in each tissue examined by TLC using two solvent systems. The results are presented in Table 3.

TABLE 3. R_f VALUES OF CHLORPROMAZINE METABOLITES IN LIVER TISSUES

Organic phase	Patient II (no side-effects)		Patient VI (hyperpigmented)		Reference compound		Comment
	SS:A*	SS:B*	SS:A	SS:B	SS:A	SS:B	
Heptane	0·50	0·45	0·50	0·46	0·51	0·47	nor_1CP
(unconjugated	0·33	0·67	0·32	0·68	0·33	0·70	CP
nonphenolic	0·65	0·54	0·64	0·54	0·65	0·56	nor_2CP
metabolites)			0·42	0·59			?†
	0·15‡	0·26‡	0·11‡	0·27‡	0·14	0·28	nor_1CPO
	0·07‡	0·53‡	0·07‡	0·55‡	0·07	0·58	CPO
	0·25‡	0·32‡	0·26‡	0·34‡	0·25	0·37	nor_2CPO
			0·18‡	0·49‡	0·18	0·50	Perphenazine
			0·03‡	0·39‡	0·03	0·40	Perphenazine 5-oxide
Dichloromethane	0·48	0·15	0·44	0·17	0·45	0·18	$nor_1$7-OHCP
(unconjugated	0·33	0·38	0·28	0·38	0·30	0·40	7-OHCP
phenolic	0·62	0·25	0·58	0·24	0·59	0·25	$nor_2$7-OHCP
metabolites)	0·20‡	0·04‡	0·18‡	0·04‡	0·20	0·04	$nor_1$7-OHCPO
	0·10‡	0·10‡	0·07‡	0·10‡	0·10	0·12	7-OHCPO
Dichloromethane	§	§	0·43	0·16	0·45	0·18	$nor_1$7-OHCP
(aglycones of	§	§	0·28	0·38	0·30	0·40	7-OHCP
hydrolyzed			0·56	0·24	0·59	0·25	$nor_2$7-OHCP
fraction)			0·07‡	0·10‡	0·10	0·12	7-OHCPO

* SS:A, solvent system A: ethanol (95%)–ethyl acetate–acetic acid–water (17:56:17:10); SS:B, solvent system B: butanol–ethanol (absolute)–diethylamine (12:6:1).

† A *levo*mépromazine metabolite according to color of spot.

‡ Trace.

§ Barely detectable.

In all tissues, the unconjugated and nonphenolic group of metabolites consisted of CP, nor_1CP, nor_2CP and traces of the corresponding sulfoxides. The predominant metabolite was nor_1CP. In all tissues, the phenolic group of metabolites consisted of 7-OHCP, $nor_1$7-OHCP, $nor_2$7-OHCP and traces of 7-OHCPO and desmonomethyl-7-hydroxychlorpromazine sulfoxide ($nor_1$7-OHCPO). The predominant metabolite was $nor_1$7-OHCP. The metabolites desdimethyl-7-hydroxychlorpromazine sulfoxide ($nor_2$7-OHCPO)* and chlorpromazine-*N*-oxide (CPNO) were not detected in any of the tissues. Aglycones of conjugated metabolites (group 3) were detected in liver, lung, kidney and pancreas and were identified as $nor_1$7-OHCP, $nor_2$7-OHCP and 7-OHCPO. The limit of detection of CP metabolites by TLC was 0·2 μg/g wet tissue. Qualitative examination was carried out for all available tissues from each patient. However, only the data from two tissues are listed in Table 3, since nearly identical

* Unavailable as reference compound; see Materials and Methods.

results were obtained for all tissues. In the case of patient VI who was hyperpigmented, careful examination of the liver extracts by TLC indicated the presence of three unknown components yielding color reactions characteristic of phenothiazine compounds, but unlike those of known CP metabolites. The patient had been receiving *levo*mépromazine and perphenazine until 4 months prior to death (Table 1). Two of the spots corresponded to perphenazine and its sulfoxide in Rf values and color reactions. The third spot corresponded to a *levo*mépromazine derivative according to its color reaction, and may have been a metabolite of *levo*mépromazine. The quantities of these 3 components in the unconjugated and nonphenolic group of CP metabolites were very low and were not thought to affect significantly the assays of CP metabolites derived from liver tissue of patient VI (Table 2).

DISCUSSION

As applied to the visceral tissues examined, the method is rapid and reproducible. Cysteine hydrochloride, known as a free radical scavenger, preserves the labile 7-OHCP metabolites added to control tissues or present in the experimental autopsied tissues. It prevents the decomposition of 7-OHCP metabolites, which in the absence of cysteine is manifested by the appearance of secondary peaks in the absorption spectra of 7-OHCP, which interfere with the assay.

CP, CPO and their demethylated derivatives as well as the corresponding metabolites of 7-OHCP are determined quantitatively as their respective ion radicals. For the formation of their ion radicals, two different oxidizing agents are used. Hydrogen peroxide and a 50% sulfuric acid medium are used with CP derivatives.[14] The ion radicals of 7-OHCP and its demethylated derivatives, however, are unstable in the presence of excess hydrogen peroxide and another oxidant was therefore required. Ferric chloride in 25% sulfuric acid is suitable for the oxidation of this group of metabolites.[15] The latter oxidant is not suitable for the quantitative measurement of 7-OHCPO; however, only trace amounts of 7-OHCPO and nor$_1$7-OHCPO occur in tissues.

In the visceral tissues examined in the present study, trace amounts of CP glucuronides are present in liver, lung, kidney and pancreas. Incubation of the tissues *per se*, or with trypsin, papain and pronase, produces only traces of 7-OHCP and its demethylated derivatives. These aglycones are obtained optimally by enzymatic hydrolysis with β-glucuronidase. Incubation is carried out with Ketodase (15 per cent by volume) at pH 4·5 and 37° for 24 hr, after removal of all unconjugated derivatives of CP and 7-OHCP; 90 per cent of the aglycones present are obtained by this procedure. The remaining 10 per cent are obtained after a second incubation period of 24 hr with an additional 10 per cent by volume of Ketodase. As the level of CP-7-*O*-glucuronides is very low (e.g. 1·6 μg/g wet lung tissue of patient III), a single incubation period of 24 hr is used. Unfortunately, CP glucuronides are unavailable as reference compounds and hence no recoveries can be carried out. According to qualitative analysis by TLC, the minor amounts of conjugates present are *O*-glucuronides and sulfates exclusively. However, the presence of other conjugates or adducts with CP metabolites can not be excluded on the basis of the present results.

According to the present study, significant levels of unconjugated metabolites are usually found only in liver and lung tissues. However, it is difficult to draw general conclusions from data derived from 6 cases. These 6 cases were the only suitable ones

obtained over a period of 2 years. The criteria for inclusion in this study were of necessity stringent. Only autopsied tissues from patients having received CP as the only chronically administered phenothiazine drug for at least 4 months and up to the time of death were studied. A further case, in which the chronic daily dose of 1000 mg CP was supplemented with 10 mg per day of trifluoperazine during the last 3 months of life, was tentatively studied. Examination of this patient's liver tissue showed that the second phenothiazine drug, administered for 3 months only and at 1 per cent of the CP dose, accumulated to nearly the same extent as CP. Since neither data on the metabolism of trifluoperazine nor suitable reference compounds are available, this preliminary observation is of clinical interest only, and bears out the necessity for stringent specifications of material to be studied.

Comparing the types and amounts of CP metabolites seen in autopsied tissues in the present study with the patterns of urinary CP excretion in comparably dosed chronic psychiatric patients,[14, 15] substantial differences are seen. In the urine of these patients, CP glucuronides usually consitute about 80 per cent of the urinary drug content, while CPO and its demethylated derivatives and small amounts of the corresponding sulfides almost account for the remaining 20 per cent. 7-OHCP, 7-OHCPO and their demethylated metabolites constitute less than 1 per cent of the urinary metabolites,[17] and the group of the deaminated CP metabolites may account for another 1 per cent of the urinary drug content. Deaminated CP derivatives or CPNO, which are present in urine, were not seen, nor have any unknown CP metabolites been detected in tissues. On the other hand, more than one-half of the drug metabolites in tissues normally consist of CP and its demethylated derivatives with only traces of the corresponding sulfoxides present. 7-OHCP, its demethylated metabolites and traces of the corresponding 7-OHCPO and nor$_1$7-OHCPO constitute the bulk of slightly less than one-half of the remaining CP derivatives in tissues, as conjugated CP metabolites are present in trace amounts only. Hence, urinary CP metabolites do not reflect the types and amounts of CP derivatives stored in tissues.

The drug analyses of patients without side-effects of CP therapy (patients I–V, Tables 1 and 2) clearly indicate that both phenolic and nonphenolic unconjugated CP metabolites are normally stored in the various body tissues. The present authors therefore conclude that no specific toxicity should be attributed to tissue levels of 7-OHCP, as postulated by Perry *et al.*[12] They do, however, consider the appearance of elevated amounts of unconjugated 7-OHCP metabolites in the urine of chronically dosed psychiatric patients as a manifestation of incipient or existing drug toxicity.[17] In a few psychiatric patients chronically dosed with CP, urinary CP excretion patterns were followed for periods of several years. A slight increase in unconjugated 7-OHCP derivatives over such initial values as 0·05 to 0·1 per cent to a range of 0·2 to 0·3 per cent of the daily CP dose preceded the appearance of hyperpigmentation of the skin by several months[18] and might have predictive value for incipient drug toxicity.

With regard to the liver tissue from patient VI, who had hyperpigmentation of the skin, qualitative analysis of the drug content according to TLC (Table 3) revealed the normal range of phenolic and nonphenolic CP derivatives, as seen in patients without hyperpigmentation of the skin. Perry *et al.*[12] reported 7-OHCP as the only drug derivative present in the liver of such a patient. However, the total amounts of phenolic and especially nonphenolic CP derivatives (Table 2) were considerably higher than the levels found in patients on a comparable drug dose (e.g. patient II) and even higher

than that of patient I who was receiving approximately 30 per cent more CP on a mg/kg basis. Compared to patient I, the hyperpigmented patient VI accumulated an almost equal amount of phenolic CP derivatives, but approximately 8 times the amount of nonphenolic CP derivatives. It appears that this high rate of accumulation and the patient's apparent inability to excrete all administered phenothiazine drugs within a reasonable time may account for the drug toxicity. (Traces of perphenazine and *levo*mépromazine were present in the liver, although these drugs were discontinued 4 months prior to her death.) Accordingly, one might speculate that hydroxylation and conjugation with glucuronic acid were impaired or that renal function was greatly decreased due to melanin-like deposits in the tubules which were found at autopsy.[19]

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