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METABOLISM OF CHLORPROMAZINE IN HUMANS

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Abstract—The isolation and characterisation of chlorpromazine metabolites from the urine of psychiatric patients is described. It is shown that chlorpromazine and its mono- and di-demethlated derivatives are hydroxylated and that these hydroxy compounds are conjugated mainly with glucuronic acid and, to a lesser extent, with sulphate. The predominant metabolite was isolated and identified as a monoglucuronide of N-desdimethyl chlorpromazine.

THE metabolic conversions undergone by chlorpromazine and related drugs which have been established unequivocally involve modification of the ring sulphur atom and dealkylation of the aminoalkyl chain. Thus, chlorpromazine[1, 2] promazine,[3] methoxypromazine,[4] mepazine[5] and thioridazine[6] are oxidised *in vivo* to the corresponding sulphoxides and in the case of thioridazine, to a disulphone as well. Oxidation of chlorpromazine to chlorpromazine sulphoxide *in vitro* has been demonstrated also. Demethylation of chlorpromazine by rats both *in vivo*,[8] and *in vitro*,[9] and the presence of the mono-demethylated derivatives of promazine and promazine sulphoxide in the urine of promazine-dosed dogs,[3] have been reported. That chlorpromazine sulphoxide and the corresponding mono- and di-methylated derivatives are urinary excretion products of humans receiving chlorpromazine has been shown recently by Fishman and Goldenberg[10, 11] and during the course of the present work.

In addition, conjugation with glucuronic acid has been presumed because of the release of less-polar phenothiazine derivatives after incubation with β-glucuronidase of urine specimens (or extracts thereof) obtained after chlorpromazine,[12–15] methotrimeprazine,[13, 14] and thioridazine[6] administration. Concomitant release of glucuronic acid from chlorpromazine metabolites also occurs under these conditions.[12] There is also evidence that the related non-phenothiazine derivative imipramine is excreted as glucuronide conjugates of 2-hydroxy-imipramine and its monodemethylated derivative.[16, 17]

Claims have been made for the presence of a free radical intermediary metabolite of chlorpromazine in human urine[18, 19] and, more recently, that N-oxides are minor urinary excretion products of chlorpromazine[20] and imipramine[21] in humans. The metabolism of unsubstituted phenothiazine, which is used as a veterinary anthelmintic, is relevant to the present study and has been reviewed by Clare.[22]

Glucuronic acid conjugates appear to form a major proportion of the total urinary metabolites of chlorpromazine and promazine although these compounds had not been isolated. This paper reports the successful isolation of some of these glucuronic acid

* This work forms part of the Ph.D. thesis of M. A. Beaven, London University, 1962.

conjugates and the characterisation of some other metabolites of chlorpromazine present in the urine of psychiatric patients.

MATERIALS

Urine specimens

Twenty-four hour urine collections from male patients receiving 150–600 mg chlorpromazine hydrochloride orally per day as sole medication were obtained from Warlingham Park Hospital, Surrey. Volunteers previously untreated with phenothiazines provided control specimens and 48 hr specimens after a single oral dose of 100 mg of chlorpromazine hydrochloride.

Reference compounds

Chlorpromazine, chlorpromazine sulphoxide and chlorpromazine sulphone and their respective N-desmono- and N-desdimethyl derivatives, all as hydrochloride salts, 2-hydroxypromazine maleate and 2-methoxypromazine were obtained through the courtesy of Smith Kline and French Laboratories, Philadelphia; promazine hydrochloride was kindly supplied by John Wyeth and Brother, Havant.

APPARATUS

Spectrophotometers

A Hilger Uvispek H 700 spectrophotometer was used for quantitative determinations and a Beckman DK 2 recording spectrophotometer was used for general qualitative work. The infrared spectrum was recorded on a Unicam SP 200 spectrophotometer.

METHODS

Paper chromatography

This was carried out on Whatman 3MM paper by downward development for 18 hr at 19 $\pm$ 2° in (1) ethylene dichloride: benzene: formic acid (88%): water (3:1:4:2, by volume) for non-polar metabolites[23] or (2) isoamyl alcohol: water: ethanol: formic acid (88%) (10:10:1·5:1, by volume)[12] for polar metabolites. A third solvent system containing benzene: acetic acid: water (2:2:1, by volume) was also used for the chromatography of non-polar metabolites. Circular chromatograms were also used. The dried chromatograms were examined in ultraviolet light and sprayed with or dipped in one or more of:

(i) concentrated hydrochloric acid containing a trace of sodium nitrite;[24]
(ii) 50% v/v sulphuric acid[2];
(iii) a solution of ferric chloride (1% w/v $FeCl_3 . 6H_2O$) in nitric acid (2·5 N);[10]
(iv) a solution of 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH, 0·35% w/v in water) followed by ferric chloride solution (0·6% w/v).[25–27]

Those reference compounds containing a sulphide sulphur atom gave positive reactions with all of these reagents; the sulphoxides gave positive reactions with all reagents except (iii); the sulphones gave a positive reaction with reagent (iv) only. The fluorescence properties varied with the oxidation state of the sulphur atom.

Other chromatograms were sprayed with:

(i) ninhydrin solution (0·1% w/v in n-butanol) to detect primary and secondary amines;
(ii) a solution of sodium nitroprusside (2% w/v) in aqueous acetaldehyde (10% v/v) followed by sodium carbonate solution (2% w/v), a specific test for secondary amines.[28]

Dragendorff reagent and platinic iodide reagent were also used but were found to be non-specific for amino groups since phenothiazine and N-ethyl phenothiazine also gave positive tests. Additional evidence for the presence of an amino group was always obtained, e.g. the solubility characteristics and the ability of the compound to form a complex with methyl orange.

R_f values, where quoted, are expressed as percentages.

Paper electrophoresis

The electrolyte solution, pH 1·9, contained glacial acetic acid (58 ml), formic acid (88%, 27 ml) and water (to 2 l.). The sample was streaked onto Whatman 3 MM paper and a current of 2m.amps/5 cm of paper applied for 12 hr after which time the paper was dried.

ASSAY METHODS

Glucuronic acid

The modified naphtharesorcinol method attributed to Paul[29] and described by Mead, Smith and Williams[30] was used with pure D-glucuronolactone as the reference substance.

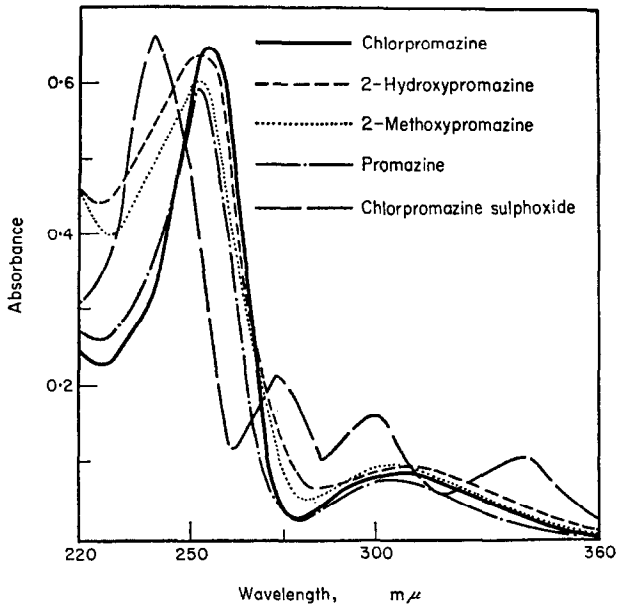


FIG. 1. Ultraviolet absorption spectra of equimolar aqueous solutions of various phenothiazine derivatives.

"Phenothiazine equivalencies" of metabolite fractions

As there are no acceptable reference compounds available against which some of the drug metabolites or extracts could be assayed, three independent assay procedures were adopted to estimate the phenothiazine content of such samples:

(1) The "phenothiazine content" was estimated spectrophotometrically assuming that the extinction coefficients of the metabolites are similar to that of chlorpromazine because it was found that the extinction coefficients of many related phenothiazine derivatives are of the same order (of approximately 33,000 in aqueous solution, see Fig. 1.)

(2) The "phenothiazine content" was also estimated by measuring the maximum extinction of a metabolite solution after colour development with an equal volume of concentrated sulphuric acid, assuming that the extinction coefficients of the coloured products from chlorpromazine and the metabolites would be similar. Reproducible results could be obtained by this method provided that the manipulations were standardised. The extinctions of the final solutions were proportional to the chlorpromazine (for instance) concentration in the initial solution of up to 1×10^{-7} mole/ml. The wavelengths of the absorption peaks varied between compounds as illustrated in Fig. 2, e.g. promazine gave 510 mμ, chlorpromazine 530 mμ, 2-hydroxypromazine 550 mμ and 2-methoxypromazine 565 mμ, but the maximum extinctions achieved using equimolar solutions varied only within 5 per cent.

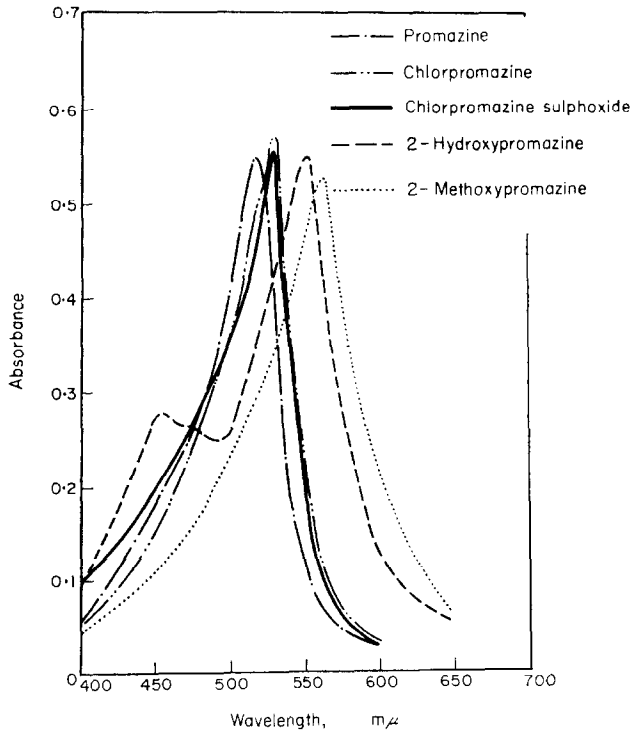


FIG. 2. Absorption spectra of solutions of various phenothiazine derivatives (all 10^{-7} mole/ml) after the addition of an equal volume of concentrated sulphuric acid under standardised conditions.

(3) The "amine content" was estimated using the methyl orange technique developed by Brodie and co-workers.[31] Glucuronic acid conjugates were first hydrolysed with acid and then neutralised. This procedure was used only when other tests indicated the absence of extraneous amines.

QUANTITATIVE ESTIMATIONS OF CHLORPROMAZINE METABOLITE FRACTIONS IN URINE

Total "phenothiazine content" of urine

Aliquot portions of urine (10 ml) were heated with hydrochloric acid (5 ml of concentrated) for 1 hr in a boiling water bath. The samples were cooled, made alkaline with concentrated ammonia solution (0·90) and extracted as below. The acid extract was made alkaline and the procedure repeated in order to reduce the non-specific background absorption found after prolonged hydrolysis. Spectrophotometric and colorimetric estimations (see above) were carried out on the final acid solutions.

The total "bound" chlorpromazine metabolites in urine were determined by applying this procedure to the urine residues obtained after ether extraction of the free chlorpromazine metabolites (cf. following section).

Free chlorpromazine metabolites

The urine was made alkaline (pH 9–10) with dilute ammonium hydroxide solution and extracted with ether (reagent grade, peroxide-free) which had been washed with normal sodium hydroxide solution, normal hydrochloric acid and water. The combined ether extracts were washed and the bases re-extracted into hydrochloric acid (0·1 N). The chlorpromazine and chlorpromazine sulphoxide concentrations were calculated from a single absorption curve of the extract following the method of Flanagan and others.[24] The total free "phenothiazine content" was estimated also by the sulphuric acid procedure.

"Chlorpromazine glucuronide" content of urine

Samples of urine (10 ml) were incubated with β-glucuronidase (see below) and the released phenothiazine derivatives extracted and assayed as described above.

Acid-labile chlorpromazine conjugates in urine

Samples of urine (10 ml) were heated with dilute hydrochloric acid (5 ml) in a boiling water bath for 7 min. After cooling, the samples were extracted and assayed as before. The results were compared with those obtained by incubating the urine with aryl sulphatase after removing the inhibiting ions (see below).

ENZYMIC HYDROLYSES

β-Glucuronidase hydrolysis

An equal volume of McIlvaine buffer, pH 4·5, was added to a urine sample or glucuronide solution (usually 10 ml) together with 30 mg (approximately 2,000 units) of β-glucuronidase (Sigma Chemical Co., St. Louis) and a drop of chloroform. The solution was incubated at 37° for 24 hr.

Aryl sulphatase incubation

Inhibiting substances were removed from urine by the method of Dodgson and Spencer.[32] After adjusting the pH value of the urine to pH 5·5 and adding an equal volume of acetate buffer (0·5 M, pH 5·5) and arylsulphatase, the mixture was incubated at 37° for 24 hr. The arylsulphatase was prepared[32] from a sample of acetone-dried limpet viscera kindly supplied by Dr. K. S. Dodgson and was freed from β-glucuronidase activity before use.

EXAMINATION OF CHLORPROMAZINE METABOLITES

Extraction of chlorpromazine metabolites from urine

It was found that both the non-polar and the polar metabolites of chlorpromazine in urine could be extracted into "wet" butanol. When water was removed from the solvent extract by distillation, the polar metabolite fraction was precipitated while the non-polar fraction remained in solution.

Thus:

The urine samples were pooled (15–18 l.), allowed to stand for several hours and then decanted into a 20 l. glass aspirator. The urine was saturated with n-butanol and then extracted continuously for at least 48 hr. Solvent (2–3 ml/min) was fed into the urine from a reservoir through a sintered glass plate (No. 4) and the fine globules dispersed by magnetic stirring. The butanol extract (7–10 l.) was aspirated continuously and after standing several hours was decanted off from any urine carried over and evaporated under reduced pressure (water pump) to about one fifth of its original volume. An amorphous powder (polar metabolites) separated out and was filtered off, washed with butanol and sodium-dried ether and dried in a vacuum desiccator. If the filter cake was allowed to dry during the washing procedure the solid became oily. The product was a pale yellow water-soluble powder.

This product was devoid of pharmacological effects when oral doses of up to 100 mg/kg were administered to rats.[33]

Partial purification of the crude polar metabolite fraction

The dry powder contained chlorpromazine derivatives and gave a positive test for glucuronic acid with naphtharescorcinol. Inorganic salts, urine pigments and other normal polar constituents of urine were also present and were removed almost completely as follows: the crude material was dissolved in dimethyl formamide and the pH adjusted to 5 with ethanolic sulphuric acid (2% w/v H_2SO_4 in dry ethanol). The solution was dried (anhydrous magnesium sulphate), filtered and the insoluble residue washed free from phenothiazine derivatives. Precipitation of the metabolite from solution was achieved by dropwise addition of an equal volume of dry ethanol followed by by an excess of dry ether. The suspension was centrifuged and the residue washed several times with ether. A cream-coloured powder was obtained when the last traces of dimethyl formamide had been removed.

Using urine from psychiatric patients the overall yields were 30–50 mg of product per 24 hr specimen. The phenothiazine content was 40–45 per cent by weight expressed as chlorpromazine base and was present as conjugates with glucuronic acid. The chief contaminants were urine pigments and a small proportion of inorganic material.

Tentative identification of the polar metabolites

The chromatographic characteristics of the polar metabolite fraction are shown in Table 1. In addition to the spot tests, β-glucuronidase hydrolysis of the eluate from each spot was accompanied by the release of ether-extractable phenothiazine derivatives. The ultraviolet absorption spectra of aqueous solutions of the eluates of spots number 1, 2, 3 and 4 were similar to chlorpromazine (Figs. 1 and 3) while that of spot number 5 was similar to chlorpromazine sulphoxide (Fig. 1). The slow-moving spots, numbers 4 and 5 were contaminated with yellow urinary pigments which

tended to interfere with the colours produced by the reagents.

From these results the following structural assignments were made:

Spot number (Metabolite number)	*Structure*
1	Chlorpromazine glucuronide
2	N-Desmonomethyl chlorpromazine glucuronide(s)
3	N-Desdimethyl chlorpromazine glucuronide(s)
4*	As number 3
5*	N-Desdimethyl chlorpromazine sulphoxide glucuronide(s)

The N-desdimethyl chlorpromazine glucuronide(s) predominated.

TABLE 1. CHROMATOGRAPHY OF CRUDE POLAR METABOLITES AND THEIR CORRESPONDING AGLYCONES

Crude Polar Metabolites Solvent system 2			Aglycones Solvent system 1
Metabolite (Spot) No.	R_f	Shape	Distance of spot from origin (cm)
1	33		6·7
2	17		
3	10		0·3
4	6*		
5	3		
Mixture			6·5 1·9 0·2
Markers: N-desdimethylchlorpromazine sulphoxide			0·3
N-desmonomethylchlorpromazine sulphoxide			3·0
chlorpromazine sulphoxide			7·0

For colour reactions, see Table 2.

* This spot was associated with the trailing edge of the spot having an R_f value of 10.

Chromatographic separation of the glucuronide metabolites

A concentrated aqueous solution containing 200 mg of crude glucuronides was streaked onto sixteen sheets of Whatman 3MM paper (23 × 48 cm) by means of a

* These spots were heavily contaminated with urinary pigments.

UNO stencil pen (No. 0). The chromatograms were developed in solvent 2 in subdued light for 24 hr, dried in air and the zones localised by means of test strips. The corresponding zones were cut out, padded together and eluted with distilled water in an atmosphere of nitrogen. The eluates were filtered and evaporated (26°) under reduced pressure in nitrogen. Butanol was added initially to assist distillation and 15 ml was added when the fluid volume was reduced to 5 ml. The metabolites (except No. 5, see later) precipitated out on further evaporation. The precipitates were removed by centrifuging, washed with a little dry ethanol and dried in a vacuum desiccator. The yields were 8 mg of metabolite 1 and 110 mg of metabolite 3.

Enzymic hydrolysis of glucuronide metabolites

Separate solutions of the partially purified polar metabolite fraction, metabolite 1 and metabolite 3 were incubated with β-glucuronidase. The resulting aglycones were extracted into ether after making the solution alkaline with ammonia and chromatographed in solvent 1 (see Table 1). Three spots were obtained on chromatography, probably corresponding with those from metabolites 1, 2 and 3. Ultraviolet absorption spectra of the aglycone eluates were similar to those of 2-methoxypromazine and to those of the parent glucuronides, see Fig. 3. Variations on changing the pH values of the solutions were small as also is the case with 2-hydroxypromazine.

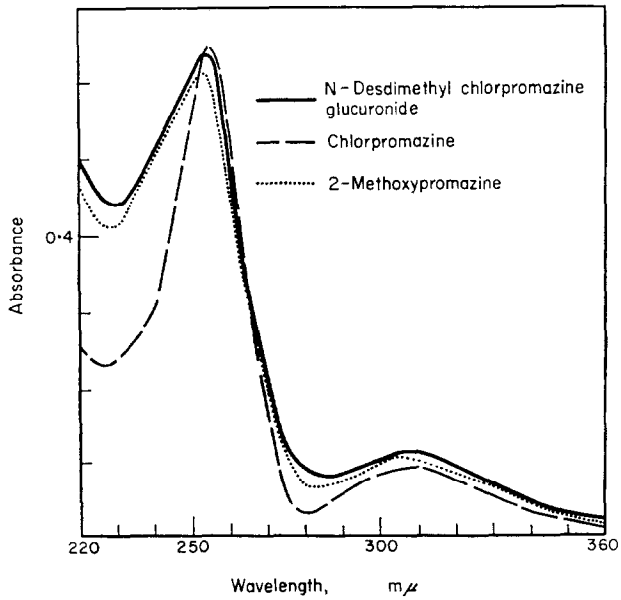


FIG. 3. Ultraviolet absorption spectra of equimolar aqueous solutions of N-desdimethylchlorpromazine glucuronide, chlorpromazine and 2-methoxypromazine.

Specific spray reagents for the detection of aromatic hydroxyl groupings failed to react with 2-hydroxypromazine under alkaline conditions. 2-Hydroxypromazine is more easily oxidised than the non-hydroxylated derivatives and could be detected, as were the aglycones, as dark grey spots on chromatograms sprayed with Tollen's reagent at concentrations at which other phenothiazine derivatives failed to react.

An attempt to isolate the aglycone from 10 mg of metabolite 1 yielded several milligrams of small red crystals which immediately darkened in colour on exposure to air and could not be characterised further.

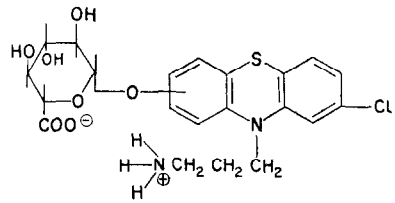
PURIFICATION OF THE GLUCURONIC ACID CONJUGATES

N-Desdimethylchlorpromazine glucuronide(s) (*Metabolite No.* 3)

Purification. This fraction was invariably contaminated with urine pigments which could not be removed either by charcoal treatment or by chromatographic separation in a number of solvent systems. Better resolution was obtained when an aqueous solution of the fraction (50 mg) was streaked onto three sheets of Whatman 3 MM paper and subjected to paper electrophoresis. The papers were dried in a subdued light and the zones located. Three bands were obtained at 12, 14 and 15 cm from the origin. The band at 14 cm gave a phenothiazine-positive reaction but was contaminated with pigment and that at 15 cm appeared to consist of a phenothiazine oxidation product. The remaining band (12 cm) was eluted with water and precipitated as described above for the chromatography. The metabolite (22 mg) was purified further by dissolving in dimethyl formamide (0·2 ml) and adding dry ethanol (1·5 ml) dropwise during several days to yield an amorphous white powder (14 mg). Only one phenothiazine-positive spot was obtained on chromatography of this material in a number of solvent systems [e.g. n-butanol–ethanol–water (15:2:5, by volume); isopropanol–pyridine–water (3:1:1, by volume)].

Properties. The metabolite was very soluble in water, dilute acids and alkalis, moderately soluble in dimethyl formamide and dimethyl sulphoxide, slightly soluble in glacial acetic acid and insoluble in the common organic solvents. On heating in a capillary tube the metabolite softened and decomposed to form a dark, viscous oil at 203–5° (uncorr.). The reactions of the metabolite to the various spot test reagents are summarised in Table 2. The equivalent weight by non-aqueous titration using a 5 mg sample was found to be 493 (calculated for N-desdimethylchlorpromazine monoglucuronide dihydrate 518); detection of the end-point of the titration is difficult and accounts for the unduly large error in this instance. Elementary analysis gave C 48·6, H 4·93 and N 5·26; $C_{21}H_{23}O_7N_2SCl,2H_2O$ requires C 48·8, H 5·20 and N 5·42.

The infrared absorption spectrum (Fig. 4) was predominantly that of the glucuronic acid moiety and was generally similar to that of morphine glucuronide reported by Fujimoto and Way[35] except for the presence of an additional peak at 12·3 μ. The compound probably exists as a zwitter ion in the solid state (see I) because the infrared spectrum indicates the presence of an ionised carbonyl group.



I

Chlorpromazine glucuronide(s) (*Metabolite No.* 1)

This metabolite was separated paper chromatographically and obtained as an amorphous white powder contaminated with some inorganic material. The solubility and

ultraviolet absorption properties of this metabolite were similar to those of the N-desdimethylchlorpromazine glucuronide(s). Insufficient material was available for elementary analysis; the metabolite has been tentatively identified as the monoglucuronide of a hydroxylated derivative of chlorpromazine on the basis of the results summarised in Table 2.

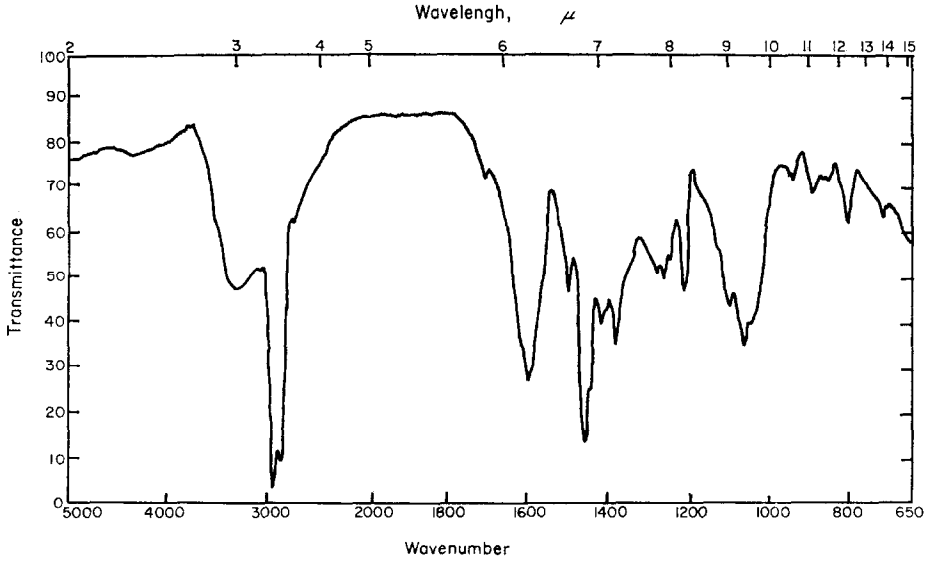


Fig. 4. Infrared spectrum of N-desdimethylchlorpromazine glucuronide (Nujol mull).

N-Desdimethylchlorpromazine sulphoxide glucuronide(s) (*Metabolite No.* 5)

Complete evaporation of the eluate from paper chromatographic separation of the glucuronide metabolites after removal of the precipitated metabolites (Nos. 1 and 3) yielded 5 mg of a water-soluble brown oily material of doubtful purity. The reactions of this metabolite are summarised in Table 2.

The ultraviolet absorption spectrum of an aqueous eluate from a paper chromatogram was similar to that of chlorpromzine sulphoxide (Fig. 1). This metabolite is considered to be a monoglucuronide (see Table 2) of a hydroxylated derivative of N-desdimethylchlorpromazine sulphoxide.

INVESTIGATION OF THE NON-POLAR METABOLITES

The butanol residues from the extracts of a total volume of 35 l. of urine were evaporated under reduced pressure to a volume of 1 l. and filtered. After partial extraction of a portion (100 ml) of the filtrate of the concentrate with hydrochloric acid (0·1 N) and re-extraction into ethylene dichloride from ammoniacal solution and chromatography in solvent 1, four phenothiazine-positive spots were obtained. The paper chromatographic characteristics of these substances were indistinguishable from N-desdimethyl and N-desmonomethylchlorpromazine sulphoxides, chlorpromazine sulphoxide and chlorpromazine.

TABLE 2. SUMMARY OF THE CHARACTERISTICS OF THE POLAR METABOLITES. FOR PRACTICAL DETAILS OF TESTS AND ASSAY PROCEDURES SEE TEXT

			Metabolite 1	Metabolite 2	Metabolite 3	Metabolite 5
Amine tests	Ninhydrin		−ve	+ve	+ve	+ve
	Dragendorff		+ve	+ve	+ve	+ve
	Nitroprusside		−ve	+ve	−ve	−ve
Phenothiazine tests	Ferric chloride		red	red	+ve	−ve
	$HCl/NaNO_2$				pink/magenta	+ve
	50% v/v H_2SO_4				magenta	
Tests for glucuronic acid	Naphtharesorcinol		+ve	+ve	+ve	+ve
	Benedict's solution		−ve		−ve	
Ultraviolet absorption data	UV absorption peaks (aqueous solutions) in mμ	major	254	254	254	
		minor	307	306	306	
Quantitative estimations	Nominal solution mg/ml		81		89	260
	concentration	mole/ml	0·159		0·185	
	glucuronic acid	mole/ml	0·150		0·193	0·52
	phenothiazine in mole/ml	1. Spectro.	0·146		0·184	0·50
		2. H_2SO_4	0·155		0·175	0·46
		3. Methyl orange	0·161		0·180	—
Ratio: phenothiazine: glucuronic acid			1:1		1:1	1:1
	Structural assignment		chlorpromazine glucuronide	N-desmonomethyl chlorpromazine glucuronide	N-desdimethyl chlorpromazine glucuronide	N-desdimethyl chlorpromazine sulphoxide glucuronide

The remaining filtered butanol extract was evaporated to yield a red-coloured oil (about 10 g). A chloroform solution of 5 g of the oil was applied to an alumina column (Type H, Peter Spence and Sons; 2 × 20 cm) and eluted with chloroform (125 ml) followed by dry ethanol (125 ml).

Two coloured bands were eluted with chloroform. One yielded 5 mg of a pungent orange-coloured oil which was insoluble in dilute solutions of acids and alkalis, soluble in most organic solvents and gave precipitates with 2,4-dinitrophenylhydrazine and sodium bisulphite and restored the colour to Shiff's reagent. Paper chromatography of the oil in solvent 3 gave a single phenothiazine spot (purple with HCl/$NaNO_2$) having an R_f value of 69. It would appear that this compound may have resulted from the deamination of chlorpromazine.

A brown-coloured band eluted with ethanol yielded 79 mg of oily material on evaporation which gave a single phenothiazine spot (R_f4) when chromatographed in solvent 3. Mild acid hydrolysis (N HCl at 100° for 5 min) caused liberation of ionic sulphate (precipitate with barium chloride) and ether-extractable phenothiazine derivatives having R_f values of 11 and 23 in solvent 3. The ultraviolet absorption spectrum of a dilute acid solution of the liberated phenothiazine(s) showed a major peak at 254 mμ and a minor peak at 304 mμ.

Acid-labile phenothiazine conjugates

In addition to the acid-labile conjugates separated chromatographically from the butanol residues, similar materials were present in fresh urine specimens from which free chlorpromazine metabolites had been removed. Dilute hydrochloric acid was added to urine to give a final hydrochloric acid concentration of 3 per cent by weight and the mixture heated in a boiling water bath for 5 min. Paper chromatography using solvent 1 of the ether-extractable phenothiazine derivatives released during hydrolysis gave three spots having R_f values and reactions similar to those of the aglycones obtained after β-glucuronidase incubation of the glucuronide metabolites, i.e. the primary, secondary and tertiary amines. Mild acid hydrolysis of a urine specimen from which free metabolites and those liberated by β-glucuronidase incubation had been extracted yielded additional ether-extractable phenothiazine derivatives.

Further evidence that ethereal sulphates are excreted in urine was obtained by incubation studies with aryl sulphatase. After incubation, the phenothiazine derivatives were extracted into ether and re-extracted into dilute hydrochloric acid (0·1 N). The ultraviolet absorption spectrum was similar to that of chlorpromazine. Similar quantities of phenothiazine derivatives were released by a mild acid hydrolysis and by aryl sulphatase incubation. Control urine specimens to which the N-desdimethylchlorpromazine glucuronide (to about 5 μg/ml) had been added were virtually unaffected by either of these procedures.

QUANTITATIVE ASPECTS OF CHLORPROMAZINE METABOLISM

The total phenothiazine metabolites excreted in the urine of normal patients within 48 hr of the administration of a single oral dose of chlorpromazine hydrochloride (100 mg) account for only 7 per cent of the dose. Of the total phenothiazine metabolites, approximately 25 per cent were ether-extractable, approximately 60 per cent were liberated by β-glucuronidase incubation and approximately 19 per cent were released

by aryl sulphatase incubation or by mild acid hydrolysis. Reports on various types of psychotic patients will be reported subsequently.

ULTRAVIOLET IRRADIATION STUDIES

Following a claim by Forrest and co-workers[18] that a compound obtained by irradiation of chlorpromazine by ultraviolet light was identical with a urinary metabolite, an aqueous solution of chlorpromazine hydrochloride was irradiated for four hours at room temperature. The ultraviolet lamp (Hanovia, fluorescence model 16) was held at a height of 10 cm above the surface of the liquid (500 ml, surface area 86 cm^2) which was magnetically stirred. No irradiation product obtained from this system other than chlorpromazine sulphoxide was comparable with any of the urinary metabolites. Preliminary work has shown that on irradiation of chlorpromazine, as the sulphate, dimerisation and dehalogenation will occur.

DISCUSSION

Investigations of the metabolism of chlorpromazine are hampered by the lack of suitable reference compounds. It was considered necessary, therefore, to devise several analytical methods for the estimation of the phenothiazine content of urine samples, extracts and solutions when significance would only be attached to the results if reasonable agreement between the different methods was demonstrated. Such correlation was obtained in all the work reported in this paper.

The lack of the various position isomers of a monohydroxylated analogue of chlorpromazine and the corresponding primary amine as reference compounds has made it impossible, so far, to confirm the exact location of the hydroxyl group of the aglycone of the N-desdimethylchlorpromazine glucuronide obtained as the major urinary metabolite of chlorpromazine in man. Nuclear magnetic resonance studies are in hand which may provide evidence of the point of attachment of the glucuronic acid moiety to the phenothiazine nucleus.

Isolation of chlorpromazine glucuronides from human urine presented a problem as no simple method was available for the isolation of small amounts of water-soluble drug conjugates from relatively large volumes of urine. The butanol extraction procedure provided "crude" chlorpromazine glucuronides in a better yield and state of purity than was achieved by any of the other methods tried. No pH adjustment of the urine was necessary and the butanol acted as a preservative.

Purification of the crude glucuronide mixture presented some difficulties because of contamination with yellow urinary pigments. The contaminants are thought to include the glucuronides of bilirubin,[34] the urobilinogens and the urobilins and perhaps also the ethereal sulphates of these substances. The nitrogen content of the crude drug glucuronide mixtures was higher than was expected on the basis of the phenothiazine content. It seems likely that similar contamination will occur with the glucuronides of other amine drugs isolated from urine and, in fact, Fujimoto and Way[35] have suggested that morphine glucuronide is strongly associated with unknown substances in urine.

The overall picture of the human metabolism of chlorpromazine as indicated by the urinary excretion products is that the compound undergoes demethylation, sulphoxidation, hydroxylation and conjugation with glucuronic acid and sulphate in the body. The predominant metabolite is N-desdimethylchlorpromazine glucuronide. A

scheme for the metabolism of chlorpromazine is illustrated in Fig. 5. Non-biological production of sulphoxide conjugates (from the sulphides) was unlikely since the pure glucuronides isolated were stable on chromatography. The products obtained after mild acid hydrolysis or by incubation with aryl sulphatase possibly correspond with the aglycones obtained after β-glucuronidase treatment of the glucuronides. Thus, it is probable that hydroxylated metabolites are conjugated with both glucuronic acid and sulphate in man. Trace amounts of neutral metabolites, possibly as aldehydes produced by deamination of the sidechain, are also excreted but unequivocal evidence of their chemical identity is lacking.

The relative proportions of the sulphide and sulphoxide derivatives in the free and conjugated fractions of the metabolites were very different: the free chlorpromazine metabolites were mainly sulphoxides and the conjugates mainly sulphides. Sulphoxidation and hydroxylation of foreign compounds in the body may occur independently at different enzymic sites or, alternatively, the former may precede the latter. *In vitro*

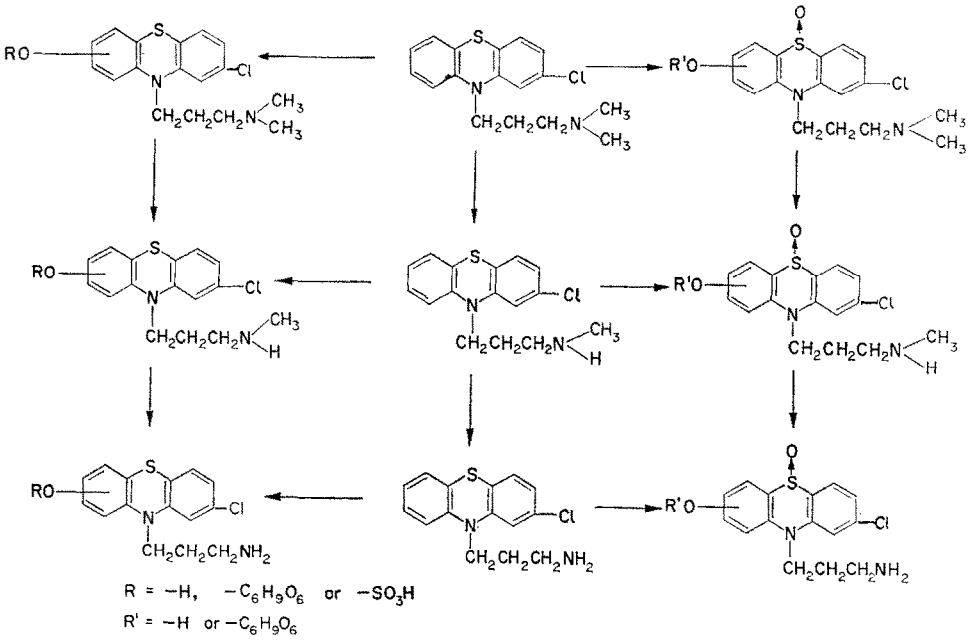


FIG. 5. Tentative scheme for the metabolism of chlorpromazine in man.

inhibition studies with β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A) on the oxidative microsomal reactions,[7] lend support to the independence of the two reactions. However, it has been postulated that a hydroxy phenothiazine derivative may be produced by intramolecular rearrangement of the sulphoxide, for example, the conversion of phenothiazine sulphoxide to 3-hydroxyphenothiazine.[36] Clare[22] has also suggested that phenothiazine sulphoxide formed in the intestine of sheep and calves from phenothiazine is metabolised in the liver to phenothiazone/leuco phenothiazone. It may be noted also that the R_f value of the major glucuronide metabolite of chlorpromazine in humans, an unoxidised sulphide, was similar to that

reported for the polar metabolite in dog urine after administration of chlorpromazine sulphoxide.[2]

The percentage of an orally administered dose of chlorpromazine excreted in the urine as phenothiazine derivatives is low. Nadeau and Sobolewski[14] recovered 5–20 per cent of the daily doses in the urine of patients and in the present study approximately 7 per cent was recovered from a normal patient within 48 hr after a single dose. Non-phenothiazine metabolites have not been considered although ^{35}S-inorganic sulphate has been reported as a metabolite of ^{35}S-labelled chlorpromazine in mice.[37] The low levels of urinary metabolites of chlorpromazine may be due to predominantly biliary excretion of the metabolites as occurs in other species or alternatively to poor absorption of the drug from the gastrointestinal tract or to poor reabsorption of the glucuronide metabolites excreted in the bile. The latter would be affected by the β-glucuronidase activity of the large intestine and the transport mechanisms involved in the large intestine and the transport mechanisms involved in the transfer of these compounds from the intestinal lumen to the plasma. It is also possible that either chlorpromazine or its metabolites may be chemically altered by the action of the natural intestinal flora with consequent effect upon the biological distribution of the compounds. Further work is necessary to elucidate these aspects of chlorpromazine metabolism.

The metabolism of chlorpromazine follows the common pathways for the metabolism of foreign compounds by the body; however, formation of a mercapturic acid derivative seems unlikely since the phenothiazine content of the urine can be otherwise accounted for after a single dose. The increased polarity of the metabolites may be reflected in the diminished pharmacological activity compared with that of the parent drug. The demethylated analogues of chlorpromazine have less sedative action than chlorpromazine[38] and the glucuronides were found to have negligible activity.[33] Chlorpromazine sulphoxide differs quantitatively and qualitatively in its pharmacological action, having only a weak sedative action in dogs[1] and man[39] and is thought to contribute little to the overall activity of chlorpromazine. The pharmacological action of the drug cannot be attributed to any one metabolite especially as the actual location of the site of the pharmacological effect is uncertain and many factors will influence the accessibility of the drug or its metabolites to this site.

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