Chlorpromazine Metabolism II: Determination of Nonconjugated Metabolites in Blood of Schizophrenie Patients

PUSHKAR N. KAUL▲, MICHAEL W. CONWAY, MAHARAJ K. TICKU, and MERVIN L. CLARK

Abstract □ A previously described dansylation method of quantitation was applied to determine the circulating levels of chlorpromazine metabolites in humans. Localization of the metabolites within the erythrocytes suggests that the whole hemolyzed blood be used for analyses. An optimum procedure of assay was developed which makes it possible, for the first time, to determine 11 metabolites in blood simultaneously. Blood level data from hospitalized chronic schizophrenic patients receiving variable doses of chlorpromazine are included.

Keyphrases □ Chlorpromazine metabolism—determination of 11 nonconjugated metabolites in the blood of schizophrenic patients, TLC, fluorometry □ Schizophrenics' chlorpromazine blood levels—determination of 11 nonconjugated metabolites, TLC, fluorometry □ TLC—separation □ Fluorometry—analysis, chlorpromazine metabolites in blood

Chlorpromazine has been in clinical use for 20 years, but the determination of its metabolites in the blood of schizophrenic patients has remained a difficult and elusive problem. This may in part be attributed to a lack, until recently, of assay methods sensitive enough to determine the low blood levels of the metabolites resulting after the usual chlorpromazine dosage. Methods developed by Curry and Brodie (1) and by Efron *et al.* (2) are capable of determining chlorpromazine and its three or four nonpolar metabolites. The mass fragmentographic method of Hammar and Holmstedt (3) using GLC coupled with mass spectrometry has shown applicability also to only a few metabolites, although chlorpromazine is known to develop more than 20 metabolites in the body. Furthermore, it is too elaborate and expensive for clinical adoption. The dansylation method (4, 5) based on photofluorometric determination, however, is capable of quantitating 11 chlorpromazine metabolites (6) in a relatively routine manner.

This paper describes some problems relevant to isolation of the metabolites prior to quantitation which might well have been additional reasons for the general failure in the attempted quantitations of circulating chlorpromazine metabolites. The optimum methodology is detailed which makes it possible, for the first time, to determine 11 chlorpromazine metabolites in a 6-ml. blood sample. Blood level data from patients receiving chlorpromazine therapy are also included.

EXPERIMENTAL

Materials—The various chlorpromazine metabolites used to develop the methodology are included in Table I. The solvents used were dimethyl sulfoxide, dioxane, cyclohexane, chloroform, tetrahydrofuran, propanol, benzene, acetone, ethyl acetate, dichloromethane, heptane, isoamyl alcohol, *n*-butanol, and *tert*-butanol, all being of either spectrograde or nanograde quality. An ultrasonic vibrator (Blackstone), fitted with a BP-2 probe and an SS-2 generator, was used to rupture the erythrocytes prior to the extraction of metabolites from the whole blood. Polyethylene terephthalate TLC plates (100 μ, Eastman) coated with silica gel without fluorescent indicator were used for fractionation of dansylated metabolites. Filter fluorometers (Turner models 110 and 111) fitted with high sensitivity doors were used for fluorescence determinations.

Methods—The individual metabolites were quantitated by the previously described dansylation procedure (6). In a few analyses, where large and known concentrations of a metabolite were used to study a set of parameters such as extractibility into solvents or the effect of shaking on the extractibility, the quantitation was carried out by reacting the extracted metabolite with 5% ferric chloride solution in 50% sulfuric acid and subsequent spectrophotometric estimation at 528 nm. The individual concentrations of various metabolites in the blood of patients were obtained by following the *Procedure of Assay*.

Procedure of Assay—(*a*) A 3-ml. unknown blood sample in a screw-necked glass centrifuge tube is adjusted to pH 9.5 with 1 *N* sodium hydroxide solution. After adding 9 ml. benzene, the tube is capped with a Teflon-coated screw cap and mechanically shaken at 150 c.p.m. for 15 min. to extract the demethylated metabolites and their 7-hydroxy congeners, as well as the 7- and 8-hydroxy chlorpromazines. The tube is centrifuged at 2000 r.p.m. for 5 min., and 7 ml. of the organic extract is transferred to another tube. An additional 5 ml. of benzene is added for the second extraction, and 5 ml. of the extract is transferred to the tube containing the first extract. The combined extracts (12 ml.) are evaporated to dryness under nitrogen at 45° and treated with 0.25 ml. of 0.5 *M* phosphate buffer at pH 12 and 0.25 ml. of dansyl chloride solution (2.5 mg./ml.) in dioxane. The reaction tube is capped and incubated at 45° for 30 min. After cooling to room temperature, the reaction mixture is extracted with 2 ml. of ethyl acetate. The extract is evaporated to

Table I—Extractibility of Various Chlorpromazine Metabolites from Blood Based on Single Extraction into Various Organic Solvents

Metabolite Compound	μMoles Added	Percent Recovered —by Organic Solvent[a]— Benzene	Ethyl Acetate	Propanol in Benzene
7-Hydroxy chlorpromazine	0.1493	81.0	53.0	17.5
8-Hydroxy chlorpromazine	0.1493	92.5	40.0	12.0
Monodesmethyl chlorpromazine	—	—	—	—
Didesmethyl chlorpromazine	0.1547	72.4	51.7	25.8
7-Hydroxy chlorpromazine sulfoxide	0.1424	19.0	40.5	75.0
Monodesmethyl chlorpromazine sulfoxide	—	—	—	—
Didesmethyl chlorpromazine sulfoxide	0.1466	73.6	66.2	85.0
7-Hydroxymonodesmethyl chlorpromazine	—	—	—	—
7-Hydroxydidesmethyl chlorpromazine	0.1629	61.2	63.3	22.0
7-Hydroxymonodesmethyl chlorpromazine sulfoxide	0.1484	—	40.0	61.9
7-Hydroxydidesmethyl chlorpromazine sulfoxide	0.1548	4.0	23.0	46.0

[a] The volume of organic phase used was three times the blood volume.

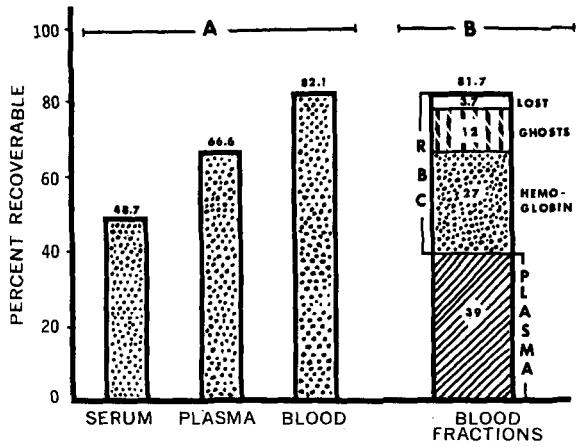

Figure 1—*Comparative recoveries of added chlorpromazine metabolites from serum, plasma, whole blood, and blood fractions. Key: A, didesmethyl chlorpromazine; and B, monodesmethyl chlorpromazine.*

dryness under nitrogen and reconstituted in 0.1 ml. of ethyl acetate for spotting on the TLC plates.

(*b*) Another 3-ml. blood sample is processed likewise, but benzene–propanol (7:3), instead of benzene, is used to extract all the sulfoxides.

For separation of all the dansylated metabolites, 10–20 μl. of the final ethyl acetate solutions from each of the two tubes (*a* and *b*) is spotted on three plates. One plate, spotted with (*a*), is developed in benzene–cyclohexane–methanol (80:20:1) to fractionate the demethylated metabolites and their hydroxy derivatives. The second plate, spotted with (*b*), is developed with benzene–cyclohexane–ethyl acetate (1:1:3) to fractionate all sulfoxides except one. The third plate, spotted separately with (*a*) as well as (*b*), is developed with benzene–acetone (2:3) to separate the 7- and 8-hydroxy chlorpromazines in the (*a*)-spot and the 7-hydroxy chlorpromazine sulfoxide in the (*b*)-spot. Also spotted on the plates are the appropriate blanks and a mixture of known amounts of dansylated standard metabolites. The standard spots serve as a control to check the deviation from the standard curves obtained by processing known amounts of the metabolites added to blood through the entire procedure. The individual spots are cut out, eluted in dioxane, and subjected to fluorescence analysis.

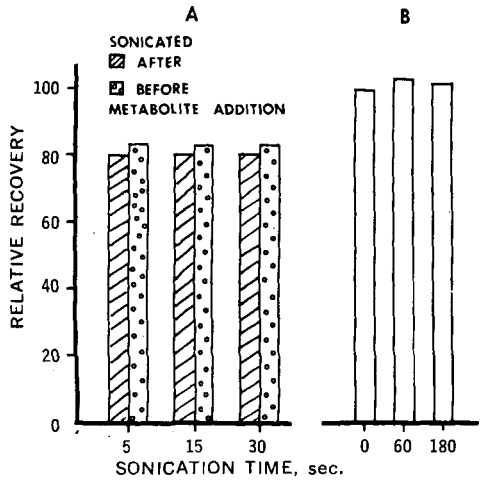

Figure 2—*Effect of sonication on the stability of free and conjugated chlorpromazine metabolites. Key: A, monodesmethyl chlorpromazine sulfoxide added to blood before and after sonication for varying times; and B, recovery at various sonication times of free 7-hydroxymonodesmethyl chlorpromazine in patient urine also containing the conjugated form of the same metabolite. Any hydrolysis due to sonic energy would have yielded significantly higher recoveries after sonication.*

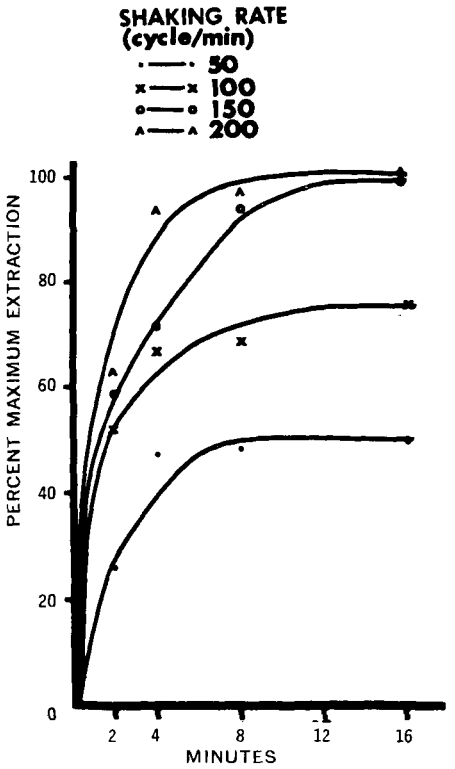

Figure 3—*Influence of rate and duration of shaking on the extractibility of 7-hydroxydidesmethyl chlorpromazine.*

Patient Data—The final test of the developed methodology was carried out by applying the procedure of assay to unknown blood samples obtained from chronic schizophrenics receiving chlorpromazine therapy. The total daily doses of 400–1500 mg. were administered in divided amounts twice daily, and the blood samples for analyses were withdrawn 2 hr. after the morning dose.

RESULTS AND DISCUSSION

Development of Assay Procedures—Satisfactory single-extraction recoveries (>80%) of nonpolar metabolites were obtained from plasma as well as serum to which known amounts of metabolites had been added. However, a drug and its metabolites are distributed in the whole blood rather than the serum or plasma alone. When known amounts of didesmethyl chlorpromazine were added to the whole blood *in vitro* and incubated at 37° for 30 min., the aliquots of plasma as well as serum (prepared by adding calcium and thrombin) obtained from this blood yielded on extraction only 66.6 and 48.7% recoveries, respectively (Fig. 1). Compared to these values, an equivalent volume of the whole blood, which was equal to only half the volume from which the plasma or serum was obtained, on extraction yielded 82.1% recovery of the added metabolite.

Erythrocyte Localization—When plasma and erythrocytes of a 10-ml. blood sample preincubated with monodesmethyl chlorpromazine were separately analyzed, 39% was recovered from the plasma and 42.7% from the erythrocytes. The combined total recovery matched well with the recovery obtained by extracting 10 ml. of the whole blood, which was 83%. When erythrocytes of a 10-ml. blood sample were isolated, washed, hemolyzed, and fractionated into hemoglobin and ghosts for separate analyses, 27% of the total amount recoverable from the whole blood was found in the hemoglobin (cell contents). The ghosts, washed three times with distilled water, yielded an additional 12% on analysis. The unaccountable 4% was perhaps lost in the washings which were not analyzed. The results of the recoveries from the various blood fractions are shown in Fig. 1.

Sonication—In view of the possibility that some proportion of the chlorpromazine metabolites circulating in the patient's blood might be lodged in the erythrocytes, the whole human blood hemolyzed by

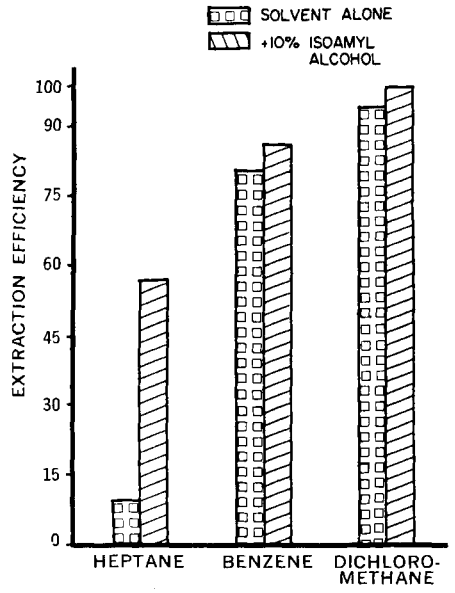

Figure 4—*Relative extractibilities of total nonconjugated chlorpromazine metabolites (mostly various demethylated compounds) from the urine of a patient receiving 600-mg. daily oral dose, as shown by heptane, benzene, and dichloromethane, with and without the inclusion of isoamyl alcohol. Spectrophotometric method described under* Methods *was used for assay.*

sonication following metabolite addition was used to study the recoveries of added metabolites. Sonic energy used to hemolyze the red cells did not appear to destroy the metabolite molecules or hydrolyze their conjugates. Also, the duration of sonication had no detectable effect on the extractibility of the metabolites from the blood. Figure 2 represents the data supporting these conclusions.

Extractibility of Metabolites—The manner and rate of shaking during immiscible solvent extraction in the case of chlorpromazine metabolites appeared to be more critical than one usually encounters in dealing with the organic compounds. Shaking on a Vortex shaker, used previously in preliminary studies on biologic fluids (6), caused emulsification and erratic extraction. Horizontal mechanical shaking at 150 c.p.m. for 15 min. was found adequate for consistent extractibility with the least emulsification difficulties (Fig. 3).

A study of the comparative extraction efficiency of various organic solvents revealed that one of the reasons for failures in quantitating the chlorpromazine metabolites in the blood may well have been the use of unsatisfactory organic solvents for extracting the metabolites.

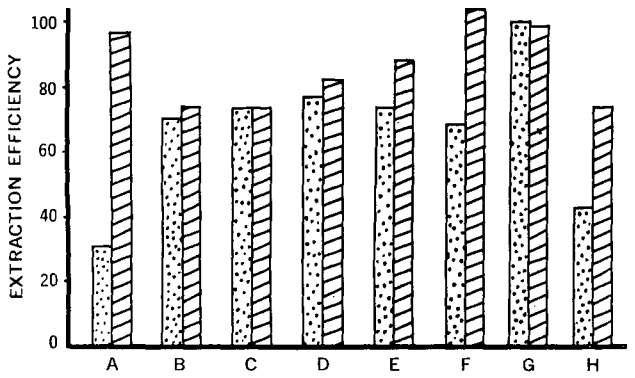

Figure 5—*Extractibility of chlorpromazine metabolites from aqueous solutions of known concentrations as shown by dichloromethane, A, alone and in combination with various polar adjuvants: B, 15% isoamyl alcohol; C, 30% isoamyl alcohol; D, 15% n-butanol; E, 15% tert-butanol; F, 15% ethanol; G, 15% n-propanol; and H, 15% dimethyl sulfoxide. Key: ▭, 7-hydroxymonodesmethyl chlorpromazine sulfoxide; and ▨, 7-hydroxymonodesmethyl chlorpromazine.*

Table II—Effect of Solvent on Recovery following Reconstitution prior to TLC Spotting of the Evaporated Ethyl Acetate Extract of the Dansylated Metabolite—Dansyl-7-hydroxydidesmethyl Chlorpromazine

Reconstitution Solvent (100 μl.)	Percent Maximum Recovered	Inference
Benzene	62.0 90.0 67.0	Poor and erratic
	$\bar{X}$ = 73.0	
Dioxane	81.0 76.0 84.0	Poor
	$\bar{X}$ = 80.3	
Ethyl acetate	98.0 102.0 100.0	Satisfactory and consistent
	$\bar{X}$ = 100.0	

Figure 4 indicates that heptane alone or mixed with isoamyl alcohol (1–3) is a poor solvent for extracting nonconjugated demethylated metabolites. For relatively more polar metabolites, such as the hydroxy sulfoxides in aqueous solutions or urine, inclusion of 5% isoamyl alcohol in dichloromethane has been recommended (7), but Fig. 5 shows that 30% isoamyl alcohol is not as good as 15% *n*-propanol.

While dichloromethane is a suitable solvent for extracting chlorpromazine metabolites from aqueous solutions and urine, it proved unsuitable for the blood. Screening a number of organic solvents which are practically useful for blood revealed that benzene and ethyl acetate were suitable for extracting the demethylated compounds, but benzene was better for the hydroxy compounds. For the sulfoxides, however, inclusion of 30% *n*-propanol in benzene was found necessary. Table I gives the percent extractibility of various

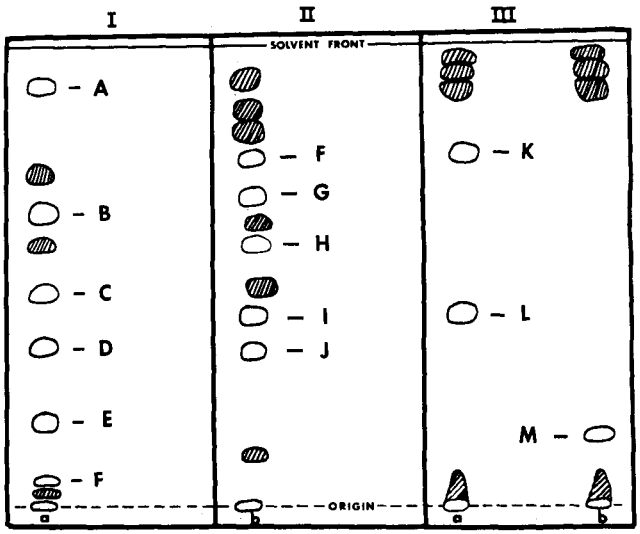

Figure 6—*Overlays representing separation of various dansylated chlorpromazine metabolites on thin-layer chromatograms developed in optimum solvent systems: I, benzene–cyclohexane–methanol (80:20:1); II, benzene–cyclohexane–ethyl acetate (1:1:3); and III, benzene–acetone (2:3). Key: a, benzene extract of the blood; b, propanol–benzene extract of the blood; ▨, various unknown fluorescing spots due to reagent and blood blanks; ○, various known fluorescing dansylate derivatives of A, dansyl chloride; B, monodesmethyl chlorpromazine; C, 7-hydroxymonodesmethyl chlorpromazine; D, didesmethyl chlorpromazine; E, 7-hydroxydidesmethyl chlorpromazine; F, dansylamide; G, 7-hydroxymonodesmethyl chlorpromazine sulfoxide; H, 7-hydroxydidesmethyl chlorpromazine sulfoxide; I, monodesmethyl chlorpromazine sulfoxide; J, didesmethyl chlorpromazine sulfoxide; K, 8-hydroxy chlorpromazine; L, 7-hydroxy chlorpromazine; and M, 7-hydroxy chlorpromazine sulfoxide.*

Table III—Metabolite Concentrations (Nanograms per Milliliter Blood) of Chronic Schizophrenic Patients Receiving Chlorpromazine

Metabolite	Patient F.S. 400 mg. MWF	B.A. 400 mg. Daily	I.G. 500 mg. Daily	J.H. 600 mg. Daily	C.D. 600 mg. Daily	B.G. 900 mg. Daily	C.H. 1000 mg. Daily	L.M. 1500 mg. Daily
Monodesmethyl chlorpromazine	32	25	6	7	25	8	83	53
Didesmethyl chlorpromazine	83	67	104	50	67	37	71	40
Monodesmethyl chlorpromazine sulfoxide	28	ND[a]	7	22	ND	30	25	133
Didesmethyl chlorpromazine sulfoxide	ND	ND	10	47	ND	50	30	162
7-Hydroxymonodesmethyl chlorpromazine	ND	83	71	81	88	92	167	421
7-Hydroxydidesmethyl chlorpromazine	42	73	108	43	41	170	98	503
7-Hydroxymonodesmethyl chlorpromazine sulfoxide	87	ND	ND	3	ND	ND	ND	13
7-Hydroxydidesmethyl chlorpromazine sulfoxide	67	ND	ND	ND	156	83	115	ND
7-Hydroxy chlorpromazine	ND	107	ND	66	213	160	83	108
7-Hydroxy chlorpromazine sulfoxide	ND	50	ND	ND	53	37	46	23

[a] ND = nondetectable quantity, which is less than 3–30 ng. of the metabolites, depending upon the quantum yields of their dansylates; the hydroxy chlorpromazine dansylates show the lowest yield.

metabolites from the blood into organic solvents, based on single-extraction recoveries. The three metabolites on which the recovery data are not included in the table were found in other experiments to yield good recoveries (>70%) in benzene and/or benzene–propanol mixture.

Dansylation Reaction—Quantitation of relatively higher concentrations of chlorpromazine metabolites in urine, serum, and plasma according to the previously described single-extraction and dansylation procedure (6) offers no special difficulty. However, in dealing with human blood containing relatively low concentrations of the metabolites, additional extractions as well as spotting of a larger volume of the dansylation mixture were necessary. This, however, yielded blank dansylated spots, which appeared at the same place on the TLC plates as the monodesmethyl chlorpromazine. In addition, the 7-hydroxy and the sulfoxide derivatives of monodesmethyl chlorpromazine were also somewhat obscured. These interfering blank spots were not present in the original dansyl chloride reagent but appeared during the dansylation reaction. They could not be eliminated by changing the buffer type in the reaction mixture. Use of dioxane as the reaction solvent yielded fewer interfering spots as compared to acetone (8). The decrease in the dansylation time also reduced the intensity of the interfering spots.

Further refinement was achieved when the dansylation mixture (0.5 ml.) at the end of the reaction was treated with ethyl acetate to extract the dansylated metabolites relatively free from salts and any other solids which otherwise interfered in the quantitative spotting procedure. Although 1 ml. of ethyl acetate gave maximum extraction, usually 2 ml. was added and the same volume was removed (2.25 ml. available) for evaporation under nitrogen and reconstitution in the smallest possible volume (0.1 ml.) such that a maximum amount could be spotted. No detectable loss was observed during the evaporation process. Of the various solvents tested for optimum and precise reconstitution, ethyl acetate yielded the best results as evidenced by reproducible data; dioxane and benzene were poor and erratic (Table II).

Chromatographic Fractionation—The interfering reagent spots were finally moved to a different R_f value by the use of appropriate developing solvents, making it possible to quantitate all the metabolites without any interference. Of the 35 solvent systems tested, the following appeared to be the best for optimum fractionation: benzene–cyclohexane–methanol (80:20:1) for the demethylated compounds and their hydroxy derivatives, benzene–cyclohexane–ethyl acetate (1:1:3) for the demethylated sulfoxides and their hydroxy derivatives, and benzene–acetone (2:3) for the hydroxy chlorpromazine derivatives and their sulfoxides. Figure 6 represents overlays of the chromatograms, showing separation of various dansylated derivatives of chlorpromazine metabolites in the optimum solvent systems employed in these studies.

Estimation of Fluorescence—The influence of eluting solvents on the quantum yields of fluorescing substances was reported (9, 10). From the data in Fig. 7 representing the relative fluorescence yields in different solvents, it can be seen that the dansylated metabolites yield higher fluorescence values in dioxane than in benzene or methanol. This is in line with the reported findings on other compounds (9, 10) insofar as dioxane is concerned but not benzene.

Standard Curve—Figure 8 includes representative plots of the dansyl derivatives of a few metabolites, showing the blood concen-

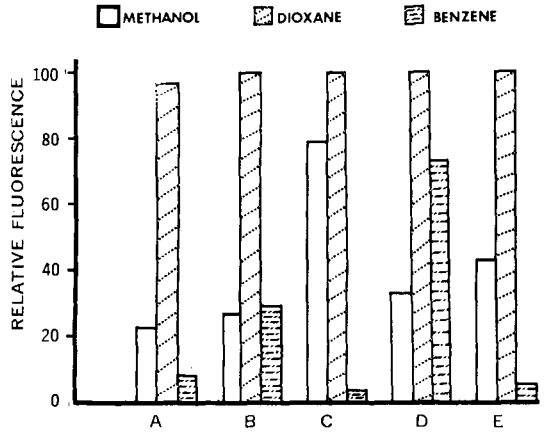

Figure 7—*Influence of eluting solvents on the fluorescence yields of various dansylated products of: A, 7-hydroxy chlorpromazine; B, didesmethyl chlorpromazine; C, didesmethyl chlorpromazine sulfoxide; D, 7-hydroxydidesmethyl chlorpromazine; and E, 7-hydroxydidesmethyl chlorpromazine sulfoxide.*

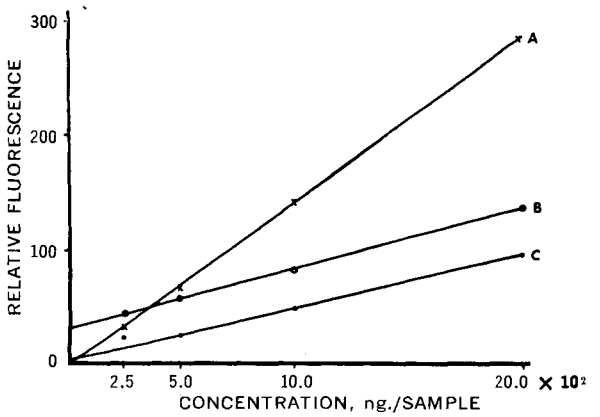

Figure 8—*Standard plots for representative metabolites added to the blood and carried through the* Procedure of Assay. *Key: A, 7-hydroxymonodesmethyl chlorpromazine; B, 7-hydroxydidesmethyl chlorpromazine sulfoxide; and C, 7-hydroxy chlorpromazine sulfoxide.*

tration–fluorescence correlation over a range of metabolite concentrations. Replicate analyses in most cases yielded values within ±5%. The total procedure shows a reliable precision, if the usual accuracy and practices warranted for fluorometric work are observed.

For quantitation of circulating levels of chlorpromazine and its metabolites attempted so far (1–3), either plasma or serum has been generally used. However, the present investigations indicate that at least the demethylated metabolites, when incubated with the whole blood, partially localize in the erythrocytes. The use of the whole hemolyzed blood for extraction of the metabolites is, therefore, necessary, and sonication appears most suitable for effecting the hemolysis. Although the extent to which different metabolites localize in the erythrocytes may vary with the type and its circulating concentration, assaying the whole blood should nonetheless yield higher recoveries of the otherwise low amounts of metabolites normally present in patient blood following chlorpromazine administration.

Sonication and its duration had no detectable effect on the stability of the metabolite molecules. Their extractibility was also not decreased, which might be expected to occur due to the possibility of increased adsorption (11), since the sonicated samples would contain a larger number of macromolecules resulting from disintegration of the blood cells.

Variations in the polarities of chlorpromazine metabolites, and thereby their extractibility patterns, appear to be important. The optimum extraction of all the sulfoxides was possible only by using a benzene–propanol (7:3) mixture. To obtain maximum recovery of the metabolites present in the blood, double extraction was used for both the sulfoxides and the nonsulfoxides, which are best extracted into benzene. Attempts to combine the benzene extract and the benzene–propanol extract for subsequent steps of the *Procedure of Assay* invariably yielded losses in the benzene extractible metabolites, *i.e.*, the demethylated and their hydroxy compounds. That is why it was necessary to evaporate and dansylate the two extracts separately.

Further investigation of this problem of loss revealed that the benzene–propanol mixture, required to extract the sulfoxides from the blood, also extracted some nonphenothiazine material which was apparently responsible for the loss of metabolites contained in the benzene extract. This interfering material appeared to be proteinaceous in nature, since treatment of the benzene–propanol extract with trichloroacetic acid considerably alleviated the loss. Although trichloroacetic acid helped remove the interfering proteinaceous material, separate processing of the two 3-ml. blood samples was preferred since phenothiazines are known to be sensitive to acid pH. Sulfoxides did not undergo this loss during the evaporation process.

All steps in the *Procedure of Assay* were adopted after examining the influence of various parameters involved. The composition of solvent systems for TLC development is very critical. For example, varying the methanol proportion by as little as 0.25% in the benzene–cyclohexane–methanol (80:20:1) mixture gave considerable differences in the fractionation patterns of the dansylated metabolites *versus* the blank spots. The three solvent systems used to enable effective separation and final quantitation of all 11 dansylated chlorpromazine metabolites were arrived at after screening 35 solvent systems.

Patient Data—Unknown blood samples from chronic schizophrenics receiving chlorpromazine therapy were assayed by the procedure of assay for their metabolite concentrations. The total daily doses of 400–1500 mg. were administered in divided amounts twice daily, and the blood samples for analyses were obtained 2 hr. after the morning dose. Table III gives the concentration of various metabolites found in the samples. None of the samples investigated contained a detectable amount of the 8-hydroxy chlorpromazine metabolite.

SUMMARY

Several hitherto unresolved problems encountered in isolating and quantitating chlorpromazine metabolites in the blood of schizophrenic patients were investigated. Optimum procedure of assay using only a 6-ml. blood sample was developed. This has made it possible, for the first time, to quantitate circulating levels of 11 chlorpromazine metabolites. The methodology is particularly useful for adoption in clinical laboratories.

REFERENCES

(1) S. H. Curry and B. B. Brodie, *Fed. Proc.*, **26**, 761(1967).

(2) D. H. Efron, L. E. Gaudette, and S. R. Harris, *Agressologie*, **9**, 103(1968).

(3) C. G. Hammar and B. Holmstedt, *Anal. Biochem.*, **25**, 532 (1968).

(4) P. N. Kaul, M. W. Conway, and M. L. Clark, *Nature*, **226**, 372(1970).

(5) I. S. Forrest, S. D. Rose, L. G. Brookes, B. Malpern, V. A. Bacon, and I. A. Silberg, *Agressologie*, **11**, 127(1970).

(6) P. N. Kaul, M. W. Conway, M. L. Clark, and J. F. Huffine, *J. Pharm. Sci.*, **59**, 1745(1970).

(7) D. E. Johnson and C. F. Rodrigues, reported in *Psychopharmacol. Bull.*, **6**, 44(1970).

(8) "Progress in TLC and Related Methods," vol. 1, A. Niederwieser and G. Pataki, Eds., Humphrey Science Publishers, Ann Arbor, Mich., 1970, pp. 95–144.

(9) R. F. Chen, *Arch. Biochem. Biophys.*, **120**, 609(1967).

(10) N. Seiler and M. Wiechmann, *Z. Anal. Chem.*, **220**, 109 (1966).

(11) D. L. Sorby, E. M. Plein, and J. D. Benmaman, *J. Pharm. Sci.*, **55**, 785(1966).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 13, 1971, from the *College of Pharmacy, University of Oklahoma; Division of Clinical Pharmacology, Department of Medicine, University of Oklahoma Medical Center; and Central State Griffin Memorial Hospital, Norman, OK 73069*

Accepted for publication December 20, 1971.

Supported by NIMH Grant MH 11666-06 and by research funds of the Oklahoma University Research Institute.

The authors thank Dr. A. Manian of the National Institute of Mental Health for supplying the test samples of various chlorpromazine metabolites, and they also appreciate the technical assistance rendered by Sheila Whittle, Luke San, and Craig Darby.

▲ To whom inquiries should be directed.