M. J. Sweeney, G. A. Poore, and S. H. Larsen, *ibid.*, **21**, 463(1968).
(7) S. B. Carter, T. J. Franklin, D. F. Jones, B. J. Leonard, S. D. Mills, R. W. Turner, and W. B. Turner, *Nature*, **223**, 848(1969).
(8) D. N. Planterose, *J. Gen. Virol.*, **4**, 629(1969).
(9) R. H. Williams, L. D. Boeck, J. C. Cline, D. C. DeLong, K. Gerzon, R. S. Gordee, M. Gorman, R. E. Holmes, S. H. Larsen, D. H. Lively, T. R. Mathews, J. D. Nelson, G. A. Poore, W. M. Stark, and M. J. Sweeney, *Antimicrob. Ag. Chemother.*, **1968**, 229.
(10) F. E. Gainer and R. L. Hussey, *J. Chromatogr.*, **54**, 446 (1971).
(11) F. E. Gainer and H. J. Wesselman, *J. Pharm. Sci.*, **59**, 1157 (1970).
(12) T. Noto, M. Sawada, K. Ando, and K. Koyama, *J. Antibiot.*, **22**, 165(1969).
(13) F. Kavanaugh, *Advan. Appl. Microbiol.*, **2**, 65(1960).
(14) T. Noto, Y. Harada, and K. Koyama, *J. Antibiot. (Tokyo)*, **23**, 96(1970).
(15) P. W. Clutterbuck and H. Raistrick, *Biochem. J.*, **27**, 654 (1933).
(16) J. H. Birkinshaw, A. Bracken, E. N. Morgan, and H. Raistrick, *ibid.*, **43**, 216(1948).

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Chlorpromazine Metabolism V: Disposition of Free and Conjugated Metabolites in Blood Fractions of Schizophrenic Patients

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

Abstract □ Disposition of the circulating levels of 10 chlorpromazine metabolites and their conjugates in the blood fractions of chronic schizophrenic patients was determined. The erythrocytes were found to sequester nearly 50% of the total blood concentration of the free metabolites, whereas the platelets contained only insignificant amounts. Of particular significance was the localization of the conjugated metabolites in the red cells. The implications of sequestration of the metabolites and their conjugates by the erythrocytes are discussed.

Keyphrases □ Chlorpromazine metabolism—disposition of free and conjugated metabolites in blood fractions of schizophrenic patients □ Erythrocyte sequestration—disposition of free and conjugated metabolites of chlorpromazine in blood of schizophrenic patients □ Blood distribution, erythrocyte sequestration—chlorpromazine metabolites, schizophrenic patients □ Schizophrenics' chlorpromazine blood distribution—disposition of free and conjugated metabolites, erythrocyte sequestration

Although metabolism of chlorpromazine has been studied extensively in the past decade (1–5), very little is known about the disposition of the drug and/or its metabolites in the blood. Only recently has it been possible to determine the blood levels of a majority of the metabolites (6). Earlier failures in consistently quantitating the circulating levels of the metabolites have been, at least in part, due to nonavailability of sensitive and precise assay methodology. Another possible reason for such failures might have been that investigators used plasma or serum samples for measuring the blood levels, which would tend to be low if the blood cells sequestered the drug and its metabolites.

For clinical studies correlating therapeutically available levels of a drug (and/or its metabolites) with its administered dose, it is important to consider the total concentration in the whole blood rather than only in the plasma. Both the protein-bound and the free (nonprotein-bound) drug molecules present in the cells as well as the plasma usually exist in a steady-state equilibrium of the type proposed in Fig. 1. The extent of delivery of the therapeutically active molecules (drug and/or its metabolites) at the biophase is dependent on the free plasma levels. The duration of this delivery, however, should be dependent not only on the free plasma levels but also on the bound plasma levels as well as on the free and bound concentrations localized in the blood cells. Therefore, to study the contribution of the blood cells in monitoring the therapeutic availability of a drug and/or its metabolites, a detailed disposition of these molecules in the blood fractions is necessary.

Huang and Ruskin (2) could not detect any chlorpromazine-like material in erythrocytes of patients receiving chlorpromazine. Hammar and Holmsted (3) also failed to detect any nonconjugated metabolites in the erythrocytes. Zingales (7), however, was the first to detect qualitatively the conjugated nonphenolic metabolites of chlorpromazine in the plasma and in erythrocyte washings and hemolysates, but to date no quantitative data have been reported.

In previous studies dealing with the development of the dansylation assay (8) and its application to blood (6),

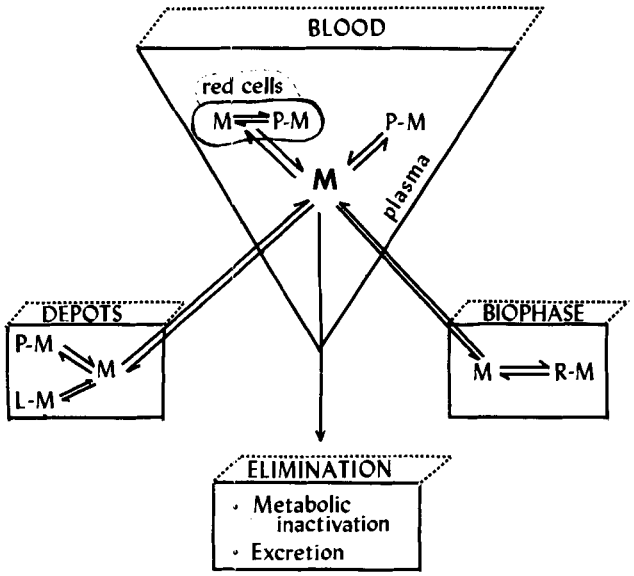

Figure 1—*Steady-state equilibrium of therapeutically active molecules (M) in the body. Key: P-M, the protein-bound molecules in plasma, cells, or other tissues; L-M, the lipid-bound or dissolved molecules; and R-M, the biophase receptor-complexed molecules.*

the demethylated metabolites were studied and found to be significantly localized in the erythrocytes. It was suggested that a possibility existed also for the other metabolites to be sequestered by the blood cells (6).

This paper reports on the distribution of both the free and the conjugated metabolites of chlorpromazine in various blood fractions. Attempts to quantitate the metabolites localized in the leukocytes failed, since it was not possible to separate accurately these cells from erythrocytes. The available fractionation method (9) invariably caused hemolysis. However, the platelets, although separable, contained only insignificant quantities of various metabolites.

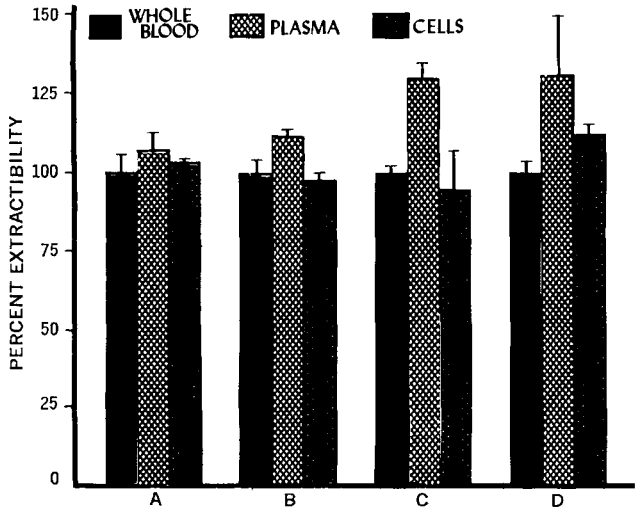

Figure 2—*Relative extractibility of various chlorpromazine metabolites from the whole blood, plasma, and cells. Key: A, didesmethyl; B, 7-hydroxydidesmethyl; C, 7-hydroxymonodesmethyl sulfoxide; and D, monodesmethyl sulfoxide. All are derivatives of chlorpromazine. Recoveries from 3 ml. blood were taken as 100%. The observed higher recoveries from the plasma fractions may be due to the presence of less protein content in these fractions prepared in the manner described in the* Methods *section.*

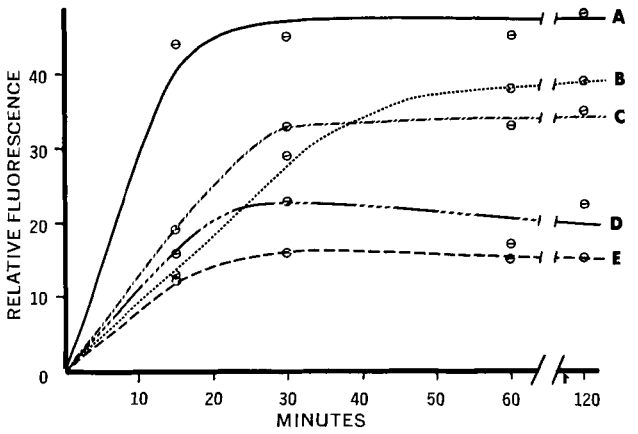

Figure 3—*Sequestration with time of various metabolites of chlorpromazine by the blood cells. Key: A, 7-hydroxy; B, didesmethyl; C, 7-hydroxydidesmethyl; D, monodesmethyl sulfoxide; and E, 7-hydroxymonodesmethyl sulfoxide. All are derivatives of chlorpromazine.*

EXPERIMENTAL

Materials and Methods—The standard metabolites, analytical and other equipment, solvents, *etc.*, used were those described previously (6). An enzyme solution[1] was used as the source of β-glucuronidase to hydrolyze the conjugated metabolites. The blood samples used for studying the distribution of added chlorpromazine metabolites were collected in EDTA Vacutainer tubes from healthy human volunteers and used immediately. The unknown blood samples were obtained from chronic schizophrenic patients receiving a 400–1600-mg. total daily dose of oral chlorpromazine divided into a morning dose and an evening dose. The samples were collected 2 hr. after the morning dose. All analyses on these samples were carried out in duplicate.

Processing of Blood and Assay—The free (nonconjugated) metabolites of chlorpromazine were determined by an earlier described method (6). For quantitation of the conjugated metabolites, separate aliquots were enzymatically hydrolyzed under standard conditions[2] and the liberated metabolites were determined (6) as the total in that sample. The difference between the total and the free levels gave the concentration of the conjugated metabolites.

Bulk blood samples (20–30 ml.), containing known amounts (usually 1–3 mcg./ml.) of added metabolites, were incubated at 37° for 30 min. in a shaker[3]. The incubated samples were centrifuged at 500 r.p.m. for 10 min. to separate the platelet-rich plasma from the erythrocytes and the leukocytes. Based on the hematocrit value of the original blood sample, aliquots of the separated plasma corresponding to 3 ml. whole blood were assayed to represent the metabolite content of plasma plus platelets. Also, a portion of the platelet-rich plasma was centrifuged at 2000 r.p.m. for 10 min. to separate the platelets. These were washed with saline, diluted appropriately with saline to yield a volume equal to the original platelet-rich plasma volume from which the platelets were obtained, and assayed in 3-ml. aliquots.

The erythrocytes and leukocytes that separated after the first centrifugation at 500 r.p.m. were recentrifuged at 2000 r.p.m. The packed cells were washed twice, resuspended in saline to yield the original blood volume, and assayed in 3-ml. aliquots. Since the localization of chlorpromazine metabolites in platelets was found to be relatively insignificant, in all subsequent experiments the blood samples were fractionated only into plasma and cells (erythrocytes, leukocytes, and platelets together).

The conjugated chlorpromazine metabolites, isolated from a patient's urine[4], were added to freshly collected blood and incubated at 37° for 1 hr. The blood was fractionated into cells and plasma, which were reconstituted as before and assayed for free and total metabolites.

[1] Glusulase, Endo.
[2] Details to be published.
[3] Dubnoff.
[4] By a method to be published.

Table I—Percent Recovery of Added Chlorpromazine Metabolites from Various Blood Fractions[a]

Metabolites[b]	Nanomoles Added	Percent Recovery[c] Plasma	Cells[d]	Platelets	Total
M-1	0.90	21.70	67.98	13.10	102.78
M-2	0.98	19.83	64.34	—	83.97
M-3	0.31	36.00	69.00	—	105.00
M-4	1.03	21.37	69.33	12.08	102.78
M-5	0.98	28.66	62.40	7.69	98.75
M-7	0.89	27.60	57.34	12.50	97.44
M-10	0.98	43.58	60.58	—	104.16
M-8	0.92	35.33	67.20	—	102.53
M-6	0.85	37.47	50.18	15.38	96.03

[a] Standard curves, used to determine the concentrations of the metabolites in the addition-recovery and unknown samples, also include the blood banks that were relatively high for the sulfoxides and low for the other metabolites. In all tables, nondetectable (ND) means less than 10 ng./ml. for the sulfoxides and the hydroxy chlorpromazine and less than 4 ng./ml. for the rest of the metabolites. [b] The metabolites of chlorpromazine are identified as follows: M-1, 7-hydroxy; M-2, monodesmethyl; M-3, 7-hydroxymonodesmethyl; M-4, didesmethyl; M-5, 7-hydroxydidesmethyl; M-6, 7-hydroxy sulfoxide; M-7, 7-hydroxymonodesmethyl sulfoxide; M-8, 7-hydroxydidesmethyl sulfoxide; M-9, monodesmethyl sulfoxide; and M-10, didesmethyl sulfoxide. [c] The recovery from 3 ml. whole blood was taken as 100%. [d] Cells represent erythrocytes and leukocytes.

The unknown blood samples from patients were fractionated similarly into cells and plasma. Each fraction was diluted with saline to the original blood volume (based on hematocrit value) and assayed in 3-ml. aliquots for free and total metabolites.

RESULTS

Extractibility from Blood Fractions—The relative extractibility of various chlorpromazine metabolites from blood and its fractions varied. Figure 2 shows the percent recovery of representative chlorpromazine metabolites from the whole blood, the plasma, and the cells. The solvents and conditions for extraction were those described earlier (6). In general, the order of extractibility was: plasma > cells > whole blood. The differential factors of extractibilities obtained in these experiments were used to correct the values of metabolite recoveries in all addition and unknown blood samples.

Time Course of Erythrocyte Sequestration—The added metabolites appear to combine with cells rapidly, reaching a saturation plateau within 15–30 min. (Fig. 3). Therefore the incubation time used in all addition experiments was 30 min.

Free Metabolite Localization—*Addition Experiments*—The percent recoveries of various chlorpromazine metabolites from plasma, cells (red and white), and platelets are given in Table I.

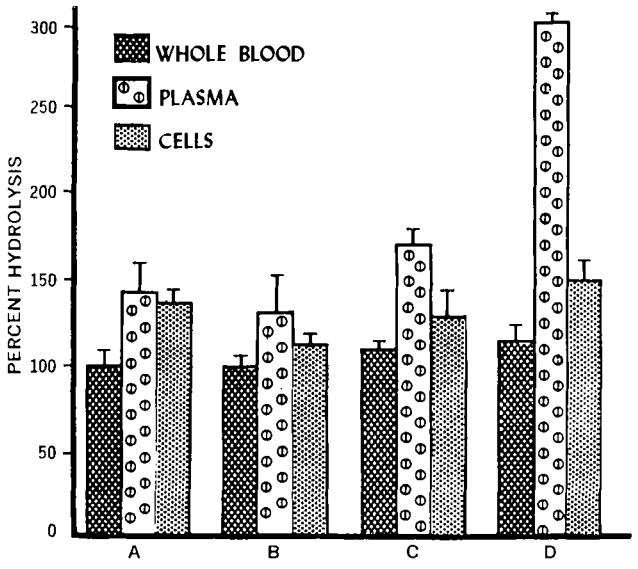

Figure 4—*Relative enzymatic hydrolyses of the conjugates of representative metabolites when present in the blood and its fractions. Key: A, 7-hydroxy; B, 7-hydroxydidesmethyl; C, 7-hydroxymonodesmethyl sulfoxide; and D, 7-hydroxydidesmethyl sulfoxide. All are conjugated derivatives of chlorpromazine.*

Significant concentrations (>50%) of the metabolites were localized in the cell fraction.

Patient Data—Table II includes the free metabolite concentrations found in whole blood, plasma, and cells of the blood samples obtained from chronic schizophrenic patients receiving oral chlorpromazine therapy. These results appear to be in agreement with the *in vitro* addition data. However, there seems to be no clear correlation between the administered dose of chlorpromazine and the levels of its metabolites.

Conjugated Metabolites—*Hydrolysis Rates*—Known amounts of the isolated conjugates of chlorpromazine metabolites (6) were added separately to the blood and its fractions (cells and plasma), and the enzymatic hydrolysis was performed on the samples. Figure 4 shows the relative degrees of hydrolyses of various representative conjugated metabolites of chlorpromazine when in plasma, cells, and whole blood. Generally, the order of hydrolyses rates appeared to be: plasma > cells > whole blood.

Addition Data—Table III gives the distribution of the added conjugates in the cells and plasma. All of the assayable conjugates were distributed in the cell fraction, but the relative proportions compared to the plasma concentrations were smaller in all cases.

Table II—Distribution of Free Chlorpromazine Metabolites in the Blood Fractions of Schizophrenic Patients[a]

Patient	Daily Dose, mg.	Sample	Metabolite[b] M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10
B.L.	400	Blood	ND[c]	ND	ND	150	333	ND	ND	82	34	36
		Plasma	ND	ND	ND	100	217	ND	ND	26	ND	ND
		Cells	ND	ND	ND	83	183	ND	ND	34	ND	ND
C.M.	600	Blood	400	ND	ND	183	426	ND	ND	234	41	70
		Plasma	50	ND	ND	83	213	ND	ND	46	ND	ND
		Cells	285	ND	ND	104	176	ND	ND	75	33	52
E.P.	1000	Blood	300	66	ND	229	92	140	ND	59	50	84
		Plasma	140	ND	ND	83	39	83	ND	ND	ND	ND
		Cells	165	ND	ND	133	46	50	ND	41	ND	ND
L.M.	1200	Blood	293	134	175	75	229	ND	55	42	50	44
		Plasma	100	42	71	22	117	ND	20	20	ND	ND
		Cells	150	108	108	50	140	ND	47	24	ND	ND
C.H.	1600	Blood	253	ND	233	392	250	433	ND	83	58	78
		Plasma	161	ND	92	125	117	200	ND	25	ND	ND
		Cells	200	ND	192	292	200	250	ND	50	42	53

[a] The values of plasma and cells are expressed so as to represent the concentrations present in nanograms in volumes of these blood fractions obtained from 1 ml. of blood. The values of whole blood are also those contained in 1-ml. volume. [b] See Table I, Footnote *b*, for identification of chlorpromazine metabolites. [c] ND = nondetectable quantity.

Table III—Distribution of Added Conjugates in Plasma and Packed Erythrocytes

Metabolite[a]	Concentration, mcg./ml.						Ratio of Conjugates (Plasma–Cells)
	Plasma			Cells (Packed Volume)			
	Free[b]	Total	Conjugate	Free	Total	Conjugate	
M-1	ND	5.000	5.000	ND	1.133	1.133	4.41
M-3	ND	5.250	5.250	ND	0.633	0.633	8.29
M-4	ND	0.999	0.999	ND	0.333	0.333	3.00
M-5	ND	8.333	8.333	ND	1.666	1.666	5.01
M-7	0.208	1.666	1.458	0.192	0.233	0.041	35.57
M-8	0.250	5.000	4.750	0.250	2.345	2.095	2.31
M-9	0.275	6.000	5.725	0.125	1.750	1.625	3.52
M-10	0.592	1.666	1.079	0.595	1.460	0.865	1.23

[a] See Table I, Footnote *b*, for identification of chlorpromazine metabolites. [b] Assay for free metabolites was necessary since the isolated conjugated metabolites used in these addition experiments invariably contained small amounts of free metabolites.

The plasma–cell concentration ratio varied for different conjugates but was always greater than one.

Patient Data—Table IV includes the data on disposition of the conjugated chlorpromazine metabolites in the blood fractions of chronic schizophrenic patients. Also included are the concentrations of the individual free metabolites. There appeared to be no correlation between the patient data and the *in vitro* addition data on the conjugated metabolites. Nor was there any apparent correlation between the dose of chlorpromazine given to the patient and the relative concentrations of the conjugates in the cells or plasma.

DISCUSSION

The detailed studies on the disposition of various chlorpromazine metabolites in the blood fractions presented in this paper further substantiate the previous contention (6) that the whole blood, rather than serum or plasma, should be used to determine the circulating levels of these metabolites. The contribution of metabolite sequestration by the blood cells toward the therapeutic availability of chlorpromazine and its metabolites is important, especially when evidence is accumulating that some metabolites of this drug are pharmacologically as active as the parent drug (10), if not more so.

The present data indicate a fairly large interpatient variation in the pattern of chlorpromazine biotransformation. One patient, for example, receiving 1000 mg. chlorpromazine daily showed negligible blood levels of the 7-hydroxymonodesmethyl metabolite; but two other patients, receiving 400- and 1600-mg. daily doses, contained fairly high concentrations of this metabolite in their blood (Table IV). Similar variations in the metabolic elimination rates of other drugs, such as phenylbutazone, dicumarol, and ethanol, have been well documented (11). However, it remains to be seen whether there is any correlation between these metabolic variations and the variations observed in the clinical response to chlorpromazine.

The presence of significant concentrations of free metabolites in the red cells indicates that there may be preferential binding sites in the cells, since on the basis of total protein content the plasma proteins are of much higher concentration than the cell proteins. For the same reason, the relative binding affinity of these cellular receptors for the metabolites appears to be higher than that of the plasma proteins to which at least chlorpromazine has been shown to be bound to an extent of 99% (12).

Since saline washings do not readily displace the metabolites from the cells, it appears that the metabolites either enter largely into the cells or, if adsorbed, are complexed with the cell membrane as proposed for chlorpromazine by Seeman (13, 14). The erythrocyte membrane structure, as observed under an electron microscope, remains largely intact even at the time of hemolysis (14). Therefore, during hemolysis the drug molecules, if incorporated into the membrane, would remain with the cell membrane fraction. If this membrane incorporation holds, the binding would be fairly strong so as to resist displacement by washing, but one would not expect any significant concentrations in the content of the red cells. In these studies, however, relatively high concentrations of the sequestered metabolites were found in the hemolysates. It is quite possible that chlorpromazine is localized only on the cell membrane, whereas metabolites find entry into the cell. Further studies to establish the exact location of chlorpromazine and its metabolites are currently in progress in these laboratories.

Maren *et al.* (15) observed that the carbonic anhydrase inhibitor, acetazolamide, required 1 hr. for optimum appearance in the erythrocytes. Since this enzyme is located inside the cell, the inhibitor may be presumed to have entered the cell. The acetazolamide concentration in the erythrocytes was independent of plasma concentration. Also, it could not be easily displaced from the cells by washing.

The chlorpromazine metabolites would appear to resemble acetazolamide because these compounds are also preferentially localized in the erythrocytes and not easily displaced by washings. However, the rate of their sequestration by the cells is faster (15–30 min.) than that of acetazolamide. Just how this entry into the cells takes place and the exact nature of binding are not known.

The conjugated metabolites were also found in the cell fraction. This is somewhat contrary to the general belief that conjugates, which are less lipid soluble, more polar, and therefore readily ex-

Table IV—Distribution of Free and Conjugated Chlorpromazine Metabolites in Nanograms per Milliliter of Plasma and Erythrocytes of Schizophrenic Patients Receiving Chlorpromazine

Metabolite[b]	Patient B.L.[a]				Patient E.P.				Patient C.H.			
	Plasma		Cells		Plasma		Cells		Plasma		Cells	
	Free	Conjugated[c]	Free	Conjugated	Free	Conjugated	Free	Conjugated	Free	Conjugated	Free	Conjugated
M-1	ND	ND	ND	ND	296	51	368	0	ND	ND	276	285
M-2	ND	ND	ND	ND	ND	ND	11	0	84	192	90	439
M-3	42	46	93	119	ND	ND	ND	ND	150	198	261	0
M-4	184	121	203	41	159	363	290	119	111	40	111	154
M-5	367	0	447	0	ND	247	109	304	999	339	965	378
M-6	ND	ND	ND	ND	154	43	91	39	ND	90	ND	15
M-7	ND	ND	ND	ND	ND	ND	ND	ND	ND	74	ND	90
M-8	30	12	41	162	ND	201	ND	ND	ND	ND	ND	ND
M-9	ND	ND	ND	ND	28	9	33	0	ND	ND	ND	ND
M-10	ND	ND	ND	41	ND	46	33	10	ND	ND	ND	ND

[a] Patients: B. L., dose 400 mg./day, hematocrit 41%; E. P., dose 1000 mg./day, hematocrit 46%; and C. H., dose 1600 mg./day, hematocrit 50%. [b] See Table I, Footnote *b*, for identification of chlorpromazine metabolites. [c] Conjugated metabolites were obtained by subtracting free levels from the total. Zero values mean that the total concentrations were the same as free.

creted, do not penetrate biological membranes (16). It is, however, known that specialized transport mechanisms for endogenous glucuronides exist in the kidney and the liver (17). In view of the present findings relative to erythrocyte sequestration, it is tempting to suggest that the same or similar transport mechanisms may be involved in actively transporting the conjugated metabolites across the erythrocyte and possibly even other biological membranes.

Supporting this contention is the ubiquitous existence of β-glucuronidase in tissues, the reason for which remains unexplained. Could it be that the active transport system controlling the transfer of glucuronides across the cell membranes is coupled with the β-glucuronidase system? It is conceivable that following the active transport of the conjugated molecules, for example at the biophase, there is an instantaneous hydrolysis and thus liberation of the drug or metabolite molecules which may then exert their pharmacological effect. Studies now in progress are aimed at investigating the validity of the belief that conjugation may have important biological significance in membrane transport of pharmacologically active molecular species. The preliminary data on human subjects indicate that the urinary elimination of the conjugated chlorpromazine metabolites was significantly inhibited by penicillin which is known to be actively secreted by the tubular cells.

REFERENCES

(1) S. S. Walkenstein and J. Seifter, *J. Pharmacol. Exp. Ther.*, **159,** 283(1959).

(2) C. L. Huang and B. H. Ruskin, *J. Nerv. Ment. Dis.*, **139,** 381(1964).

(3) C. G. Hammar and B. Holmsted, *Experientia*, **24,** 98 (1968).

(4) I. S. Forrest, L. G. Brooks, G. A. Deneau, and L. B. Mallett, *Pharmacologist*, **12,** 273(1970).

(5) E. Usdin, *CRC Critical Rev. Clin. Lab. Sci.*, **2,** 347(1971).

(6) P. N. Kaul, M. W. Conway, M. K. Ticku, and M. L. Clark, *J. Pharm. Sci.*, **61,** 581(1972).

(7) I. Zingales, *J. Chromatogr.*, **44,** 547(1969).

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

(9) J. W. Hampton, *Amer. J. Med.*, **48,** 406(1970).

(10) A. A. Manian, D. H. Efron, and A. F. Goldberg, *Life Sci.*, **4,** 2425(1965).

(11) E. S. Vessel, T. Passamanti, F. E. Greene, and J. G. Page, *Ann. N. Y. Acad. Sci.*, **179,** 752(1971).

(12) S. H. Curry, *J. Pharm. Pharmacol.*, **22,** 193(1970).

(13) P. Seeman, *Biochem. Pharmacol.*, **15,** 1753(1966).

(14) P. Seeman, presented at First International Symposium, Vienna, Austria, June 17–20, 1968.

(15) T. H. Maren, B. Robinson, R. F. Palmer, and A. E. Griffith, *Biochem. Pharmacol.*, **6,** 21(1960).

(16) B. B. Brodie, H. Kurz, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **130,** 20(1960).

(17) L. Petters, in "Urinary Excretion of Drugs, First International Pharmacological Meetings," B. B. Brodie and E. G. Erdös, Eds., **6,** 179(1962).

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Micelle Formation and Testosterone Solubilization by Sodium Glycocholate

LEO MARTIS*, NATHAN A. HALL*, and ARVIND L. THAKKAR†▲

Abstract □ Surface tension and light-scattering studies on aqueous solutions of sodium glycocholate gave CMC values of 0.0129 and 0.0138 mole/l., respectively. Light-scattering studies in 0.1 *M* NaCl gave a weight-average micellar weight of about 1950, indicating that four monomers of glycocholate aggregate to form the primary micelle. Preferential broadening of the angular methyl signals in the NMR spectra suggests that glycocholate micelles are formed by back-to-back hydrophobic association of the steroid nuclei. The signal due to the glycine methylene protons in the hydrophilic region of the molecule showed little broadening. Solubility studies showed that testosterone is solubilized by glycocholate below the apparent binary CMC. The solubilizing capacity is quite low. The complex association between testosterone and glycocholate suggests mixed micelle formation.

Keyphrases □ Sodium glycocholate—micellar properties, determination of CMC, solubilization of testosterone □ Micelle formation, sodium glycocholate—determination of CMC, solubilization of testosterone □ Testosterone—solubilization by sodium glycocholate □ Solubilization—testosterone by sodium glycocholate

Pharmaceutical systems have involved surfactants for many years. Such agents show grossly similar chemical characteristics, typified by hydrophobic and hydrophilic portions of their molecules. Bile salts, however, differ both in chemical structure and colloidal behavior from the conventional surface-active molecules. The formation of micelles in the case of bile salts is not as abrupt as in the case of ordinary association colloids. Ekwall *et al.* (1) showed that the micelle formation for different bile salts passes through introductory association stages. The concentrations at which these steps take place are determined mainly by the number of hydroxyl groups in the bile acid molecules. Small (2) and Carey and Small (3) studied the properties of bile salt solutions and reported that trihydroxy bile salts form very small micelles at all concentrations while dihydroxy bile salts