CONCLUSIONS

The present method for the simultaneous determinations of ASA and SA in plasma is simple and relatively rapid, since at least 20 samples can be analyzed in 5 hr. The stability of ASA under the sample and storage conditions enable determinations to be made at the analyst's convenience. The advantage of this method over the differential SA method for the determination of low concentrations of ASA in the presence of large amounts of SA is clearly shown in Fig. 2. Forty minutes after the administration of 650 mg. of ASA, the SA levels usually reach 40–50 mcg./ml., and shortly thereafter one must be able to determine as little as 0.4 mcg. ASA/ml. in the presence of the above vast excess of SA. It is only with a GLC method that the biological half-life of ASA can be accurately determined. Also, the recently proposed alternative method of Cotty *et al.* (5) may not be any more useful than the differential methods due to relative errors indicated in the published results. The methods presented in this paper are now being used to study the pharmacokinetics of ASA and SA in man and to investigate the influence of dosage forms on the absorption of ASA.

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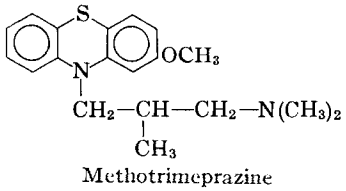
Estimation of Methotrimeprazine in Brain and Correlation of Brain Levels with Pharmacologic Activity

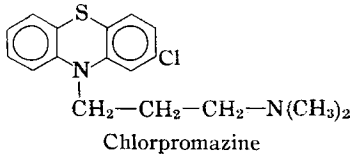
By ABDEL-HALIM M. AFIFI* and E. LEONG WAY

A method for estimating methotrimeprazine in brain by ultraviolet spectrophotometry was developed which is based on extracting the compound from alkalinized tissue with 1 per cent butanol in cyclohexane and determining its absorbance in acid at 250 mμ. A high degree of specificity for methotrimeprazine was conveyed to the procedure by subjecting the organic solvent extract of tissue to several buffer washes. Following intravenous injection of the compound in mice, methotrimeprazine was found to be metabolized rapidly during the first hour, after which its rate of disappearance slowed considerably. Brain levels were maximal at 1 hr. and were still appreciable at 4 hr. The onset and duration of pharmacologic effects as measured by a loss in coordinated motor ability varied directly with the concentration of drug in the brain, suggesting that the parent compound is principally responsible for mediating the response.

THE PHENOTHIAZINE DERIVATIVE, methotrimeprazine maleate (levomepromazine, and R.P. 7044),[1] until recently an investigational drug in the United States,[2] has been used for some time in Europe by psychiatrists as an alternative for chlorpromazine. The structures of the two agents are shown for comparison.



Methotrimeprazine



Chlorpromazine

The pharmacology of the compound has been studied extensively by Courvoisier *et al.* (1) who reported that its actions are similar to those of chlorpromazine but it is more potent than the latter with respect to sedative and hypothermic properties and capability of potentiating the

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The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.
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[1] Marketed as Nozinan by Specia, Paris, France, and Veractil by May & Baker, Dagenham, Essex, England.
[2] Since the writing of this paper, this drug has been marketed in the U.S. as Levoprom by Lederle Laboratories, Pearl River, N.Y.

depressant effects of other CNS agents. The analgetic effect of methotrimeprazine was noted by Sigwald *et al.* in the clinic (2).

More recently Lasagna *et al.* (3) reported that when injected methotrimeprazine appeared to be as effective as morphine, milligram for milligram, in the alleviation of postpartum pain, and that 15 mg. of methotrimeprazine gave as much relief as 75 mg. of meperidine in the management of pain during childbirth (4). The therapeutic potential of such a potent nonaddicting analgetic with minimal respiratory depressant properties prompted the study of the metabolism of the compound. The present report, using the mouse, is the first in a series concerning the biologic disposition of methotrimeprazine. A study in humans has been previously reported by Allgén *et al.* (5).

EXPERIMENTAL

Estimation of Methotrimeprazine in Mouse Carcass and Brain—The procedure used by Salzman and Brodie (6) for chlorpromazine was adapted and modified for methotrimeprazine. The method is based on determining the compound by ultraviolet spectrophotometry after its extraction from alkalinized tissue homogenate with a suitable organic solvent. The homogenate is obtained by mincing 1 part of tissue with 4 parts of 0.1 *N* H_2SO_4 in an electric blender.

Assay Procedure—Pipet 5 ml. of tissue homogenate into a 40-ml. glass-stoppered extraction tube; add 1 ml. of 10% NaOH and 25 ml. of cyclohexane containing 1% *n*-butanol. Shake for 5 min. and separate the two layers by centrifugation at 2500 r.p.m. Transfer 23 ml. of the organic phase to another 40-ml. extraction tube containing 10 ml. of 0.2 *M* phosphate buffer, pH 6.4. Shake for 5 min. and separate by centrifugation. Remove the buffer layer and add another 10 ml. of fresh buffer and repeat the washing process twice more. Transfer 20 ml. of the organic phase to a tube containing 4 ml. of 0.1 *N* H_2SO_4. Shake for 5 min., centrifuge, and remove the organic phase completely by aspiration. Transfer about 3 ml. of the aqueous acid phase to a quartz cell and determine its absorbance at 250 mμ in a Beckman DU spectrophotometer. The amount of methotrimeprazine recovered from a sample containing the drug is read from the corresponding standard curve derived from known amounts of methotrimeprazine added to each tissue homogenate.

Appraisal of Specificity of the Method—Countercurrent distribution (7) and partition behavior at varying pH values (8) were employed to characterize the ultraviolet absorbing material extracted from the carcasses of mice given methotrimeprazine.

Countercurrent Distribution—Two mice were injected intravenously with 15 mg./Kg. methotrimeprazine and sacrificed after 1 hr. The total carcass was diluted 1:5 with 0.1 *N* sulfuric acid and homogenized thoroughly in an electric blender. The homogenate was made alkaline with sodium hydroxide and extracted three times with 5 times its volume of cyclohexane containing 1% *n*-butanol. The extracts were pooled and divided into two parts. One part was used directly for countercurrent distribution and the other portion was washed three times with 0.2 *M* phosphate buffer, pH 6.4, before being subjected to countercurrent transfer. In each experiment an eight plate transfer separation was carried out in a system consisting of equal parts of 1% *n*-butanol in cyclohexane and 0.2 *M* phosphate buffer, pH 4, moving the lower aqueous phase. At the end of the distribution, 1 ml. of 10% sodium hydroxide was added to each tube. After shaking and centrifuging, an aliquot of the organic solvent was transferred to another tube containing 4 ml. of 0.1 *N* sulfuric acid, and the acid layer was analyzed for methotrimeprazine in the usual manner. A distribution curve was obtained by plotting the fraction of the total amount of methotrimeprazine present in each tube against the number of the tube, and the experimental distribution curve was fitted according to the method of Way and Bennett (9).

Partition Behavior—The alternative technique for appraising specificity of the procedure consisted of determining the distribution ratios of authentic methotrimeprazine in an organic solvent–water system at various pH values of the aqueous phase, and comparing them with those obtained for the extracted substance recovered from mice sacrificed after receiving methotrimeprazine. The carcass was homogenized as before in 4 parts of 0.1 *N* H_2SO_4, and after the addition of sufficient alkali, the homogenate was extracted with five times its volume of 1% *n*-butanol in cyclohexane. Duplicate 10-ml. aliquots of the organic solvent extract were washed three times with 0.2 *M* phosphate buffer of pH 6.4 and then equilibrated with 4 ml. of 0.1 *N* sulfuric acid. Other 10-ml. samples of the organic solvent extract were similarly washed and then equilibrated with equal volumes of 0.2 *M* phosphate buffer solutions of pH 7, 6, 5, or 4, respectively. After removal of the buffer phase, the organic solvent layer was shaken with 4 ml. of 0.1 *N* H_2SO_4. The organic solvent layer was discarded and the amount of methotrimeprazine in the acid aqueous phase was determined. The results were compared with those obtained from a similar treatment of authentic methotrimeprazine in aqueous solution.

Rate of Metabolism—The rate of disappearance of methotrimeprazine was studied in 23 intact mice. Methotrimeprazine, 15 mg./Kg., was injected into the tail vein of Swiss-Webster mice weighing 30 ± 5 Gm. The drug was dissolved in isotonic solution to give a concentration of 2 mg./ml. At fixed time intervals of 15, 30, 60, 120, 240 min., and 24 hr., at least two and generally four mice were individually homogenized and assayed for methotrimeprazine as described previously; the results are expressed as per cent of drug recovered.

Brain Concentrations of Methotrimeprazine—Mice were injected subcutaneously with methotrimeprazine and then sacrificed by decapitation at 15, 30, 60, 90, 120, 180, and 240 min.; two mice were used at each time interval. The brains were extirpated, weighed, diluted 10 times with 0.1 *N* sulfuric acid, and analyzed for methotrimeprazine in the usual manner.

Onset and Duration of Pharmacologic Effects—The time-response relationship of methotrimeprazine was assessed in mice in conjunction with brain concentration determinations. Two tests for central

nervous system activity were used: "analgesia," by the tail flick method of D'Amour and Smith (10); and coordinated motor ability, by the method of Dunham and Miya (11).

For analgetic activity, the reaction time for mice to remove their tails from a thermal stimulus, provided by a beam of light focused 1.5 cm. from the blackened tip of the mouse tail, was determined prior to drug injection and every 20 min. after subcutaneous administration of methotrimeprazine at doses of 2, 4, and 8 mg./Kg. A cut-off time of 5 sec. was used to prevent damage to the tail.

To test coordinated motor activity, the time an animal remained on a rotating rod was determined. One end of a wooden rod, 48 in. long and 1.2 in. in diameter, was connected to the motor shaft of an electric kymograph. The two ends of the rod were inserted into ball bearings supported by suitable clamps at a height of 28 in. above the table top to discourage animals from jumping off the roller. The speed selector was set so that the rod made 15 r.p.m. In order that multiple tests could be performed simultaneously, cardboard disks were placed along the rod at suitable distances to divide it into five compartments. Untreated animals after conditioning can maintain their balance on the rotating rod for at least 20 min. Performance trials were conducted on 10 mice at 15, 30, and then every 30 min. after subcutaneous injection of 4 mg./Kg. of the drug. The time each animal remained on the rotating rod was recorded up to a limit of 1 min. An animal remaining on the rod at the end of this observation period was assigned a relative activity of 1.0 and any perfomance value of less than 60 sec. was expressed as a fraction. The average relative activities and the mean deviations were calculated and plotted against time.

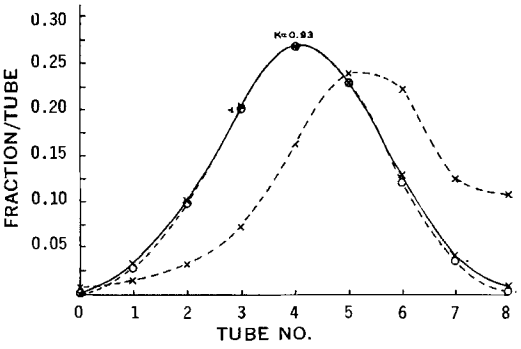

Fig. 1—Countercurrent distribution curves of the pooled extracts from the homogenized carcasses of two mice injected with methotrimeprazine.

$$System = \frac{0.01 \text{ n-butanol in cyclohexane}}{0.2 \text{ M phosphate buffer pH } 4}$$

Key: X---X, *unwashed solvent extract of mouse homogenate;* X—X, *washed solvent extract of mouse homogenate;* O---O, *theoretical.*

TABLE I—DISTRIBUTION RATIOS[a] OF AUTHENTIC METHOTRIMEPRAZINE AND OF APPARENT DRUG RECOVERED FROM MICE RECEIVING THE COMPOUND. THE SOLVENT PAIR CONSISTED OF 1% *n*-BUTANOL IN CYCLOHEXANE AND BUFFERS OF VARYING pH

Sample	pH 7	6	5	4
Added to water	0.98	0.93	0.73	0.35
Recovered from total mouse carcass	0.96	0.92	0.74	0.40

[a] $\frac{\text{solvent concentration post equilibration}}{\text{solvent concentration pre-equilibration}}$

RESULTS

Precision and Specificity of the Procedure—The application of single extraction techniques in conjunction with ultraviolet spectrophotometry appeared to be quite satisfactory for estimating methotrimeprazine in the mouse carcass or brain. Based on the standard curve obtained for methotrimeprazine extracted from water, the recovery of methotrimeprazine added to the tissue homogenates at concentrations ranging from 5 to 20 mcg. of drug per Gm. of wet tissues exceeded 90%. The blank correction was negligible, amounting to less than 0.1 mcg. of drug equivalent for brain and to less than 0.5 mcg. for the carcass.

The procedure was found to possess a high degree of specificity for methotrimeprazine. The countercurrent distribution experiments indicate that specificity is conveyed to the procedure by washing the solvent extract of tissue with a suitable buffer. As seen in Fig. 1, the distribution curve of the unwashed extract did not yield a curve with the distribution characteristics of a single substance. On the other hand, with the washed solvent extract of mouse homogenate, the experimental distribution curve with a maximum at tube 4 exhibited the characteristics of a single substance. With the latter curve, the calculated values of each tube for a partition ratio, K, of 0.93 yielded a theoretical curve which fitted almost perfectly with the experimental curve. When the K of methotrimeprazine added to a mouse homogenate was determined for the same system used in the countercurrent distribution, it was found to be 0.90. Thus, there is a strong evidence indicating that the substance characterized by countercurrent distribution is actually methotrimeprazine and that the introduction of a three-buffer washing removes interfering substances and renders the method highly specific for methotrimeprazine. The major fraction in the unwashed extract of total mouse homogenate appears to be biotransformation products and only about 30% of the ultraviolet absorbing material represents unchanged methotrimeprazine.

The partition behavior of biologically recovered methotrimeprazine at varying pH was also virtually identical to that of authentic methotrimeprazine. As can be noted in Table I, the distribution ratios of the recovered material in a solvent buffer pair system of varying pH were highly similar to those for authentic methotrimeprazine, and the probability for two different compounds to have the same distribution ratios under such rigidly defined conditions appears rather remote.

Rate of Metabolism in Intact Mice—Figure 2 represents a plot of the average per cent of the dose of methotrimeprazine recovered against the time interval after injection of the drug. The heavy line indicates the calculated mean recovery for each interval and the broken lines the range. Considerable varia-

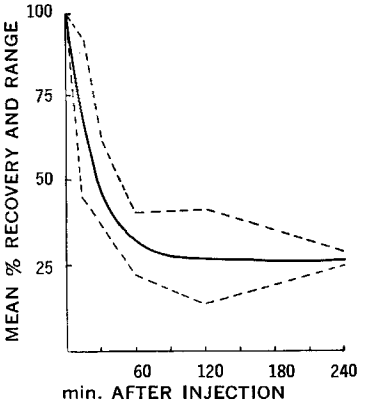

Fig. 2—Rate of metabolism of methotrimeprazine in intact mice after 15 mg./Kg. intravenously. Key: ———, mean obtained on two to four animals at six different time intervals; ----, range.

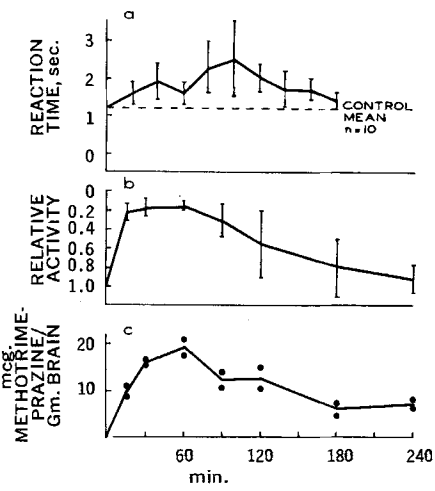

Fig. 3—Time response curves for mice receiving methotrimeprazine and assessed for (a) *"analgetic" activity (15 mg./Kg. dose);* (b) *loss of motor coordinating ability (4 mg./Kg. dose); and* (c) *brain levels of the drug (4 mg./Kg. dose).*

TABLE II—RESPONSE OF MICE TO THERMAL STIMULUS (TAIL FLICK METHOD)

Dose, mg./Kg.	Mice, No.	Reaction Time, sec., Mean ± m.d.— Time After Injection, min. 20	40	60
2	13	1.6 ± 0.3	2.1 ± 0.8	2.6 ± 1.1
4	14	2.5 ± 0.9	2.5 ± 0.9	2.7 ± 1.1
8	14	2.1 ± 0.9	2.6 ± 0.8	3.1 ± 1.1
15[a]	10	1.6 ± 0.3	1.9 ± 0.5	1.6 ± 0.3

[a] Values at 80, 100, 120, 140, 160, and 180 min. were, respectively, 2.3 ± 0.7, 2.5 ± 1.0, 2.0 ± 0.4, 1.7 ± 0.5, 1.7 ± 0.3, and 1.4 ± 0.2 sec.

tion in the rate of metabolism of methotrimeprazine was noted among the individual animals during the first 2 hr. The variation is less marked at 4 and 24 hr. after the drug injection. It can be noted that the initial part of the curve falls steeply and that after 60 min. about 70% of the injected dose has already disappeared. However, after the initial rapid decline the rate of disappearance of methotrimeprazine tends to level off, and the curve flattens out between 1 and 2 hr. After 24 hr., 7 and 8% methotrimeprazine was still recovered from two mice, respectively. Contamination of body levels by any drug in bladder urine was negligible since the amount of the drug present in the bladder urine at various time intervals in 8 of 23 mice was barely detectable, and the total 24-hr. excretion reflected less than 1% of the injected dose.

Methotrimeprazine "Analgesia"—Table II shows the mean values of the reaction time and their mean deviations obtained by injecting 2, 4, 8, and 15 mg./Kg. methotrimeprazine in 51 mice. The mean value of the control reaction time of the untreated mice was found to be 1.2 ± 0.04 sec.

While there appears to be a slight increase in the reaction time at the three dose levels and at 20, 40, and 60 min. after the administration of the drug, this increase is more apparent than real. The individual values varied considerably and the intensity of the responses correlated poorly with increasing doses of the compound. In fact with the highest dose, 15 mg./Kg. of methotrimeprazine, the reaction time was found to be almost the same or even less than that of lower dosages. Figure 3, a, represents the reaction time in seconds obtained with this dosage over a 3-hr. period with animals tested at 20-min. intervals. The mean deviations plotted in the same figure demonstrate the marked variation at each time interval. Despite the fact that little "analgesia" was noted, the mice appeared heavily sedated and exhibited little physical activity even at the lowest dose (2 mg./Kg.) of methotrimeprazine.

Coordinated Motor Ability—Figure 3, b, represents the average values of the relative activity of 10 mice when given 4 mg./Kg. of methotrimeprazine subcutaneously. It is apparent from the curve that the coordinated motor ability of the animals was reduced about 80% during the first hour. The mice gradually recovered, and by the end of the 4-hr. period there was only a 5% reduction in the relative activity of the mice. Subjectively, however, the animals still appeared very sedated and moved only when prodded.

Brain Levels of Methotrimeprazine—Figure 3, c, represents the concentration of methotrimeprazine as mcg./Gm. brain over the 4-hr. period after the administration of 4 mg./Kg. subcutaneously. The concentration in the brain increases gradually with time and reaches a peak level at 60 min. and then the drug concentration slowly declines. Significant amounts of methotrimeprazine were still present in the brain at the end of the 4-hr. period. The brain concentrations appeared to correlate with the coordinated motor ability of the mice to remain on the rotating rod.

DISCUSSION

The prolonged sedation and rather persistent tissue levels of methotrimeprazine observed in the mouse are in agreement with what is known concerning the duration of action and rate of metabolism of phenothiazine compounds in other animal species.

The psychotropic effects of chlorpromazine in humans have been reported to be maintained for 1 to 2 weeks after its discontinuation (5), and excretion of the drug in trace amounts in the urine may last for weeks (5, 12). Salzman and Brodie reported a relatively slow rate of biotransformation of chlorpromazine in dogs, and attribute the slow rate partly to extensive localization of the drug in various organs with consequent lesser immediate availability of the drug to metabolizing enzymes (6).

The studies on the organ distribution of methotrimeprazine in the rat (13) indicate that there is also rapid uptake of the drug by organ depots, especially parenchymatous tissues, and this is consistent with findings on most basic compounds (15). In the case of methotrimeprazine, organ levels of the drug may still be appreciable 12 hr. after its administration. Ultimately, however, the drug is extensively biotransformed. Less than 1% of the unchanged drug is excreted in the urine, but a variety of metabolic products, including sulfoxide metabolites, are detectable in varying amounts (13); similar findings have been reported for humans (5).

To what extent the biotransformation products of methotrimeprazine may contribute to the sum total of pharmacologic effects remains a question. The prolonged half-life of the drug indicates that adequate amounts of the original substance are available for eliciting drug effects. A reasonable degree of parallelism was noted for the curve for concentration of drug in the brain and that for coordinated motor ability. Such a correlation suggests that the parent substance is principally mediating the pharmacologic effects under observation, but whether loss of motor coordination is accompanied by analgesia cannot be answered by our experiments.

The failure to obtain a good correlation with "analgesia" resulted from the inability to establish a clear-cut response with methotrimeprazine by the test procedure. This is not surprising and was in part anticipated. Agents other than the narcotic analgetic type yield erratic effects in prolonging the tail reaction time to thermal stimulus and in our hands methotrimeprazine proved to be no exception to the rule. In view of the established effectiveness of the compound as a potent analgetic clinically (2–4), the tail flick procedure obviously would be a poor screening method for other compounds in this series. These findings are not consistent with an earlier report (14), and we have no explanation for the discrepancy. There is still clearly a need to establish a suitable test procedure for surveying non-narcotic type analgetics.

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Effect of γ Radiation on Selected Pharmaceuticals

By LAWRENCE J. RASERO, JR.*, and DONALD M. SKAUEN

Aqueous solutions of chlorobutanol, theophylline, and sodium carboxymethylcellulose (CMC) were irradiated with γ radiation from a ^{60}Co source. Solutions were irradiated in glass and polyethylene containers. Chlorobutanol solutions were analyzed for appearance, pH changes, chlorobutanol degradation, and production of chlorine, acetone, and hydrogen peroxide. Theophylline solutions were analyzed for appearance, pH changes, theophylline degradation, and production of hydrogen peroxide. CMC solutions were analyzed for viscosity changes. All systems showed pronounced changes, even at low radiation levels.

THE ABILITY of γ radiation to destroy microorganisms without an appreciable temperature rise in the substrate offers an attractive means of sterilizing pharmaceuticals. The possibility of side effects must be considered, however, as γ radiation has the capacity to create highly reactive chemical species in any matter through which it passes. This can lead to an alteration of the physical properties of materials and to the production of numerous chemical changes.

Willis (1) and Proctor and Goldblith (2) have published review articles on the effects of radiation on pharmaceuticals. Although much valuable information has appeared in the literature, the majority of the published reports are confined

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