Biochemical Pharmacology, 1963, Vol. 12, pp. 1429–1435. Pergamon Press Ltd., Printed in Great Britain.

THE EXCRETION AND METABOLISM OF ^{35}S–LABELED THIORIDAZINE IN URINE, BLOOD, BILE, AND FECES*

SAMUEL EIDUSON and EDWARD GELLER

Neuropsychiatric Institute, of the Center for the Health Sciences at the University of California, and Neurobiochemistry Laboratory, Veterans Administration Center, Los Angeles, Calif., U.S.A.

(*Received* 7 *May* 1963; *accepted* 8 *July* 1963)

Abstract—After administration of a single oral dose of ^{35}S-ring-labeled thioridazine to neuropsychiatric patients, the following observations were made:

1. Approximately 30% of the original dose is excreted in the urine.
2. Approximately 50% of the original dose is excreted in the feces.
3. Over 60% of the drug passes through the bile fluid.
4. Only about 3% of the original dose remains in the serum at peak levels which are reached after 3 to 4 hr after administration.
5. Radioautograms and chromatograms of a urinary extract indicate a congruity between radioactive metabolites and acid chromogens and that urinary metabolites differ qualitatively from those found in feces and bile.
6. Chloroform extracts over 97% of the urinary radioactivity. This would be in accord with the observation that little or no glucuronide-conjugated material appears in the urine after oral administration of thioridazine.

IN previous reports[1,2] we have noted our observations on the amount and rate of excretion of a phenothiazine-type tranquilizer, thioridazine (Mellaril), using a spectrophotometric method. It was clear, however, that this method measured only part of the phenothiazine-like metabolites in the urine, those that were adsorbable on the ion-exchange resin used [Amberlite-IRC 50 (H^+)], and not conjugated forms like glucuronides. It was noted, however, that the data on thioridazine excretion showed little, if any, glucuronide conjugation.

In order to test these matters more accurately and to be able to measure very low quantities of phenothiazine material excreted in various biological fluids, ^{35}S-ring-labeled thioridazine was used. The data obtained with the isotopic drug on urine, feces, serum, and bile are the subject matter of this report.

METHODS

The spectrophotometric method employed was that reported previously.[1] All ^{35}S was converted to magnesium sulfate for determination of radioactivity.[3] A Packard Tri-Carb scintillation counter was used for all measurements. The certified standard solution of sulfuric acid-^{35}S, added as an internal standard to each sample to assess efficiency so that absolute radioactivity could be calculated, was obtained from Nuclear-Chicago Corp.

* This study was supported in part by Grant MY 3968 from the U.S. Public Health Service and a grant from Sandoz Pharmaceuticals. We wish to acknowledge gratefully the encouragement and kind assistance with the patients by Dr. Sidney Cohen, Chief, Psychosomatic Service, Veterans Administration Center, Los Angeles, Calif. We wish to thank Sandoz for the supply of ^{35}S-Mellaril.

Urine. To prepare a urine sample for counting, a 0·5-ml aliquot was placed in a counting vial. To this was added 0·5-ml Pirie's reagent* and the uncovered vial placed in a hole in a solid aluminum block such that the top of the block covered the bottom half of the vial. The block was then slowly heated on a hot plate, the current to which was controlled by a Variac, to about 260°. The temperature was maintained until all of the sample was oxidized to a fine white powder (1 to 2 hr). If complete oxidation did not occur the vials were taken out, cooled, and either an additional amount of Pirie's reagent or 1 to 4-ml concentrated nitric acid was added and the vials reheated in the aluminum block. After complete oxidation was achieved, the blocks containing the samples were cooled to 150°. At this time 1 ml glycerol at a temperature of 100° was added to the vial containing the sample, and the temperature was maintained at 150° until the white powder in the vial was completely dissolved. Enough scintillation mixture was added to make a total volume of 10-ml.† The samples were then counted in a Packard Tri-Carb scintillation counter.

Feces. Sufficient distilled water was added to the weighed sample to give a fine suspension after homogenization in a Waring Blendor. A 2-ml aliquot of the suspension was extracted four times with 4-ml chloroform. The chloroform extracts were combined and evaporated to dryness in an air stream and the dry residue taken up in 0·1-ml ethanol. Scintillation mixture, 9·9-ml, was added and the sample counted. A 0·5-ml aliquot of the original fecal suspension and the nonchloroform-extractable material were treated as indicated above for urine samples.

Serum. Serum samples of 0·5-ml were treated as were the urine samples.

Bile. Same as for urine samples.

A neuropsychiatric patient having an indwelling catheter was the subject of the urine and feces experiments; a different patient with a bile duct cannula was the subject for the bile fluid studies; a third patient was used for the serum (and urine) studies. The ^{35}S-ring-labeled thioridazine‡ was administered orally as a water solution mixed with cherry syrup. The first patient received 112 μc in a total dose of 400 mg of drug. The bile-fluid and the serum-study patients each received 100 μc in a 200-mg total dose.

RESULTS AND DISCUSSION

Table 1 indicates how much of the thioridazine-like substances in the urine was adsorbed by the Amberlite-IRC 50 (H^+) and how much was lost from the resin by the washing procedure used. The data in the upper portion of the table indicate that less than 12% of the total radioactive sulfur was not adsorbed by the resin after two extractions. It is reasonable to assume that further treatment with resin would have adsorbed additional amounts of thioridazine-like material. The nonadsorbable fraction most probably consists of conjugated metabolites, such as sulfates[4] or glucuronides,[1,5] any free radicals formed,[6] and any radioactive sulfur derived from a rupturing of the thioridazine nucleus. The lower portion of the table indicates that less than 3 per cent was lost while the resin was washed, in our procedure. Following the same

* Pirie's reagent: 3 volumes of concentrated nitric acid was added to 1 volume of 60% perchloric acid. One-fourth of this mixture was removed, saturated with magnesium nitrate (50 to 60 g/100 ml), and mixed with the remaining acid mixture.

† The scintillation mixture consisted of 25 mg POPOP [1. 4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene], and 750 mg PPO (2,5-diphenyloxazole) in 250 ml toluene.

‡ The ^{35}S-labeled thioridazine, chromatographed in 3 different solvent systems, had the same R_f value and gave the same color reaction with sulfuric acid as did authentic unlabeled thioridazine.

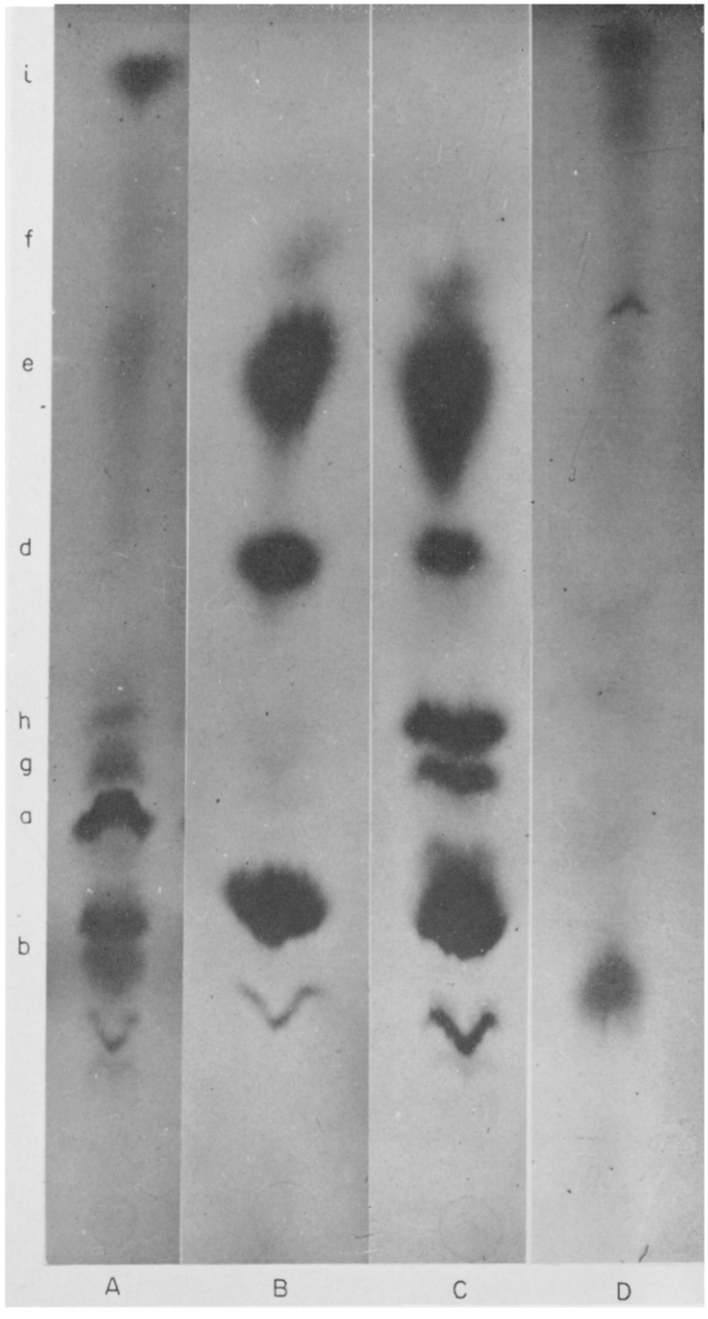

FIG. 1. An autoradiograph of a unidimentional ascending chromatogram (Solvent I) of urine obtained during the first 24 hr after i.p. injection of ^{35}S-sulphur mustard A, ^{35}S-thiodiglycol, B, ^{35}S-2-chloroethyl-2′-hydroxyethylsulphide, C, and A after treatment with hot concentrated hydrochloric acid for 24 hr.

facing page 1330

procedure but using only a standard aqueous solution of ^{35}S-labeled thioridazine, we have observed that at least 99 per cent of the drug was adsorbed by the resin. The free thioridazine is very tightly held to the resin since we found only 0·15 per cent was removed by our washing procedure. Further, we have observed that chloroform extracted over 97 per cent of the radioactivity from urine of a subject given labeled thioridazine. This indicates that no more than 3 per cent of the thioridazine-like meta-

TABLE 1. AMOUNTS OF THIORIDAZINE-LIKE SUBSTANCE ADSORBED ON RESIN AND LOST IN WASHING

Sample		Original counts (%)
Urine counts/ml	522	100
Counts/min/ml remaining after 1st resin treatment of urine	157	28
Counts/min/ml remaining after 2nd resin treatment of urine	62	12
Total counts adsorbed on resin	41,760	
Total counts appearing in 1st cold-water wash	2,180	5·2
Total counts appearing in hot-water wash	1,402	3·4
Total counts appearing in 6th and 7th cold-water wash	276	0·7
		9·3
Counts calculated as lost during urine drain from resin*		−6·7
Total loss		2·6

* Calculated as difference between the counts per milliliter originally in the urine sample and those in the supernatant after adsorption by the resin.

bolites in the urine could have been glucuronides. These data are in accord with those we previously reported on the lack of glucuronide formation after oral thioridazine administration.[1] In addition, these data are in sharp contrast to the observations that we (and others) have reported on the excretion of large amounts of glucuronides following chlorpromazine administration. These data suggest that some of the phenothiazine class of drugs like thioridazine differ from others, like chlorpromazine, in that little or no glucuronide is excreted after ingestion of the former, whereas large amounts of the conjugate are excreted after oral administration of the latter.

A comparison was made on the same patient, at the same time, between the excretion of thioridazine as determined spectrophotometrically and as determined by radioactivity measurements. These data are plotted in Fig. 1 (see also Fig. 2). The measurement of radioactivity includes not only those metabolites measured by the spectrophotometric method but also those substances not adsorbed by the resin, as well as those substances adsorbed by the resin but possibly not giving color with the acid reagent. The scales for the curves have been adjusted to bring out their general concordance, and it is apparent that the peaks and troughs of both curves are similar. There does not appear to be a marked diurnal variation in the excretion of thioridazine-like metabolites. About 30 per cent of the originally ingested isotopically-labeled drug was found to be excreted in the urine. These data also indicate that small but appreciable amounts of metabolites were excreted, at various intervals, at least as late as sixteen days after administration of the drug.

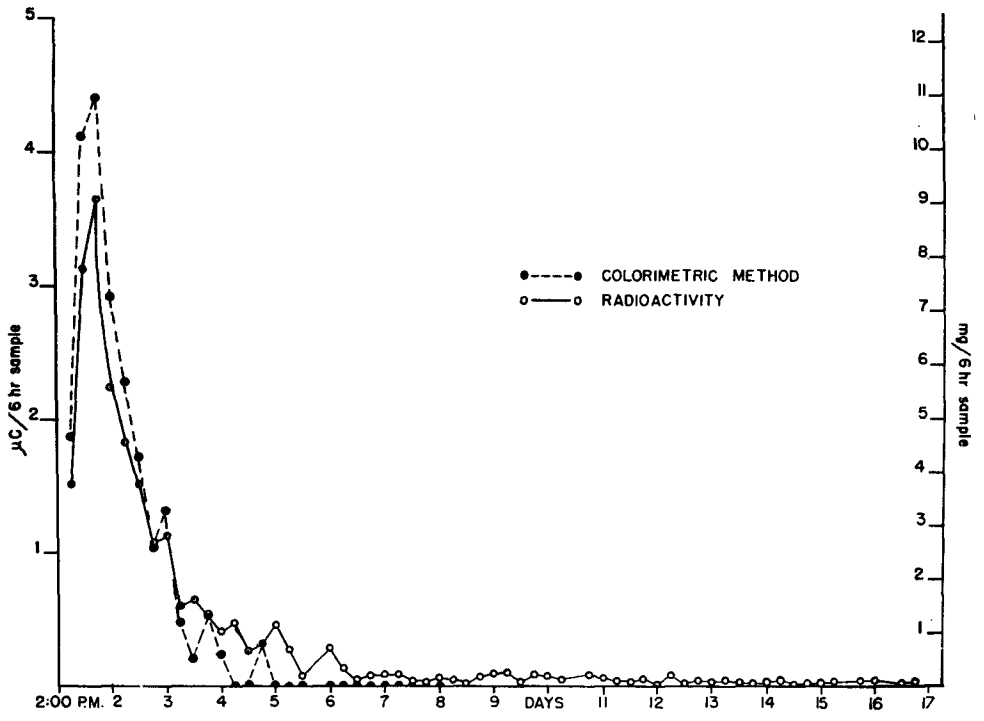

FIG. 1. Urinary excretion of ^{35}S-ring labeled thioridazine.

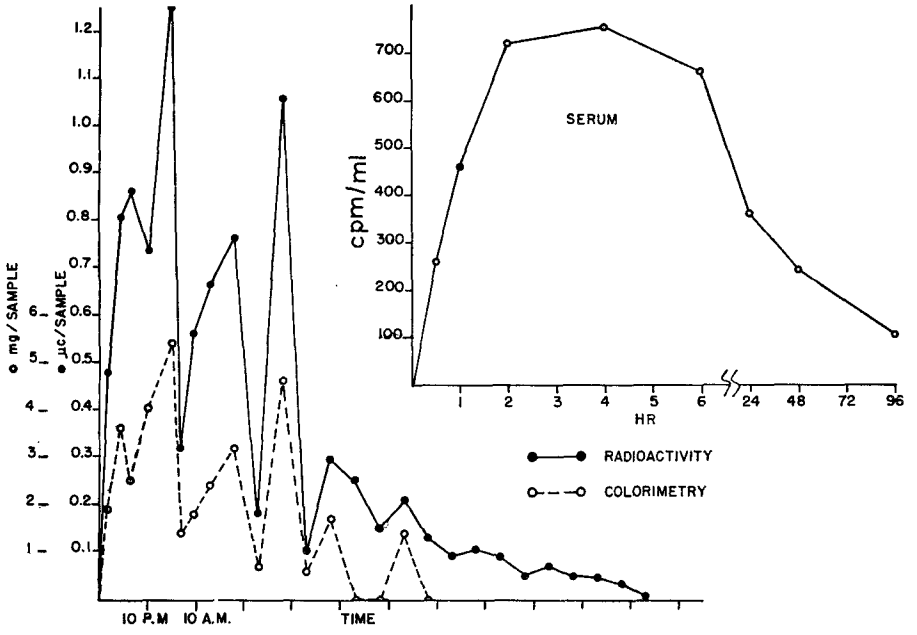

FIG. 2. Appearance of ^{35}S-ring-labeled thioridazine metabolites in urine and serum from the same patient.

When measurement of ^{35}S-labeled drug appearing in the feces of the same patient was determined, it was observed that over 50 per cent of the originally ingested dose appeared in the feces during the same interval that urine was measured. Thus, about 15 per cent of the radioactive dose is unaccounted for, but this probably could be found in tissue storage or could have been lost in sweat, tears, and (perhaps) saliva. It is also reasonable to assume that if the collection of samples was carried out for a much longer period of time, a greater percentage of original radioactivity would be accounted for.

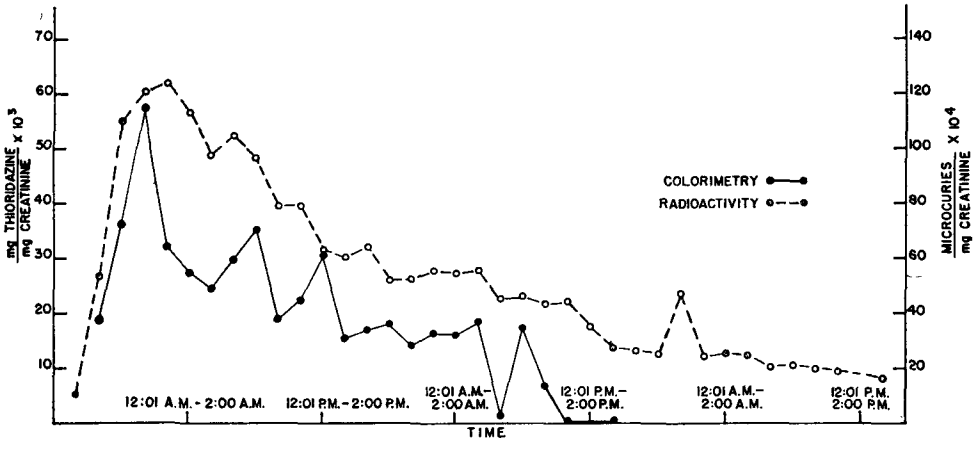

FIG. 3. Excretion of ^{35}S-ring-labeled thioridazine metabolites per gram creatinine.

Fig. 2 shows the 2-hr urinary excretion rate of thioridazine metabolites in a different patient. The urinary fluctuations are large, as we have observed previously, and it should be noticed that the peaks and troughs of the curve demonstrating the excretion of radioactive metabolites follow closely those we obtained using our colorimetric method.

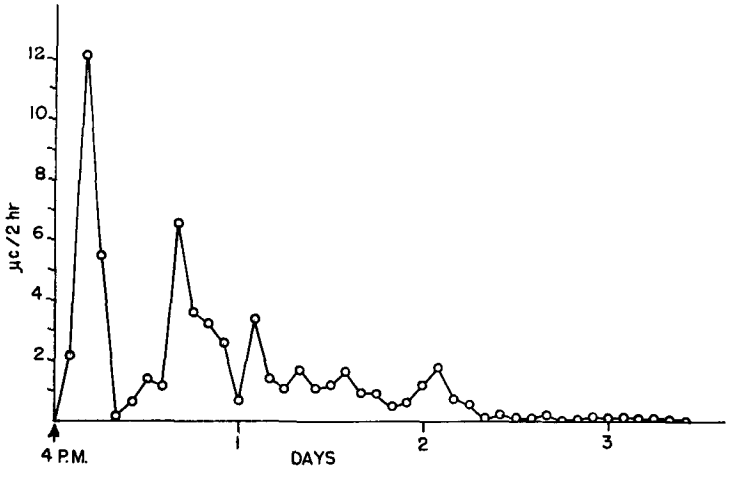

FIG. 4. ^{35}S-thioridazine metabolites in bile fluid.

The figure also shows the radioactivity of venous blood samples from this patient at various times after ingestion of the radioactive dose of tranquilizer in a total of 200 mg of drug. Calculation of the data obtained from the serum indicated that even at the peak levels only about 3 μc (assuming a volume of blood of 5 liters) appeared in the serum. Again, we were here measuring the total radioactivity of the serum irrespective of the nature of the phenothiazine metabolites. The quantity in each blood sample was below the limits of detection of the colorimetric method. These data clearly indicate that an extremely small amount of drug is found in the serum at any instant in time.

In urinary determinations, many workers prefer to order their data on a per gram creatinine basis to attempt to account for large variations in urine volume. In Fig. 3 the urinary data are plotted to relate the amount of thioridazine metabolites to the creatinine content of the urine. It is evident that marked fluctuations still occur and are not much different from these data related to 24-hr urine volume per se.

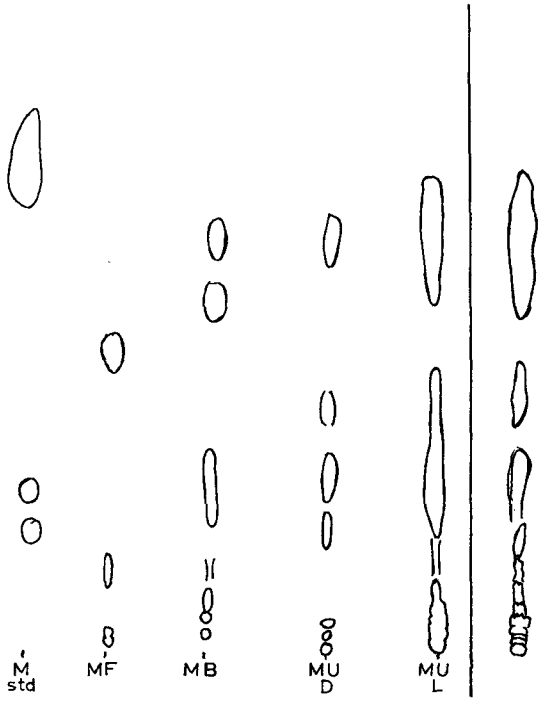

FIG. 5. Chromatogram and radioautogram of chloroform extracts of urine, bile, and feces. The left portion of this figure is the chromatogram; the right portion is a radioautogram of that part of the chromatogram marked MU L. The difference in intensity of spots on the chromatogram is due to the application of unequal amounts of material on the paper. Solvent system, ethylene dichloride:benzene:formic acid:water (3:1:4:2). Spray reagent, 50% sulfuric acid.

M std, chloroform solution of authentic thioridazine.
MF chloroform extract of feces from Patient L.
MB, chloroform extract of bile fluid from Patient B.
MU D, chloroform extract of urine from Patient D.
MU L, chloroform extract of urine from Patient L.

Measurement of the biliary content of thioridazine metabolites was carried out on a patient given an oral dose of 200 mg thioridazine containing 100 μc of labeled drug. Bile fluid was collected every 2 hr. The data in Fig. 4 indicate that the peak drug content occurred about 4 hr after ingestion of drug. A total of 59 μc, 59 per cent of the original radioactive dose, appeared in the bile fluid over the period studied. The fluctuations in concentration of labeled thioridazine metabolites were similar to those observed in the urine.

Because of the possibility of metabolic variation in the body fluids investigated, the chloroform extracts of urine, bile, and feces were compared chromatographically. A diagrammatic representation of a descending chromatogram after acid spray (and a radioautograph of one section) is shown in Fig. 5. Some spots derived from the chloroform extracts of bile and of feces appear to be quite different from those of the urine. Authentic thioridazine was used as a reference, and the two spots on the standard strip appear as an artifact of the procedure. It is apparent that there was no radioactive spot that did not give the expected color reaction of the phenothiazine nucleus with the acid spray.

REFERENCES

1. S. Eiduson, E. Geller and R. D. Wallace, *Biochem. Pharmacol.* **12**, 1437 (1963).
2. S. Eiduson and R. D. Wallace, *Trans., Second Research Conf. on Chemotherapy in Psychiatry*, Veterans Administration, **2**, 88 (1958).
3. H. Jeffay, E. O. Olubajo and W. R. Jewell, *Analyt. Chem.* **32**, 306 (1960).
4. A. H. Beckett, M. A. Beaven and A. E. Robinson, *Abstracts*, First Internat. Cong. on Action, Mechanism, and Metabolism of Psychoactive Drugs Derived from Phenothiazine and Structurally Related Compounds, Sept. 7–8, 1962, Paris.
5. T. H. Lin, L. W. Reynolds, F. M. Rondish and E. J. Van Loon, *Proc. Soc. expt. Biol. (N.Y.)* **102** 602 (1959).
6. I. S. Forrest, F. M. Forrest and M. Berger, *Biochim. biophys. Acta* **29**, 441 (1958).