Determination of Iodide in Metabolic Studies of ^{125}I-Labeled Compounds

Y. Y. SHUM * **and J. E. SINSHEIMER** ˣ

Received January 18, 1978, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication August 7, 1978. *Present address: Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI 48106.

Abstract □ Methods capable of measuring inorganic iodide in the presence of other highly polar metabolites were developed in support of studies concerning the metabolism of ^{125}I-labeled compounds. The methods included separation of iodine on a weakly basic resin paper followed by γ-counting, methyl iodide exchange, and reverse isotopic dilution.

Keyphrases □ Iodide—analyzed in urine by paper chromatography–γ-counting, methyl iodide exchange, and reverse isotopic dilution □ Chromatography, paper—followed by γ-counting, analysis of iodide in urine □ Methyl iodide exchange—analysis of iodide in urine □ Reverse isotopic dilution—analysis of iodide in urine

Methods for the measurement of inorganic iodide were required in studies concerning the metabolism of ^{125}I-labeled compounds. It was desired to have chromatographic separation procedures followed by γ-counting and also independent chemical confirmation of such chromatographic results. For example, these methods had to be capable of measuring iodide in the presence of other highly polar metabolites such as the glucuronides, sulfates, and mercapturic acid derivatives formed during the metabolism of 4-iodobiphenyl (1).

Chemical determinations of iodide in biological systems appeared not to be applicable to these studies. The limit of detection of the electrometric titration method (2) is 25 μg/50 ml of urine in systems containing relatively large amounts of organically bound iodide. In several procedures, the iodide was oxidized to iodine, followed by colorimetric determination (3), γ-counting (4), or titration with sodium thiosulfate (5). Iodine in urine or filtrates of incubation mixtures containing iodine-131 in the presence of organically bound iodine also was measured by passing such a solution through a silver chloride column (6–8). The activity of the silver iodide formed at the top of the column was counted with excellent recovery, but the presence of easily oxidizable iodo compounds could be a problem.

Several acidic and basic alcohol–water solvent systems were applied to paper chromatographic separations of iodide in biological systems (4, 6, 9–16). The 1-butanol–0.5 *N* ammonia–ethanol–water system (16) was found to be applicable to metabolic studies. In addition, a resin paper separation, a methyl iodide exchange procedure, and a reverse isotopic dilution method were developed. This paper describes these three procedures for iodide measurements in biological systems. These procedures are being applied for metabolism studies of ^{125}I-4-iodobiphenyl in rats in which the daily yield of iodide in urine represents a maximum of 3% of the radioactivity of an original dose of 400 mg/kg (400 μCi).

EXPERIMENTAL

Resin Paper Chromatography—Weakly basic resin papers[1] were developed to 16.0 cm by an ascending method in tanks lined with filter paper and saturated with butanol–acetone–concentrated ammonium hydroxide–water (65:20:10:5). Radioactivity was detected by γ-counting[2] 0.5-cm strips of the chromatogram. In urine samples showing multiple peak activity, the resin paper chromatogram corresponding to inorganic iodide was extracted with methanol. The extract was concentrated and redeveloped in the same system for improved separation of the inorganic iodide.

Methyl Iodide Exchange—A volume of 50.0 μl of rat urine was transferred with a microliter pipet to a 10 × 75-cm screw-capped culture tube and dried under a gentle nitrogen stream. To the residue were added 100 μl of acetone and 100 μl of methyl iodide, both freshly distilled before use. The tube was capped, and the sample was allowed to exchange overnight at room temperature. The exchanged radioactive methyl iodide was removed along with acetone by a slow nitrogen stream, which had passed through a solution trap containing 2.0 ml of 5% NaOH.

The dried residue was dissolved in 200 μl of acetone and counted in a γ-counting system. The difference in radioactivity before and after exchange was used to calculate the inorganic iodide concentration in the urine (17). The amount of ^{125}I-label present in a sample was calculated by dividing the difference in activity before and after methyl iodide exchange by the factor 0.926, as established from known concentrations of iodine-125.

Reverse Isotopic Dilution—To 3.0 ml of rat sample urine was added about 1 g of carrier sodium iodide, accurately weighed, to give a homogeneous solution. An excess volume of saturated lead nitrate was added to the solution to precipitate iodide as lead ^{125}I-iodide, which was filtered and recrystallized in large volumes of boiling water. The radioactive lead iodide was oxidized to elemental iodine by addition of a slight excess of sodium nitrite (~0.5 g) in a 2.5 × 20.3-cm test tube. Concentrated nitric acid was added dropwise until the release of nitrous acid ceased.

The iodine crystals were trapped on the inside wall of the test tube, which had a cooling coil around the outside. Purification of the radioactive iodine crystals was by repeated sublimations until a constant specific activity was obtained.

RESULTS AND DISCUSSION

To develop a rapid analytical method to separate inorganic iodide from other highly polar metabolites, a number of reported TLC systems (18, 19) were tested and found to be unsatisfactory. Separation by paper electrophoresis in various buffer systems could be achieved, but quantification of the results was poor. In pH 3 phthalate buffer, even at the minimal constant voltage (15 v) or current (15 mamp) required for satisfactory iodide-ion mobility (0.25 cm/hr), there was appreciable oxidation of the iodide.

With a weakly basic polyamine anion-exchange resin[3] (0.2 g dry weight), it was possible to separate iodide from polar metabolites. The ^{125}I-ions in urine samples (10,000 cpm) from rats dosed with ^{125}I-4-iodobiphenyl were retained at the top of the resin column while all other radioactive compounds from the hydroxylation of the iodobiphenyl and their conjugates were removed by elution with 0.8% HCl in methanol. γ-Counting of iodide activity bound to the resin particles, however, did not give consistent results, probably because of the difficulty in controlling the geometry of counting the resin particles.

The information gained from this resin column led to the use of chromatography on resin-loaded papers. A single-phase solvent system of butanol–acetone–concentrated ammonium hydroxide–water (65:20:10:5) was used with a weakly basic resin paper. It was speculated that an ammonia–iodide interaction (20) would be useful to separate iodide from other polar urinary metabolites. In urine sample chromatograms, a peak

[1] WB 2 resin paper, Reeve Angel, Clifton, N.J.

[2] Model 1185 γ-counting system, Searle, Chicago, Ill. The limit of counting was set for 10,000 cpm or 1 min, and the counting efficiency for iodine-125 was 76%.

[3] IR-45, Mallinckrodt, St. Louis, Mo.

0022-3549/79/0200-0258$01.00/0

at R_f 0.39 corresponded to the single peak appearing in the ^{125}I-control chromatograms. The presence of iodide in the peak was confirmed by methanol extraction of the peak and rechromatography in the USP paper system (21) used to determine the purity of the ^{125}I-label. Both a reference sample of sodium ^{125}I-iodide[4] and the concentrated extracted material had R_f values of 0.71 in the paper system.

The resin paper system in the metabolism study of iodobiphenyl in rats (1) provided a satisfactory separation of inorganic iodide (R_f 0.39) from other urinary metabolites (polar conjugates, R_f 0.16; iodobiphenyl and 2- and 4-hydroxy-4′-iodobiphenyl, R_f 0.73). The same system failed when it was used for liver homogenate studies of the biodehalogenation of 4-iodobiphenyl, 4-iodonitrobenzene, and 4-iodoaniline (22). The system was incapable of separating iodide after the addition of trichloroacetic acid, which was required for the workup of these *in vitro* samples. However, a reported paper partition system (16) was used for this purpose and also provided excellent separations for iodide in the presence of other metabolites in urine samples.

The results of the analysis of six urine samples by both the resin paper and partition systems differed by less than 3% in total urinary activity. In general, while the paper partition system gave better defined separations in complex mixtures of polar metabolites, the resin paper system was less time consuming and results could be achieved in the time required for conventional TLC systems.

To confirm further the accuracy of iodide determinations by the paper and resin paper chromatographic systems, alternative quantitative techniques were sought. Existing methods with adequate sensitivity all involve quantitations of total iodide concentrations after chemical manipulations, including electrometric titrations, oxidations, adsorption, and extractions, of the original biological samples, which may introduce artifacts. Limitations due to these artifacts have been discussed (3, 6, 23).

A reverse isotopic dilution method was one of two procedures devised to avoid these artifacts. Since isotopic dilution methods depend on specific activity as the criterion for quantitation instead of on total content in the processed samples, any loss of iodide resulting from multistep handling would not affect the final determination. After addition of cold sodium iodide to radioactive urine samples, precipitation with lead nitrate was used to provide an initial isolation of inorganic iodide. The lead iodide formed could not be reproducibly counted directly because of the apparent quenching effect of lead, so the lead ^{125}I-iodide was oxidized to iodine. The iodine crystals were purified by repeated sublimations to a constant specific activity. This specific activity, multiplied by the weight of cold iodide added, was used as a measure of the ^{125}I-label in the urine sample.

Evaluation of this procedure with duplicate samples of sodium ^{125}I-iodide (73,000 and 80,000 cpm) content gave an average of 95.6% recovery with a difference of 0.6%. A constant specific activity was obtained after three sublimations and remained constant for the additional three sublimations tested. The method also was applied to urine samples (243,000–1,000,000 cpm/5 ml) from rats dosed with ^{125}I-4-iodobiphenyl (400 mg/kg; 400 μCi), with a constant specific activity being obtained after three or four sublimations and remaining constant for at least the three additional sublimations tested.

[4] New England Nuclear (17 Ci/mg); used within 6 months of calibration.

A second chemical analytical method used an exchange reaction between methyl iodide and the ^{125}I-label and was based on studies (17, 24) of rates of exchange of organic iodides with radioactive inorganic iodide. In the present studies, when the exchange procedure was applied to iodide in urine samples, it was important to establish anhydrous conditions; acetone was an excellent solvent for the exchange. Thirty exchange experiments with known quantities (500–14,000 cpm) of sodium ^{125}I-iodide (17 Ci/mg) added to control rat urine indicated that 92.6 ± 2.23% of added activity was removed after methyl iodide exchange.

A comparison of the exchange and reverse isotopic procedures for iodide determination was made for the first 4-day urine excretion after the administration of ^{125}I-4-iodobiphenyl (400 mg/kg; 400 μCi) to rats. The two procedures agreed within 3.4% of the total daily urinary radioactivity and were equally effective in measuring iodide in metabolic systems.

REFERENCES

(1) Y. Y. Shum, Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1977.

(2) J. H. Wilkinson, W. E. Sprott, C. H. Browden, and N. F. Maclagan, *Biochem. J.*, **56,** 215 (1954).

(3) M. K. Johnson, *ibid.*, **98,** 44 (1966).

(4) J. R. Tata, J. E. Rall, and R. W. Rawson, *Endocrinology*, **60,** 83 (1957).

(5) H. G. Bray, J. C. Caygill, S. P. James, and P. B. Wood, *Biochem. J.*, **90,** 127 (1964).

(6) L. G. Plaskett, *ibid.*, **78,** 652 (1961).

(7) K. Fletcher, *ibid.*, **67,** 136 (1957).

(8) *Ibid.*, **67,** 140 (1957).

(9) D. W. Slingerland and R. K. Josephs, *Endocrinology*, **62,** 853 (1958).

(10) J. B. Stanbury, *J. Biol. Chem.*, **228,** 801 (1957).

(11) E. Yamazaki and D. W. Slingerland, *Endocrinology*, **64,** 126 (1959).

(12) F. C. Larson and E. D. Albright, *J. Clin. Invest.*, **40,** 1132 (1961).

(13) J. R. Tata, *Biochem. J.*, **77,** 214 (1960).

(14) J. Gross, C. P. Leblond, A. E. Franklin, and J. H. Quastel, *Science*, **111,** 605 (1950).

(15) J. Roche, M. Jutisz, S. Lissitzky, and R. Michel, *Biochim. Biophys. Acta*, **7,** 257 (1951).

(16) R. Grasbeck, B. A. Lamberg, and F. Bjorkstén, *Acta Endocrinol.*, **34,** 113 (1960).

(17) P. Beronius and A.-B. Gabrielsson, *Anal. Chem. Acta*, **40,** 352 (1968).

(18) C. E. Mellish, *Panel Anal. Control Radiopharm.*, **1970,** 115.

(19) V. Shen, K. R. Hetzel, and R. D. Ice, in "Technical Bulletin 32," Gelman Instrument Co., Ann Arbor, Mich., 1974.

(20) "Thin-Layer Chromatography," E. Stahl, Ed., Springer-Verlag, New York, N.Y., 1965.

(21) "The United State Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 462.

(22) J. E. Sinsheimer, T. Wang, S. Röder, and Y. Y. Shum, *Biochem. Biophys. Res. Commun.*, in press.

(23) J. D. Acland, *Nature*, **170,** 32 (1952).

(24) P. Beronius, *Acta Chem. Scand.*, **15,** 1151 (1961).