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Note

Simple method for the determination of hormonal iodine and exogenous iodine from X-ray contrast media drugs

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The organic iodine diagnostic drugs, commonly used in clinical practice as X-ray radiopaque agents, are compounds in which the iodine atoms are bound to an aromatic nucleus of the Ar–I structural type. The bond is very stable and resists biotransformation reactions well.

Clear-cut separation of exogenous iodine from hormonal iodine has never been achieved, despite numerous reports on the interference of radiopaque agents with thyroxine determinations [1–5].

This report describes a simple and very sensitive procedure, which permits precise quantification of the radiopaque agents and hormonal iodine, without any reciprocal interference, in a single analytical determination. The procedure is based on the selective elution of the thyroxine followed by precise elution of the contrast medium from a mini-column bed of anionic resin, followed by spectrophotometric determination of iodine according to the Sandell–Kolthoff reaction. To obtain these results, the column eluents, reaction reagents and conditions of the original Pileggi and Kessler procedure [6] had to be substantially modified.

This procedure can easily be applied to the study of the rate and extent of excretion of organoiodate contrast media or of other drugs of similar structure.

EXPERIMENTAL

Radiopaque organic iodine compounds

Diatrizoate salts (sodium or meglumine), iodamide and iotroxate meglumine were supplied by Shering (Milan, Italy). Iothalamate salts (sodium or meglumine), iodamide, iopamidol and iodoxamate mixed salts (sodium or meglumine) were supplied by Bracco Industria Chimica (Milan, Italy).

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Chemicals

Potassium iodate (99.9% RPE-ACS grade) for the preparation of iodine standard stock solutions was purchased from Farmitalia Carlo Erba (Milan, Italy). All other chemicals used were of analytical grade and commercially available.

Chromatographic columns

Disposable polypropylene chromatographic mini-columns (25 mm × 8 mm I.D.) were supplied by Sibar (Perugia, Italy) and were packed with Dowex 1 × 2 (50–100 mesh) supplied by Bio-Rad Labs. (Richmond, CA, U.S.A.) (bed height 20 mm). The flow-rate was ca. 0.5 ml/min.

Samples

Blood samples were obtained from patients of the Department of Surgical Pathology (University Hospital of Perugia, Perugia, Italy) to whom the radiopaque agent had been given 24–30 h before the operation. The first blood sample was taken 6–10 h after intravenous administration of the contrast medium. Blood samples were collected every day until the patients left the hospital. Each sample was tested three times and the points plotted represent the average of the results.

Reagents

Reagent 1, 0.1 *M* sodium hydroxide; reagent 2, 0.2 *M* sodium acetate–2-propanol (1:1, v/v); reagent 3, 2.5 *M* acetic acid; reagent 4, glacial acetic acid; reagent 5, 8 *M* acetic acid; reagent 6, methanol–2 *M* sulphuric acid–70% perchloric acid (1:0.5:0.5, v/v); reagent 7, 2 *M* sulphuric acid; reagent 8, potassium bromide (5.2 g) and potassium bromate (1.1 g) in 1000 ml of deionized water; reagent 9, 0.015 *M* sodium arsenite; reagent 10, 0.032 *M* cerium ammonium sulphate in 0.5 *M* sulphuric acid; reagent 11, iodide standard stock solution (50 μg/ml I^-) prepared by dissolving 84.310 mg of potassium iodate in 1000 ml of distilled water; reagent 12, 0.1 *M* mercury(II) acetate.

Preparation of the standard reference solutions

Reagent 11 (1.0 ml) was pipetted into a 100-ml volumetric flask and brought to volume with deionized water. Then 2.0, 4.0, 6.0 and 8.0 ml of this diluted reagent 11 were pipetted into a series of 100-ml volumetric flasks. The I^- value of the four solutions is equivalent to 20.0, 40.0, 60.0 and 80.0 μg per 100 ml, respectively, for the calibration curve of the radiopaque organic iodine agents (and to 2.0, 4.0, 6.0 and 8.0 μg per 100 ml, respectively, for the calibration curve of the hormonal iodine).

Chromatographic separation

The mini-column was washed with 3.0 ml of reagent 1. Then 1.0 ml of plasma, diluted with 3.0 ml of reagent 1, 10.0 ml of reagent 2, 1.0 ml of reagent 3 and 0.5 ml of reagent 4 were added in sequence. All eluates were discarded. Thyroxine was eluted with 6.0 ml of reagent 5 (eluate 1). The mini-column was washed with 5.0 ml of distilled water and the iodinated contrast agent was eluted with 6.0 ml of reagent 6 (eluate 2).

Acid bromination

Acid bromination was carried out on the standards, blanks and samples with reagents 6 and 8, as detailed in Table I. Each tube was mixed well, and the level of the solution in each tube was marked. The tubes were incubated for 30 min at 110°C in the oven, then cooled under running water. Each solution was restored to its original level by adding deionized water. In the meantime hormonal iodine was determined (if required) according to the procedure outlined in Table II.

Colorimetric determination

Both sets of tubes were incubated for 5 min at 37°C. Reagent 9 (1.0 ml) was added to each tube, then at regular intervals of 30 s (or 1 min) 1.0 ml of reagent 10 was pipetted into each tube. Immediately after each addition each tube was mixed well and incubated again at 37°C. Exactly 20 min after addition of reagent 10 to the first tube, three drops (0.015 ml) of reagent 12 were added in exactly the same sequence and with the same time interval used for the addition of reagent 10. Each tube was immediately shaken to stop the reaction. All the solutions (blanks, samples and standards) were read at 430 nm against water. Two calibration curves were drawn with the two sets of standards: one for the exoge-

TABLE I

VOLUMES OF REACTANTS USED IN BROMINATION OF CONTRAST MEDIA

	Sample	Blank	Std. 20	Std. 40	Std. 60	Std. 80
Eluate 2	0.30 ml	–	–	–	–	–
Std. 20 μg I^- per 100 ml	–	–	1.0 ml	–	–	–
Std. 40 μg I^- per 100 ml	–	–	–	1.0 ml	–	–
Std. 60 μg I^- per 100 ml	–	–	–	–	1.0 ml	–
Std. 80 μg I^- per 100 ml	–	–	–	–	–	1.0 ml
Water	3.0 ml	3.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent 6	2.7 ml	3.0 ml	3.0 ml	3.0 ml	3.0 ml	3.0 ml
Reagent 8	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml

TABLE II

VOLUMES OF REACTANTS USED IN BROMINATION OF THYROXINE

	Sample	Blank	Std. 20	Std. 40	Std. 60	Std. 80
Eluate 1	3.0 ml	–	–	–	–	–
Std. 2 μg I^- per 100 ml	–	–	1.0 ml	–	–	–
Std. 4 μg I^- per 100 ml	–	–	–	1.0 ml	–	–
Std. 6 μg I^- per 100 ml	–	–	–	–	1.0 ml	–
Std. 8 μg I^- per 100 ml	–	–	–	–	–	1.0 ml
Reagent 5	–	3.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Reagent 7	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 8	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml	0.25 ml

nous iodine and the other for the hormonal iodine (if required). The values of the samples were read on the proper calibration curve.

RESULTS AND DISCUSSION

This study tested some radiopaque organic compounds used in urography and cholecystography and/or cholangiography. Among the compounds used for urography and cholecystography there were ionic monomers (diatrizoate, iothalamate, iodamide) and a non-ionic monomer (iopamidol). Among the compounds used for cholangiography there were only ionic dimers (iotroxinate and iodossamate). The chromatographic behaviour of all the molecules tested was the same.

The analytical procedure is derived from the well known T4 iodine determination of Pileggi and Kessler [6], in which the hormone is determined after separation by anion-exchange chromatography [6–11], quantitatively displacing the iodine with bromine and measuring the iodine by the catalytic method of Sandell and Kolthoff [8,10–12]. The novelty of this method is that this separation technique has been applied to determine organoiodate contrast media. This method is more precise than the protein-bound iodine (PBI) [2,4,13–16], competitive protein-binding (CPB) [15] and butanol-extractable iodine (BEI) [3] methods which do not separate the contrast media or thyroxine efficiently. In fact, with these methods, the contrast media normally interfere with the determination of T4.

With the described procedure the chromatographic separation of thyroxine and radiopaque compounds is so precise that it is possible to determine the normal levels of hormonal iodine even in the presence of maximum levels of radiopaque iodine ($60 \cdot 10^4$ μg per 100 ml of plasma). The same results cannot be achieved with other methods that employ cationic resins [4,5,17].

We were able to determine that the contrast medium was totally absorbed by the resin. No traces of iodine, after acid bromination at 110°C, were found in the eluate. Oven incubation at 110°C of the solution containing eluate 2 for acid bromination of the contrast media is a necessary step for the quantitative determination of exogenous iodine, as described in a previous paper [11].

After comparison of two equivalent series of aqueous solutions of each contrast medium, the first of which was absorbed and eluted from a mini-column, a recovery of 98–100% was found. When the contrast medium was added to drug-free sera, the mini-columns again yielded a 100% recovery. Moreover, it was found that all the added thyroxine was completely eluted before elution of the contrast medium began. Even though massive amounts of contrast media drugs were added to sera containing predetermined minimal amounts of thyroxine, no detectable interference of the contrast media with the thyroxine present was found [11].

In eluate 1, no leakage of the contrast medium was found, but if it had been eluted, it wouldn't have caused any interference because hormonal iodine is easily substituted by bromine, in the presence of sulphuric acid, whereas the more stable iodine in the contrast medium requires perchloric acid in the reacting mixture [11]. So bromination without perchloric acid gives a more precise determination of hormonal iodine since it eliminates interference by the contrast media [11].

This method is highly sensitive: less than 1 μg I^- per 100 ml of serum can be determined. For this reason even traces of contrast media can be determined quite

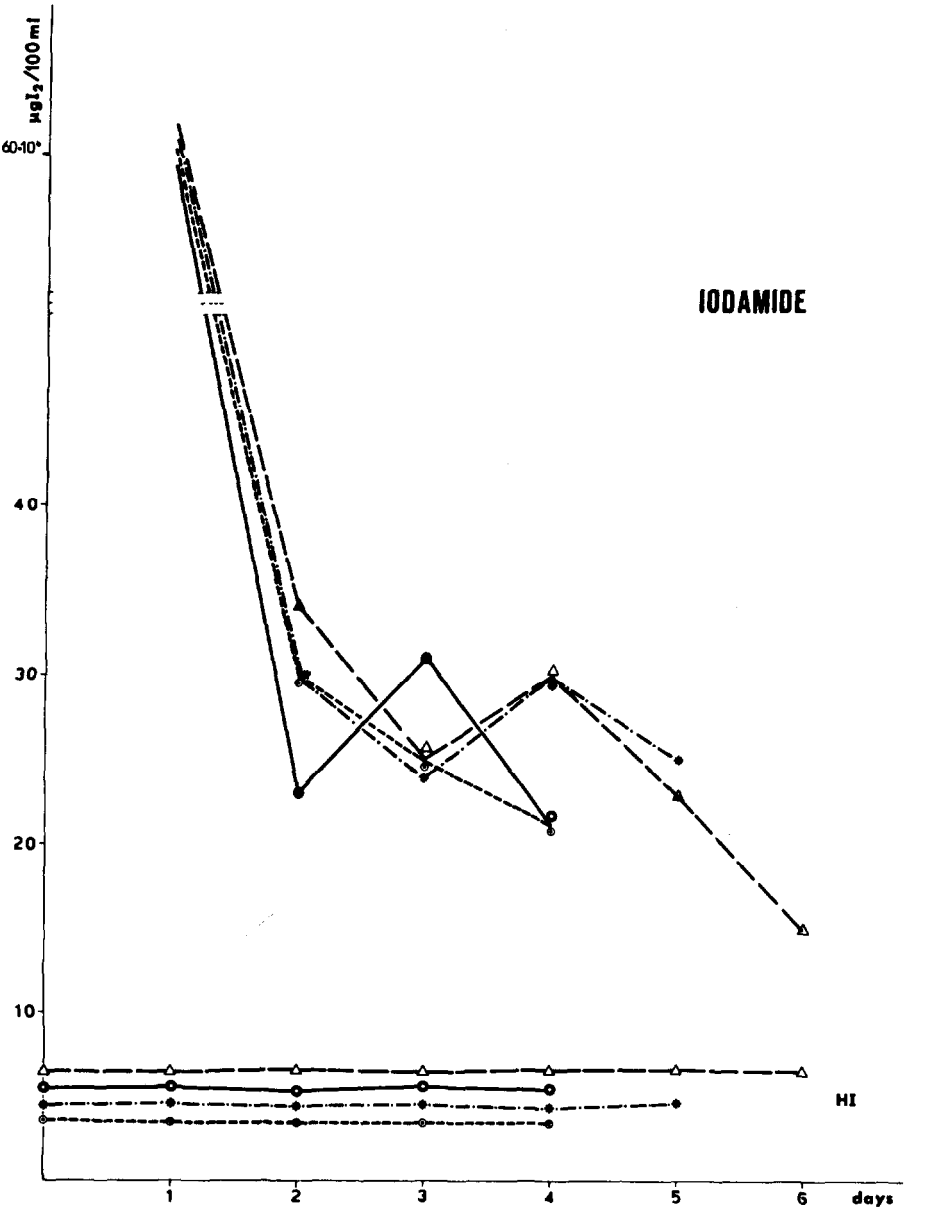

Fig. 1. Iodamide (Angiografin-Bracco) and hormonal iodine (HI) plasma levels in four patients during the first four to six days after administration of the radiopaque diagnostic agent. The amount of exogenous iodine rapidly decreases during the first 48 h; then in one case it continues to decrease and in the others there is an unexpected increase of ca. 10 μg I^- per 100 ml of plasma followed by renewed decrease. The level of thyroxine remains unchanged throughout the entire test period.

precisely with 1.0 ml of serum. If this procedure is followed carefully and accurately, consistently precise results can be obtained within ± 0.1 μg I^- per 100 ml [11].

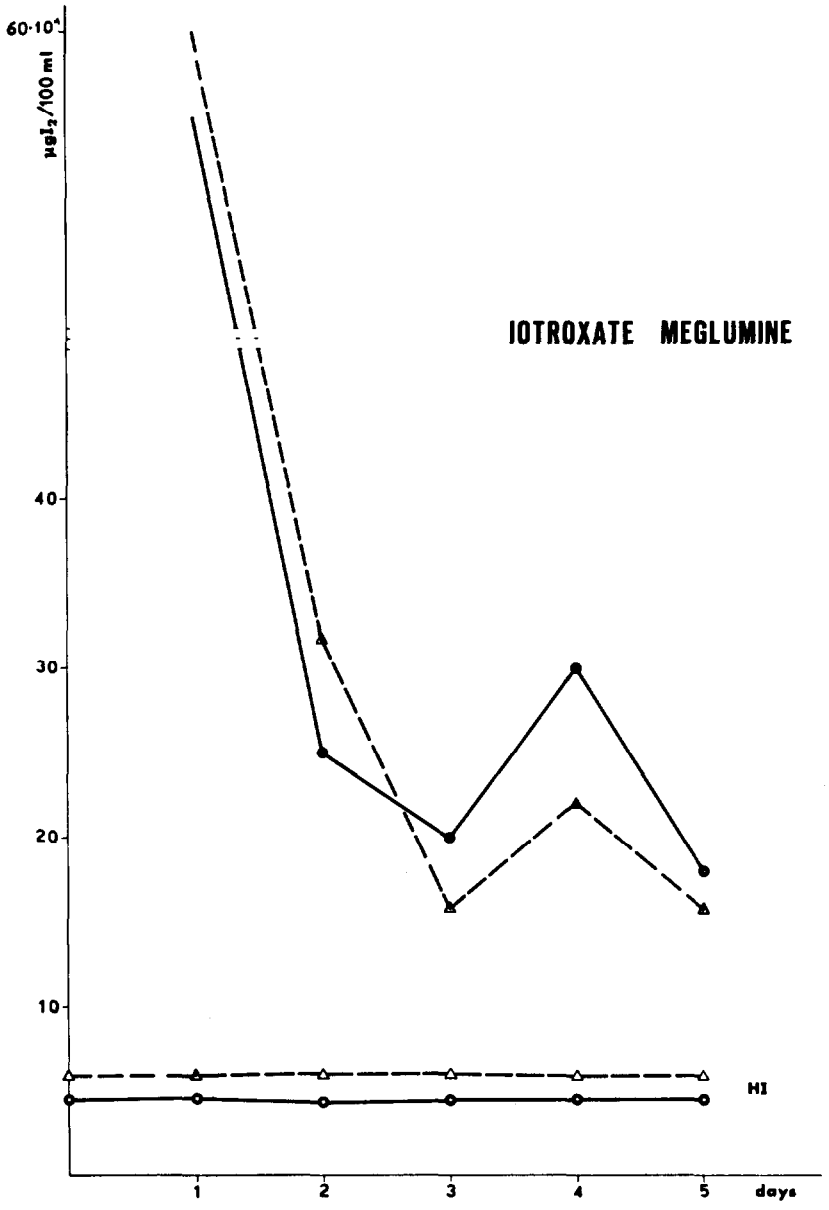

Fig. 2. Iotroxate meglumine (Chologram-Shering) and hormonal iodine (HI) plasma levels of two patients during the first five days after administration of the radiopaque diagnostic agent. Excretion of the radiopaque medium surprisingly follows the same pattern as the iodamide graph in Fig. 1, despite the fact that iodamide is rapidly excreted through the kidneys and iotroxate is excreted through the bile. The level of thyroxine remains unchanged, as in Fig. 1, throughout the entire test period.

The method can be usefully employed to investigate hormonal iodine when the radiopaque agent is present in the serum. It is a well known fact that various radiopaque iodine compounds, such as diatrizoate, must be used with caution in

patients with hyperthyroidism, or an autonomously functioning thyroid nodule, since thyroid storm has reportedly occurred in some of these patients following intravascular administration of a contrast medium [18]. The method can also be used where suspicion of the presence of these radiopaque agents could invalidate the thyroid function tests based on measurements of iodine.

It is well known that some of the contrast media are completely eliminated in ca. sixteen days (iothalamate and diatrizoate), whereas others take ca. three or four months (iodipamide), or up to several years (iophendylate) [3,4,18].

Laurberg and Boye [19] recently reported that the cholecystographic contrast agents ipodate, iocetamate, iodipamide, ioglycamate and iotroxate induced a rapid, sustained and reversible inhibition of thyroxine secretion from perfused dog thyroid lobes. On the other hand, none of the contrast media excreted through the kidneys: amidotrizoate, metrizamide, metrizoate, iodamide, diodone and ioxithalamate influenced thyroxine secretion. However, they found that diodone and all the cholecystographic contrast agents also inhibited thyroxine deiodinases from liver and thyroid.

In a group of hospitalized patients tested daily with the procedure described here, it was not possible to see any real variation of the plasma thyroxine level, for at least the four- to six-day period during which all patients were examined (as can be seen in Figs. 1 and 2).

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